Antifungal Terpenoid and Troponoid Analogues for Wood Durability Enhancement

A thesis

submitted in partial fulfilment

of the requirements for the Degree

of

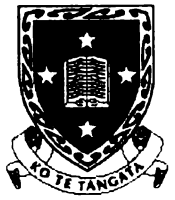
Doctor of Philosophy in Chemistry

at the

University of Waikato

by

Kirk Torr



The University of Waikato

Te Whare Wānanga o Waikato

University Of Waikato

2002

Abstract

Aspects of the development of novel analogues of terpenoid and tropolone natural products with antifungal properties against important wood deteriorating fungi are reported. Antifungal wood extractives and essential oil components provided valuable leads in chemical syntheses toward novel compounds with potential as wood bio-protectants.

Analogues of antifungal monoterpenoid and diterpenoid wood extractives were synthesised and tested for activity against wood damaging fungi. 1-Methyl-4-nonylcyclohexan-1-ol, an analogue of β-terpineol and 2-isopropyl-4-nonylphenol, an analogue of thymol were prepared from *p-n*-nonylanisole by multi-step syntheses. 2-Isopropyl-1-naphthol, a simplified analogue of totarol was prepared by C-isopropylation of β-naphthoxide. In antifungal activity assays, these terpenoid analogues showed good to moderate activity against the brown rot fungus *Coniophora puteana* but were, in general, only weakly active against the other test decay and staining fungi.

A bio-activity led investigation of oxygenated sesquiterpenoids from the foliage essential oils of three New Zealand native softwoods, totara, rimu and kahikatea, identified caryophyllene oxide, humulene oxide II, globulol and α-cadinol as major components of fungistatic oil fractions. Based on these findings simple synthetic compounds with epoxide (4-nonylcyclohexan-1,2-epoxide) and tertiary alcohol functionalities (4-ethyl-1-methyl-1,2,3,4-tetrahydronaphth-1-ol and 5,9-dimethyl-6,7,8,9-tetrahydrobenzocyclohepten-5-ol) were synthesised by multi-step pathways. Bioassays against wood decay and sapstain fungi revealed these compounds possessed efficacies equal to, or in some cases superior to, the natural sesquiterpenoids.

A range of known and novel tropolone compounds was prepared by adapted literature methods. 3-Carboxy-4-carboxymethyltropolone, prepared by oxidation of purpurogallin, was a key intermediate in the synthesis of 4-alkylcarboxytropolones, an alkyl δ-lactone tropolone, 4-alkenyl and 4-alkyltropolones and 4-styryltropolones. A new synthetic pathway to β-thujaplicin *via* oxidation of 1-methyl-3,4,6-trihydroxy-(5*H*)-benzocyclohepten-5-one was proposed and investigated, but proved unsuccessful. A series of novel 3-alkyltropolones were prepared by direct alkylation of tropolone and β-thujaplicin with diethyl acetals. 3-(1-

Formylmethyl)tropolone, prepared by condensation of *trans*-hex-2-enal diethyl acetal with tropolone, proved a useful intermediate to tropolones with secondary functional groups. A selection of these synthetic tropolones exhibited potent antifungal properties against four wood damaging fungi, in particular *Coniophora puteana*. Efficacies were comparable to and, in certain cases, superior to the commercial wood preservative tri-*n*-butyltin napththenate (TBTN). The best performing of the tropolones was 3-(1-ethyl)butyltropolone. This compound exhibited greater fungitoxicity than TBTN against the white rot and soft rot test fungi and was of comparable activity to the commercial antisapstain agent 3-iodo-2-propynyl butyl carbamate against the sapstain fungus *Sphaeropsis sapinea*.

Acknowledgements

First and foremost, I wish to thank my Forest Research supervisor, Dr Robert Franich for his guidance and support throughout this project. Robert was instrumental in defining the topic and provided valuable advice throughout. I greatly appreciated his enthusiasm for the research and his encouragement and suggestions in negotiating the challenges of chemical synthesis. I also wish to thank my two University of Waikato supervisors, initially Dr Rick Ede (now with FPP, CSIRO) and then Dr Alistair Wilkins, for their support throughout this work. I am grateful to Alistair for his helpful suggestions during writing up.

I wish to acknowledge the financial assistance provided by AGMARDT (The Agricultural and Marketing Research and Development Trust) in the form of a Ph.D scholarship. Without the financial support of Forest Research, which included employment and provisions for lab and office space, chemicals and external lab analysis, this project would not have been possible. Funding from Forest Research also allowed me to participate in the 1997 Pacific Oils Conference in Auckland and the 1999 New Zealand Institute of Chemistry Conference in Wellington. I wish to acknowledge funding for the project from the Foundation for Research Science and Technology administered through HortResearch, Hamilton as part of the programme of research headed by Dr Pat Holland (now with the Cawthorne Institute, Nelson).

Thanks must go to Dr Roger Meder and Dr Stefan Hill for assistance running NMR experiments and to Hank Kroese, Murray Robinson and Murray Smith for help running the Probe MS, GC/MS and ES/MS instruments. Hank was always willing to assist on the frequent occasions the old HP 5985 GC/MS decided to fall over. I would like to thank Diane Steward, for letting me borrow various chemicals and equipment and for her assistance in sourcing lab consumables. Thanks also to Dr Ian Suckling and Dr Bernhard Kerber for helpful discussions on synthetic chemistry and wood mycology. A special thanks to, Jackie van der Waals, Diahanna O'Callahan and Colleen Chittenden for teaching me the antifungal assays, providing fungal cultures and for allowing me to invade their lab and make use of their equipment. Thanks must go to Professor R. C. Cambie for the gift of the α-cadinol and foliage essential oils and to Kathryn Anderson, Clinton Handcock and Garth Weinberg for supply of thermomechanical pulp and commercial newsprint paper. Thanks also to Kathryn for teaching me how to prepare mechanical pulp handsheets.

I would like to thank my work colleagues at Forest Research who were a pleasure to work with, in particular Robert Franich, Dianne Steward and Hank Kroese. On a personal level, thanks to my friends, in particular Sheryl, Jason, Stu, Andy, Ruben, Dave, Kelpy, Garth and Murray who made my stay in Rotorua an enjoyable one and provided many excellent opportunities for stress relief. A special thank you to Sheryl for your friendship, support and understanding and for the excellent job proof reading this thesis.

Finally I wish to thank my family, especially my parents Bob and Carol, for their love, support and encouragement, without which I would not be where I am today.

Table of Contents

List of Figures

Chapter 4: Synthesis of Tropolone Analogues

List of Schemes

List of Tables

Page

Chapter 5: Antifungal Activity Assays

Chapter 7: Experimental

Appendices

List of Abbreviations

AA	antibiotic assay
AAC	alkyl ammonium compound
ACA	ammoniacal copper arsenate
ACB	ammoniacal copper borate
Al-HDO	N-cyclohexyldiazeniumdioxy chelates of aluminium
APCI	atmospheric pressure chemical ionisation
AR	analytical reagent
ATP	adenosine triphosphate
Av.	average
b.p.	boiling point
BSA	bis(trimethylsilyl)acetamide
BSTFA	bis(trimethylsilyl)trifluoro-acetamide
°C	degrees Celsius
ca.	*circa* (*L* about)
CCA	copper-chromium-arsenic
CCB	copper-chromium-boron
CCF	copper-chromium-fluoride
CCFB	copper-chromium-fluoroborate
CCP	copper-chromium-phosphorus
CE	Collision Energy
cf.	*confer* (*L* compare)
cm	centimetres
cm^{-1}	reciprocal centimetres
conc.	concentrated
Cu-HDO	N-cyclohexyldiazeniumdioxy chelates of copper
d	doublet
δ	chemical shift
Da	Daltons
DCC	dicyclohexylcarbodiimide
DDAC	didecyldimethyl ammonium chloride
DIP	direct insertion probe
DMAPP	dimethylallyl pyrophosphate
DMSO	dimethyl sulphoxide
e.g.	*exempli gratia* (*L* for example)
EI	electron impact
ES	electrospray
ESI	electrospray ionisation
ES/MS	electrospray mass spectroscopy
et al.	*et alia* (*L* and others)
eV	electron volts
FCAP	fluor chrome arsenate phenol
FPP	farnesyl pyrophosphate
FTIR	fourier transform infrared
g	grams
GAP	glyceraldehyde-3-phosphate
GC	gas chromatography
GC/MS	gas chromatography/mass spectroscopy
gCOSY	gradient correlation spectroscopy
GGPP	geranyl geranyl pyrophosphate
GPP	geranyl pyrophosphate

HMBC	heteronuclear multiple bond correlation
HMQC	heteronuclear multiple quantum coherence
hr	hour
Hz	hertz
i.e.	*id est* (*L* for that is)
IPBC	3-iodo-2-propynyl butyl carbamate
IPP	isopentenyl pyrophosphate
IR	infrared
J	coupling constant
K	degrees Kelvin
Kf	kahikatea oil column chromatography fraction
kV	kilovolts
L	Latin
LD_{50}	median lethal dose
LOSP	light organic solvent preservative
m	multiplet and metres
m-	meta-
$M^{\cdot+}$	molecular ion
MBT	methylene bisthiocyanate
MHz	mega hertz
MIC	minimum inhibitory concentration
min	minutes
ml	millilitres
mm	millimetres
μm	micrometres
mmol	millimole
m/m	mass to mass ratio
mol	mole
m.p.	melting point
ms	milliseconds
μs	microseconds
MS	mass spectroscopy
MW	molecular weight
m/z	mass to charge ratio
n-	normal
ν	stretching
NaPCP	sodium pentachlorophenate
NMR	nuclear magnetic resonance
o-	ortho-
p	pentet
p-	para-
PCP	pentachlorophenol
pp	pages
ppm	parts per million
psi	pounds per square inch
q	quartet
Rf	rimu oil column chromatography fraction
RI	retention index
Rt	retention time
s	singlet
sec	seconds
S_N1	substitution, nucleophilic, unimolecular
S_N1cA	substitution, nucleophilic, unimolecular, conjugated acid
t	triplet
t-	tert-
T	Tesla
TBTN	tri-*n*-butyltin naphthenate

TBTO	bis(tri-n-butyltin) oxide
TCMTB	2-(thiocyanomethylthio)-benzothiazole
TF	totara oil distillation fraction
TF8f2	totara oil distillation fraction 8, column chromatography fraction 2
THF	tetrahydrofuran
tlc	thin layer chromatography
TMCS	chlorotrimethylsilane
TMP	thermomechanical pulp
TMS	tetramethylsilane
TMSi	trimethylsilyl
TsOH	toluene sulphonic acid
UV	ultraviolet
V	volts
w.r.t	with respect to
1°	primary
2°	secondary
3°	tertiary
1D	one dimensional
2D	two dimensional

Chance favours the prepared mind

Louis Pasteur

Chapter 1: A Review of the Chemistry of Preventing Biodeterioration of Wood

1.1 Introduction

Wood is one of the world's most valuable natural resources. A versatile material by virtue of its unique physical and engineering properties, wood has been used throughout history as a construction material in a variety of end-uses. Mankind's desire for timber products led initially to large scale felling of natural forests worldwide. However, as worldwide demand for timber and wood products increased and native timbers became scarce, the forestry industry shifted its focus towards plantation forests of fast growing softwood species. Many of these plantation softwoods have low natural durability and are susceptible to deterioration by biological organisms.

The wood preservation industry grew out of the need to protect susceptible timbers from biodeterioration. Modern wood preservation technology involves impregnating wood with broadly active biocidal agents, which kill or inhibit the growth of wood damaging organisms. Treatment prolongs the service life of the timber allowing non-durable timbers to be used in situations of high biological hazard. This in turn has eased the demand on rare naturally durable species of conservational significance. In New Zealand, as in other countries of temperate climate, wood decay fungi are the most important wood deteriorators. According to Hedley (1), fungal decay accounts for 99% of the biodegradation of treated timber used in service in New Zealand. Staining fungi, which discolour unseasoned timber, can also cause significant losses in value of the timber and hence reduce profit margins. Freshly felled and unseasoned timber which is being processed, stored or transported requires protection from attack by staining fungi. Topical application of biocidal agents *via* dipping or spraying offers short-term protection against these organisms.

In today's environmentally conscious times, the major challenge facing the wood protection industry is the need to find safer alternatives to toxic biocides that have traditionally been used

in wood preservation and the control of fungal stain. Occupational health and environmental safety are sensitive issues in the wood protection industry. Many important advances towards alternative chemicals have been made building on the knowledge gained from fungal and insect pest control programmes in the agricultural sector. However, many of these second-generation chemicals still have appreciable mammalian toxicities and/or their environmental safety has not been adequately established. Ideal wood preservatives and antistaining chemicals are yet to be developed and novel methods of protecting timber against biological attack can offer attractive alternatives to current technologies. Some of the more novel approaches under investigation include chemical modification of wood, biological control and the use of natural products (for example wood extractives).

The research described in this thesis focuses on the approach of using wood extractives from naturally durable timbers as lead compounds to novel synthetic analogues with potential for wood protection. Two classes of wood extractives were investigated, terpenoids and tropolones. The research can be subdivided in four sections:

- syntheses of terpenoid analogues of known antifungal wood extractives (Chapter 2);

- a bio-activity led investigation of natural sesquiterpenoids from three native New Zealand timber species and the preparation of simple synthetic compounds based on these sesquiterpenoids (Chapter 3);

- syntheses of tropolone analogues of known antifungal wood extractives (Chapter 4);

- evaluation of the wood protection potential of the synthetic terpenoid and tropolone analogues through antifungal activity testing against common wood damaging fungi (Chapter 5).

The overall aim of this research was not the development of a novel commercial wood preservative or antistain chemical, as this was not a realisable goal. The objective was to test the validity of this novel approach to wood protection and gain some insights into chemical structures important in toxicity to wood decay and staining fungi.

1.2 Biodeterioration of Wood

1.2.1 Wood Chemistry

Central to any discussion of biodeterioration of wood is an understanding of wood chemistry. Reviews of this topic can be found in Fengel and Wegener (2), Sjostrom (3) and Hon and Shiraishi (4). In this Section some key aspects of wood chemistry are reviewed.

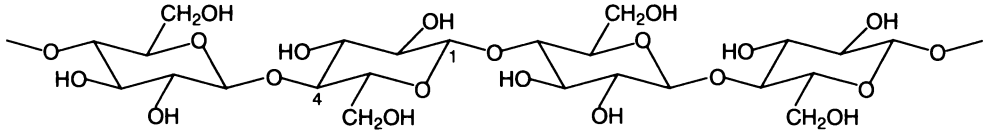
Wood is a complex composite composed of three major polymeric materials; cellulose, hemicelluloses and lignin. These bio-polymers make up the wood cell wall. Cellulose is the main structural component providing strength to the cell wall while hemicelluloses and lignin are present as matrix materials (2, 3, 4). Cellulose content in softwoods ranges from 38-52%, hemicellulose content from 16-27% and lignin content from 26-36% (4).

Cellulose is a linear polymer of anhydro-D-glucose units linked by β-1,4-glycosidic bonds (Figure 1.1). Each molecule of cellulose is composed of 8000-10000 glucose units (5). Cellulose molecules are arranged into linear bundles called elementary fibrils. Within the fibrils intermolecular hydrogen bonding aligns the cellulose molecules laterally giving rise to crystallinity. X-ray diffraction measurements indicate that approximately 70% of wood cell wall cellulose is crystalline (6). Elementary fibrils are aggregated together to give microfibrils with the spaces between the microfibrils filled by hemicelluloses and lignin.

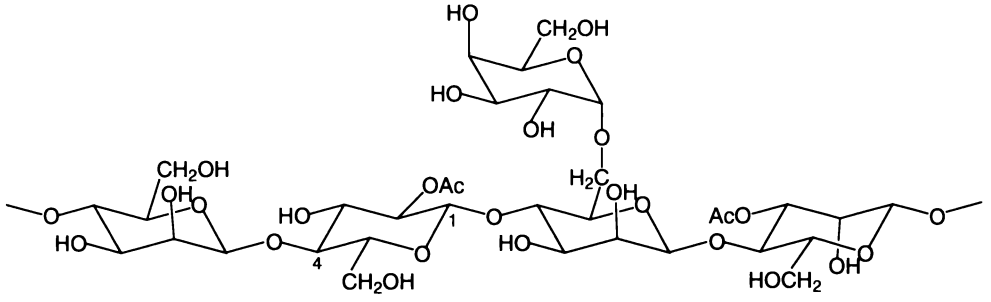
Hemicelluloses are polymers of anhydrosugar units linked by glycosidic bonds. The component monosaccharides include D-glucose, D-mannose, D-xylose, L-arabinose, D-galactose, L-rhamnose, 4-*O*-methyl-D-glucuronic acid, D-glucuronic acid and D-galacturonic acid (3, 4). Hemicelluloses, in general, are relatively short, branched polymers of up to 200 sugar units (2, 3). The major hemicelluloses found in softwoods are *O*-acetylgalacto-glucomannans (*ca.* 20%), which consist of a glucomannan backbone with acetyl and galactose residues attached (Figure 1.1) (3). Conifers also contain an arabino-4-*O*-methylglucurono-xylan, which has a xylan backbone with arabinose and 4-*O*-methylglucuronic acid substituents, in significant amounts (5-10%) (3). Other polysaccharides are present in minor amounts although arabinogalactans are found in large amounts in some species.

Hemicelluloses exist in the amorphous state in the cell wall forming a matrix which surrounds the cellulose microfibrils (2, 6).

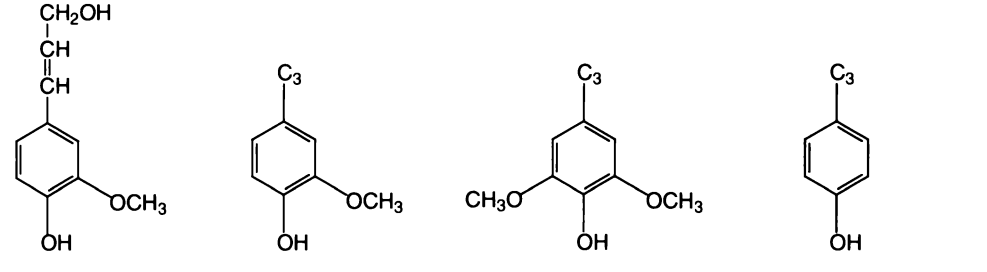
Lignin is an extremely complex, amorphous, highly branched three-dimensional polymer composed of oxyphenylpropane units. Lignin in softwoods is formed primarily by dehydrogenative polymerisation of coniferyl alcohol (Figure 1.1) and is consequently mainly comprised of polymers of guaiacyl units (Figure 1.1) with only minor amounts of syringyl and *p*-hydroxyphenylpropane units (2, 3, 4). Lignin serves as an encrusting material surrounding the cellulose microfibrils, adding strength to the cell wall (2, 4). Part of the lignin is believed to be chemically bonded to the polysaccharide polymers with lignin-hemicellulose linkages predominating over lignin-cellulose linkages (2).



Partial structure of cellulose



Partial structure of *O*-acetylgalactoglucomannan



Coniferyl alcohol Guaiacyl Syringyl Hydroxyphenylpropane

Figure 1.1: Cellulose, hemicellulose and lignin precursor components of wood.

A number of minor components including wood extractives, starch, low molecular weight sugars, pectin, nitrogenous materials and minerals are also present (2, 6). Their roles are functional rather than structural and many are important in biodeterioration of timber. Wood extractives, in particular, can greatly influence the durability of timber (Section 1.5).

1.2.2 Wood Damaging Fungi

Wood damaging organisms include wood decay fungi, staining fungi, insect borers, bacteria and marine boring animals. In terrestrial environments of temperate climate wood decay fungi are generally the most problematic. They cause the greatest damage in terms of economic losses. Decay fungi and staining fungi are the organisms of primary interest in this study and hence will be discussed in greatest detail. True fungi, Eumycota, are classified into five sub-divisions. Three of these sub-divisions, Ascomycotina, Basidiomycotina and Deuteromycotina (Fungi imperfecti) contain members that are important deteriorators of timber in service and freshly-felled green timber. Wood damaging fungi can be grouped into decay fungi and staining fungi.

1.2.2.1 Decay Fungi

Decay fungi can be categorised into three groups, brown rot fungi, white rot fungi and soft rot fungi. Brown and white rot is caused by basidiomycetes, while members of Ascomycotina and Deuteromycotina cause soft rot. Decay fungi break down the cellulose, hemicellulose and lignin polymers of the wood cell wall to simple sugars and phenylpropane units that can be absorbed and metabolised (see Section 1.2.3). Additional requirements for nutrition include trace minerals, thiamine and nitrogen. Oxygen, moisture (wood moisture content of >20%), a temperature range of 10-40°C and a pH between 2-7 (optimum 4.5-5.5) are all necessary elements for fungal decay (7). Decay fungi degrade cell wall components and hence affect the structural integrity of the timber.

Brown rot fungi include many of the most important destroyers of timber in service, species such as *Coniophora puteana* (the cellar fungus), *Serpula lacrymans* (the dry rot fungus), *Gloeophyllum trabeum* and *Oligoporus placenta* (8). Brown rot fungi preferentially degrade cellulose, leaving lignin largely intact which gives the decayed wood a dark brown

appearance. The hyphae grow mainly within the cell lumen decomposing cellulose in a diffuse and incomplete manner throughout the entire wall (9). Brown rot fungi hyphae are believed to produce a freely-diffusible extracellular non-enzymatic wood degradation system, which is able to degrade cellulose some distance from the hyphae (Section 1.2.3). Lignin within the partially degraded cell wall maintains the cell shape until finally in the late stages of decay the residual cell wall collapses giving the decayed wood a cracked and charred appearance (Figure 1.2).

Figure 1.2: Damage caused by a brown rot decay fungus on untreated timber (Photo courstesy of Forest Research).

White rot fungi are wood decaying basidiomycetes that are capable of degrading all wood cell wall components. There are two types of white rot; simultaneous rot, where all wood components are degraded at a similar rate and preferential rot, where lignin and hemicelluloses are degraded prior to cellulose being attacked. Compared to brown rot fungi, relatively few white rot fungi are important decayers of commercial timber (8). These include *Trametes versicolor*, *Phanerochaete chrysosporium* and *Pleurotus ostreatus*. White rot produces decayed timber with a lightened bleached appearance and fibrous texture (Figure 1.3). The whitened appearance is due to lignin removal and oxidative bleaching (10). Generally hardwood species are more susceptible to white rot than are softwoods (Section 1.2.3). The fungi grow principally in the cell lumen. Degradation involves progressive decomposition of the cell wall bio-polymers from the lumen outward, resulting in erosion troughs, bore holes and a gradual thinning of the cell wall (11). Decay is localised close to the hyphae and is relatively complete before fresh surfaces are attacked (9).

Figure 1.3: Damage caused by a white rot decay fungus on untreated timber (Photo by John Barran).

Soft rot fungi attack wood under conditions where the growth of the more competitive basidiomycetes is inhibited, such as timbers with high moisture contents and low aeration or preservative treated timbers (7, 8). Important deteriorators of timber include *Chaetomium globosum*, *Lecythophora hoffmannii* and *Cephalosporium acremonium* (8). Soft rot fungi break down both polysaccharides and lignin. Their lignolytic capabilities are inferior to those of white rot fungi. Attack by soft rot fungi is largely superficial (Figure 1.4). When the timber is wet the surface becomes soft and discoloured.

Figure 1.4: Damage caused by a soft rot fungus on untreated timber (Photo courtesy of Forest Research).

Soft rot fungi infest a wider range of timbers than both brown and white rot fungi, although attack of hardwoods is more prevalent (12). Soft rot fungi degrade the cell wall by creating diamond-shaped cavities and by eroding luminal surfaces (13, 14). In contrast to decay by basidiomycetes, where one fungus tends to dominate in the wood, soft rot decay is characterised by a succession of fungi (8). Some soft rot fungi are capable of detoxifying

certain wood preservatives (at marginal concentrations) rending these treatments less effective against basidiomycetes (7).

1.2.2.2 Staining Fungi

The term 'staining fungi' can be used to describe sapstain fungi and moulds that cause discolouration on freshly-felled and unseasoned timber and blue stain fungi that discolour timber in service. Staining fungi comprise members from Ascomycotina and Deuteromycotina. Staining fungi consume soluble carbohydrates, proteins and lipids found in the living parenchyma cells of wood (15). Attack is consequently restricted to sapwood, as heartwood does not contain this readily assimilable food source. Generally softwoods are more susceptible than hardwoods. Pine species are particularly vulnerable (7). Because staining fungi do not degrade wood cell wall components their action does not appreciably reduce the strength of timber. The development of fungal stain on timber is of significant economic importance to timber suppliers due to associated losses in product quality and market value. Wood infected with fungal stain may be suitable for structural purposes but is unacceptable for high value appearance products where aesthetics is important.

Sapstain fungi attack freshly-felled timber and unseasoned lumber. Colonisation *via* the ray cells can take place in softwoods that have initial moisture contents of 100-130% based on the dry weight of the wood (16). The coloured hyphae rapidly proliferate along the ray parenchyma cells resulting in internal staining characterised by wedge-shaped zones of discolouration revealed on cross-sectional surfaces (Figure 1.5). Mature hyphae contain brown melanin granules. Extensively colonised wood appears blue-black due to light diffraction (16). Melanin is believed to be produced by the fungi as a means of protection against UV light damage (17). Fungi often implicated in sapstain include *Ophiostoma* and *Ceratocystis* species and *Sphaeropsis sapinea* (15, 18).

Staining fungi can infect timbers in service, like external joinery, which periodically absorbs moisture, resulting in the development of blue stain. Typically the staining pattern involves patches of discolouration on the wood surface due to fungal mycelium and spores. The mould *Alternaria alternata* and the black yeast *Aureobasidium pullulans* have been found to cause

blue stain (15, 16). The development of blue stain on timbers in service is of relatively minor economic importance compared to sapstain on freshly-felled timber.

Figure 1.5: Discolouration on unseasoned timber caused by a sapstain fungus (Photo courtesy of Forest Research).

Moulds utilise starch and sugars in superficial parenchyma tissues. Discolouration is therefore confined to timber surfaces and is chiefly caused by the development of masses of coloured spores. Moulds are of relatively minor economic importance compared to decay and sapstain fungi. In many cases the pigmented spores can be removed by brushing or planing the wood surface, although some moulds produce a penetrating stain (15). Common wood moulds include species of *Trichoderma* and *Penicillium* (green moulds), *Gliocladium* (green-pink discolourations) and *Alternaria* and *Aspergillus* (black discolourations) (16). Moulds frequently inhabit timber in conjunction with other stain or decay fungi (7, 16). Wood moulds are more tolerant to unfavourable conditions than decay fungi, including low moisture contents (<20%), higher temperatures (>40°C) and preservative treatments (7). In some case moulds have been observed to degrade certain wood preservatives (19).

Staining fungi, in general, do not appreciably reduce the strength of the infested timber. Significant losses in toughness (resistance to sudden shock) have been observed in intensely stained pine sapwood (20). Staining fungi and moulds, like decay fungi, enhance the permeability of infected timber due to fine perforations of the cell wall and disruption of ray cells and pits (9, 16). Increased permeability can cause the timber to dry out more rapidly shifting the timber environment towards more favourable decay conditions.

1.2.2.3 Microbial Succession

The microbial deterioration of timber follows a succession of micro-organisms, which reflects the environment of the timber and the changing nutrient status and moisture conditions of the wood. Clubbe (21) proposed a model for succession of micro-organisms into wood in ground contact (Figure 1.6).

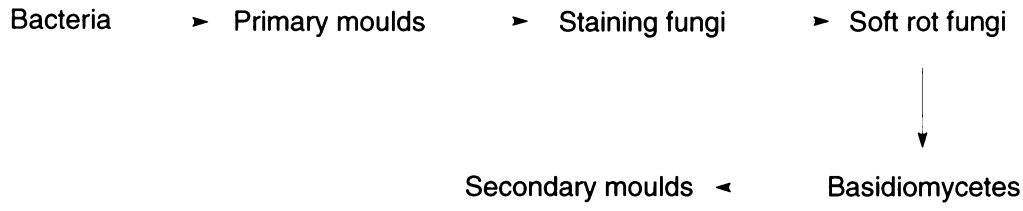


Figure 1.6: Model for the succession of micro-organisms into wood in ground contact.

Bacteria and primary moulds invade initially as they have highly competitive saprophytic ability and can best compete for available soluble nutrients. As surface carbohydrates are depleted sapstain fungi and soft rot fungi colonise and grow. The permeability of the timber increases, the wood dries out and optimum conditions for invasion by decay basidiomycetes become established. Finally, secondary moulds that utilise the breakdown products of decay fungi invade wood surfaces.

1.2.3 Chemistry and Biochemistry of Decay

Decay fungi are the principal biodeteriorators of wood in temperate climates. For this reason, some aspects of the chemistry and biochemistry of fungal decay have been addressed. The degradation of secondary cell wall cellulose, in particular crystalline cellulose, is the cause of the major strength losses observed in decayed timber (6, 10). Wood decay fungi possess a variety of degradative enzymes, all of which are too large to move freely in the cell wall (22). Hemicelluloses and lignin surround cell wall cellulose and therefore must be degraded or opened up to allow cellulase enzymes access to the cellulose. Alternatively, the decay fungus may utilise a non-enzymatic mechanism for cellulose breakdown.

1.2.3.1 Enzymatic Cellulose Breakdown

An hypothesis for cellulose breakdown proposed by Reese *et al.* (23) recognises three different types of enzymes, C_1 and C_x cellulases and β-glucosidases, which work together synergistically to depolymerise cellulose (Figure 1.7). This generalised scheme has been refined since its proposal and presently it is accepted that three major classes of enzymes, cellobiohydrolases (C_1, exocellulases), endoglucanases (C_x, endocellulases) and β-glucosidases are involved in cellulose breakdown.

Shimada and Takahashi (24), Eriksson *et al.* (25) and Eriksson and Wood (26) have reviewed mechanisms for the synergistic actions of these enzymes. Endoglucanases hydrolyse amorphous or less-ordered regions of cellulose opening up the cellulose chains to cellobiohydrolases which bind to the edges of crystalline cellulose and move along the chain cleaving cellobiose units. Additionally endoglucanases can cleave crystalline regions of cellulose at random points in the chain allowing further attack by cellobiohydrolases. The cellobiose units are converted to glucose by the action of β-glucosidases. Oxidative enzymes, such as cellobiose and glucose oxidase and cellobiose dehydrogenase, have been shown to be involved in cellulose degradation (27, 28, 29).

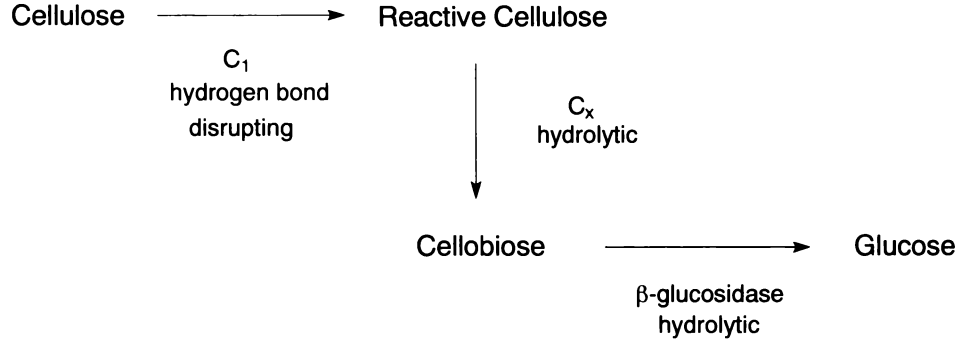


Figure 1.7: Model proposed by Reese *et al.* (23) for the enzymatic degradation of cellulose by fungi.

1.2.3.2 Enzymatic Hemicellulose Breakdown

Hemicellulases, like cellulases, are hydrolytic enzymes. Due to the large degree of heterogeneity in hemicellulose chemistry, only those enzymes involved in the degradation of the major backbones, 1,4-β-D-mannans and xylans, are mentioned. Reviews of hemicellulose degradation can be found in Eriksson *et al.* (25) and Dekker (30). Both exo- and endo-

hemicellulases exist although endo-enzymes are more common in decay fungi (9). Endo-mannanases attack the mannase backbone in an endwise manner cleaving manno-oligosaccharides, mannobiose and mannose (26). β-Mannosidases convert oligomers and dimers to mannose (30). Two types of endo-β-xylanases are known; debranching xylanases, which cleave arabinose side chains as well as the xylan backbone and non-debranching xylanases, which produce xylo-oligosaccharides of two or three residues with an attached L-arabinose (10, 30). The β-xylosidases degrade the xylo-oligosaccharides to xylose.

1.2.3.3 Lignin Degradation

White rot fungi are efficient degraders of lignin. Soft rot fungi have some lignolytic capabilities, while lignin degradation by brown rot fungi is limited to demethylation of methoxy groups and incorporation of molecular oxygen with little depolymerisation (25, 31). Lignin degradation, at present, is only partially understood due to the complexity of its structure and the number of different interunit bonds that need to be hydrolysed. Important enzymes involved in lignin degradation by white rot fungi are lignin-peroxidase, Mn-dependent peroxidases and laccase (a phenoloxidase) (10, 25).

Lignin-peroxidase, which requires H_2O_2 for activity, has been shown to perform a number of oxidation, hydroxylation and cleavage reactions on isolated lignins and lignin-model compounds. These reactions include cleavage of β-O-4 linkages, Cα-Cβ and Cβ-Cγ bonds and oxidative cleavage of aromatic rings (32, 33, 34). Mn-dependent peroxidases oxidise Mn^{2+} to Mn^{3+}, which in turn oxidises phenols in lignin to form phenoxy radicals, thus initiating degradation (10, 25). Laccase is capable of catalysing a variety of oxidation reactions on phenolic lignin end-groups including demethoxylation of guaiacyl structures to *o*-quinones structures, cleavage of side chains to form methoxybenzoquinones and Cα-Cβ cleavage of lignin dimers (35).

1.2.3.4 Brown Rot Fungi

Brown rot fungi of the Coniophoraceae family have a complete complement of cellulase enzymes, while members of other families lack cellobiohydrolases (10). In these cases oxidative mechanisms are being investigated to explain cellulose breakdown. Brown rot fungi cause rapid depolymerisation of cellulose and hemicelluloses as decay proceeds, which is

reflected in rapid losses in wood strength (6). Enzymes are too large to permeate through cell wall capillaries. Several researchers have proposed non-enzymatic, low molecular weight, degradative systems to explain observations of rapid decay occurring some distance from brown rot hyphae. Mechanisms for cellulose degradation involving H_2O_2, Fe^{2+}, oxalic acid and hydroxyl radicals have been proposed (36, 37, 38). Presumably, the low molecular weight degradative systems of brown rot fungi overcome the need for partial lignin degradation to allow the cellulases access to the cellulose. Compared to white and soft rot, brown rot decay is less affected by the type and quantity of the lignin presence in the wood.

1.2.3.5 White Rot Fungi

White rot fungi possess the full complement of cellulase and β-glucosidase enzymes. They cause a gradual decrease in the degree of polymerisation of cellulose as decay proceeds. Only a small portion of the total cellulose chain is attacked at any one time (6). Cellulose breakdown is closely coordinated to β-glucosidase activity, as alkali soluble oligosaccharides do not accumulate in the wood (6). Lignin and hemicelluloses are degraded preferentially to, or simultaneously with, cellulose depending on the fungal species and/or nutritional factors (8). White rot fungi decay hardwoods in preference to softwoods, a preference that is related to lignin composition. Highley (39) showed that softwoods rich in guaiacyl lignin were more resistant to degradation by *Trametes versicolor* than syringyl lignin rich hardwoods.

1.2.3.6 Soft Rot Fungi

Virtually all soft rot fungi possess endocellulases although many lack exocellulases. A combined oxidative system and endocellulase system has been proposed to explain the cell wall degrading ability of those soft rot fungi which are capable of causing severe damage (10). Studies on *Chaetomium globosium* have shown an initial increase in the degree of polymerisation of the cellulose and hemicelluloses, followed by a gradual decline (6). This indicates that the lower molecular weight polysaccharides are attacked prior to the polymer chains. The rate of cellulose breakdown is regulated by the rate of lignin modification, which involves demethylation, demethoxylation and some depolymerisation (26, 40). The type and quantity of lignin affects the degree of cellulose breakdown, as soft rot fungi exhibit the same preferences as white rot fungi for syringyl lignin rich hardwoods, over guaiacyl lignin rich softwoods (41).

1.2.4 Other Wood Damaging Organisms

1.2.4.1 Insects

In tropical and subtropical regions, damage caused by insect attack on timber can be more severe than that caused by decay fungi. Worldwide, the most economically important wood-destroying insects are subterranean termites (12). They occur commonly in tropical soils of Africa, Asia, North and South America and Australia. Damage caused by subterranean termites is largely internal; superficially the wood appears intact. Eventually the timber becomes so riddled with tunnels and chambers that collapse occurs. In addition to subterranean termites, drywood and dampwood termites and wood boring beetles are important insect destroyers of wood. In the case of wood boring beetles, the larval stage rather than the adult insect is responsible for the damage. In general there are three families of wood boring beetles which feed on sound dry timber: Anobiidae (e.g *Anobium puntatum*, common furniture beetle), Lyctidae (powder-post beetles) and Bostrychidae. Members from several other beetle orders (including Cerambycidae - longhorn beetles, Platypodidae and Scolytidae - ambrosia beetles) mainly attack freshly felled timber or fungal decayed wood (Curculionidae - weevils) (12, 42). Insect damage in New Zealand is restricted to buildings constructed of untreated native timbers prior to the establishment of the wood preservation industry (1).

1.2.4.2 Bacteria

Bacteria are early colonisers of wood with high moisture content and are primarily found in wood submerged in seawater, freshwater or in soil contact (12, 16). Although a wide range of bacteria have been found in wood, the two genera which have most frequently been isolated are *Bacillus* and *Pseudomonas* (16). Compared to decay fungi and insects, bacteria do relatively little damage. Attack is generally restricted to parenchyma cells and pit structures. Bacteria degradation to the pit structures increases the permeability of the timber, which can enhance the timber's susceptibility to fungal attack (9).

1.2.4.3 Marine Boring Animals

In marine environments, molluscan and crustacean borers are the major biodeteriorators of maritime timbers. Marine fungi and bacteria play a minor role. There are two groups of

bivalve molluscan woodborers: the teredinids (shipworms) and the pholads. Wood boring crusteans include the Limnoriids (gribble), the Sphaeromatids (pill bugs) and the Chelurids (12, 42). These animals destroy wood by boring and feeding (the pholads are primary plankton filter feeders). The crustaceans are able to crawl over wood surfaces and infest fresh timber, while the molluscs remain in their burrows for life.

1.3 Wood Protection

1.3.1 Principles of Wood Protection

The control of biological deterioration of wood encompasses the fields of wood preservation and post harvest protection of wood. Wood preservation involves protecting timber during its service life against damage caused by decay fungi and insects. Separate from this is the protection of freshly-felled and unseasoned timber from discolouration by sapstain and mould fungi during processing, storage and transport. In terrestrial environments of temperate climate, such as in New Zealand, fungal deterioration of timber is of primary concern. The environmental and nutritional conditions necessary for fungal growth in wood provide the means by which these organisms can be controlled. Strategies for protecting wood against fungal damage include; controlling moisture levels, controlling oxygen availability, modification of the food substrate, removal of trace nutritional elements and treatment with fungicidal agents.

Current wood preservation technology is almost solely based on the latter approach, treating with fungicides (and insecticides). This approach provides the greatest advantages in terms of inhibiting biodeterioration, cost-effectiveness, practicality and versatility. Treating with wood preservatives prolongs the service life of timbers that are naturally non-durable and allows their use in situations of high biological hazard. The ideal wood preservative would be selectively toxic towards the target organisms, inexpensive, permanently fixable in wood, chemically stable, safe and easy to apply, non-corrosive, non-toxic to the environment, compatible with finishing products and amenable to recycling or safe disposal of the product (43).

Development of fungal stain on timber is currently prevented by either kiln-drying the wood after sawn-milling or by application of prophylactic fungicides. Treating with fungicidal formulations is more economically viable than kiln drying. An ideal antistain chemical would be active against a broad range of staining fungi, capable of arresting pre-infection, inexpensive, leach resistant, chemically stable, safe to use and environmentally benign (17). Research has lead to many important advances in the fields of wood preservation and stain prevention. However, ideal systems are yet to be developed.

1.3.2 History of Wood Preservation

Wood preservation dates back to antiquity, to the ancient civilisations of Egypt, Greece, Italy, Burma and China, where attempts were made to preserve timber with various animal, vegetable and mineral oils (44). In the 16^{th} and 17^{th} centuries, the search for effective wood preservatives began in earnest, stimulated by the need for durable wooden vessels that could withstand the ravages of marine borers. The practice at the time was to sheath wooden hulls with tar and pitch, an ineffective remedy. During the period from mid 1600 to 1800, a vast number of materials were tried as wood preservatives including; oils, tars, glues, resins, rubbers, salts and waste material from a variety of industrial processes (44). No adequately effective treatment was found.

In the 18^{th} and 19^{th} centuries, the need for suitable preservatives for railway sleepers maintained the interest in wood preservation. In 1832, John Kyan invented the 'Kyanising' process which involved steeping railway sleepers in solutions of mercuric chloride. This process was surpassed in 1838 by 'Burnettising', which involved pressure impregnation of timber with zinc chloride. Because of its low cost, this process became widely accepted as the standard treatment for railway sleepers in Europe and the USA (45). Around this time developments were being made in the area of coal tar oil preservatives. Franz Moll, in 1836, was issued the first patent on the use of creosote as a wood preservative. Creosote, a black oily liquid, was obtained from the distillation of coal tar.

John Bethell, in 1838, invented the 'Bethell', or full-cell, process which used creosote as the wood preservative and was the first timber impregnation process to use periods of alternate vacuum and pressure. The use of creosote escalated with the success of the Bethell process to

become the most commonly used wood preservative worldwide from the mid 1800's to mid 1900's. The Bethell process was improved upon in the early 1900's with the Reuping and Lowry processes, so called 'empty-cell' processes, as they did not employ an initial vacuum. Lower loadings were achieved with these processes making them more economical.

Concurrent to the work on tar oil preservatives were developments in water borne preservative salts. Boucherie, in 1838, patented the treatment of unseasoned timber with copper sulphate. However, its use throughout the mid 1800's was plagued with problems of efficacy losses due to leaching. Henrich Bruning, in 1910, discovered that the addition of large amounts of chromium to $CuSO_4$ produced water insoluble Cu complexes in wood. This discovery was a major breakthrough in the development of fixed water borne wood preservatives. In 1926 Gilbert Gunn patented a $CuSO_4$ + $Na_2Cr_2O_7$ mixture called acid copper chromate which was found to be an effective wood preservative under leaching conditions. In due course it was discovered that copper was not universally effective against decay fungi and ineffectual against termites. Kamesan, in 1933, patented the first copper-chromium-arsenic preservative called Ascu. The formulation contained copper sulphate, potassium dichromate and arsenic pentoxide. This combination of chemicals was to become one of the most effective preservatives for the protection of timber against fungi, insects and marine borers.

1.3.3 Traditional Wood Preservatives

Wood preservatives currently used for the protection of timber in service can be classed as tar oil preservatives, water-borne preservatives or organic solvent preservatives.

1.3.3.1 Tar Oil Preservatives

Creosote, a brownish-black oily liquid, is a distillate of coal tar produced during carbonisation of bituminous coal. Creosote contains over 200 different compounds, the main constituents are tar acids (6-8%) (*e.g.* phenol, cresols, naphthols, xylenols); tar bases (2-3%) (*e.g.* pyridine, quinoline, acridine) and neutral hydrocarbons, such as naphthalene and anthracene. The phenolic compounds exert a biocidal effect on wood invading organisms by interrupting oxidative phosphorylation processes (46). Creosote is a very effective wood preservative; pressure treated timber has a service life of over 30 years. However, its use has declined since the 1950's in favour of cleaner, safer alternatives.

1.3.3.2 Water-borne Preservatives

Water-borne preservatives have several advantages over creosote including lack of odour and transportability as powders or pastes. The treated timber is clean and able to be painted. The disadvantage of water-borne treatments is that the treated wood swells and requires redrying.

Copper-chromium-arsenic (CCA) is by far the most popular water-borne wood preservative. Its world usage was estimated in 1988 at 100,000 tonnes, compared to a total of 15,000 tonnes for other water-borne preservatives (47). Originally, CCA was formulated as a dry powder mixture containing various proportions of $CuSO_4.5H_2O$, $K_2Cr_2O_7$ and $As_2O_5.2H_2O$. Later pastes and liquid concentrates were developed and salt-free CCA formulations containing metal oxides became favourable. Fixing of Cu and As in wood is achieved by the formation of insoluble copper chromates and chromium arsenic oxides involving the reduction of Cr^{6+} to Cr^{3+} (48). CCA ranks alongside creosote in effectiveness, with an expected service life of 30+ years in high hazard situations. Copper is the principal antifungal agent and it exerts its effects by denaturing thiol containing proteins and enzymes (46). Copper-tolerant fungi, such as *Poria* species, and insects are killed by arsenic, which inhibits their respiratory pathways (46, 49).

Concerns over the high toxicity of arsenic and the release of arsenic oxide into the atmosphere, when CCA treated timbers are combusted, has lead to the development of arsenic-free formulations. These include copper-chromium-boron (CCB), copper-chromium-fluoride (CCF), copper-chromium-fluoroborate (CCFB) and copper-chromium-phosphorus (CCP). The boron and fluoride components are not fixed and are subject to leaching. These formulations are used more widely in Europe (50). Concern over chromium, due to its carcinogenic properties, has led to chromium free formulations such as ammoniacal copper arsenate (ACA), ammoniacal copper borate (ACB) and copper fluoride boron ammonia mixtures, which are used in North America, Scandinavia and Australia respectively (49). Other cuprammonium formulations are being investigated in the USA (Section 1.3.4).

Preservatives containing a mixture of sodium fluoride, chromium trioxide, arsenic pentoxide and dinitrophenol or sodium pentachlorophenate (FCAP) have been used for treating timber for above ground applications. Zinc chloride, widely used in the early 1900's, has been

improved upon by mixing with sodium dichromate to form chromated zinc chloride which is used in the USA and Germany for treating timbers for above ground use (49).

Boric acid (H_3BO_3) and borax ($Na_2B_4O_7.10H_2O$) are used as wood preservatives. The method of application involves dipping green timber in water formulations followed by close stacking under plastic sheeting for 4-8 weeks, thus allowing the boron compounds to slowly diffuse into the timber (51). Borax and boric acid are susceptible to leaching on exposure to moisture and hence are only suitable for treating timbers that are to be used above ground and protected from weathering. Both boric acid and borax are active against decay fungi and wood boring beetles, have low mammalian toxicities and are considered to be relatively environmentally benign.

Alkyl ammonium compounds (AACs) which include tertiary amine salts and quaternary ammonium compounds, such as alkyldimethylammonium salts and dialkyldimethyl ammonium chloride have been used as wood preservatives in New Zealand. However, failure of AAC treated timbers after 9 years of service led regulatory authorities to rescind approval for their use (52).

1.3.3.3 Organic Solvent Preservatives

Organic solvent preservatives are composed of fungicidal and insecticidal compounds dissolved in a non-polar organic solvent. Most are formulated in white spirits and are described as light organic solvent preservatives (LOSPs). The preservatives can be applied by dipping, brushing, spraying or low-pressure processes, the solvent quickly evaporating after treatment (with the exception of residue alkylated aromatic compounds (53)) leaving the water-insoluble biocide. LOSPs are well suited to the treatment of joinery timbers as the treated wood does not undergo dimensional changes and can be painted and glued.

Pentachlorophenol (PCP), developed by Monsanto in 1935, is a very effective fungicide and when combined with an insecticide is suitable for treatment of joinery timbers. PCP, in ground contact timbers, is subject to gravitational migration and depletion from the treated wood into the environment. PCP, like other phenolic compounds acts as an uncoupler of oxidative phosphorylation, allowing electron transport down the respiratory chain but

preventing adenosine triphosphate (ATP) synthesis (46). The use of PCP is now banned in many counties, including New Zealand, due to concerns over the presence of toxic, environmentally persistent chlorodioxins and dibenzofurans in commercial preparations.

Organotin compounds, such as bis(tri-*n*-butyltin) oxide (TBTO) and tri-*n*-butyltin naphthenate (TBTN) (Figure 1.4) have been widely used for the last 30 years for treating timber used above ground. TBTO and TBTN are effective fungicides, particularly against brown rot fungi. They are less active against white and soft rot fungi and have limited insecticidal activity (54). The mode of action of tri-alkyltin compounds is through the inhibition of oxidative phosphorylation (46). Detoxification of TBTO through chemical degradation and the actions of wood-inhabiting fungi have been demonstrated (19, 55). TBTO has been banned in Japan due to concerns that it may bioaccumulate in seafood (a consequence of its extensive use in antifouling paints (19, 56)). In the UK, TBTO is approved for industrial use but banned from sale to the general public in do-it-yourself preservatives due to health and safety concerns (49, 56).

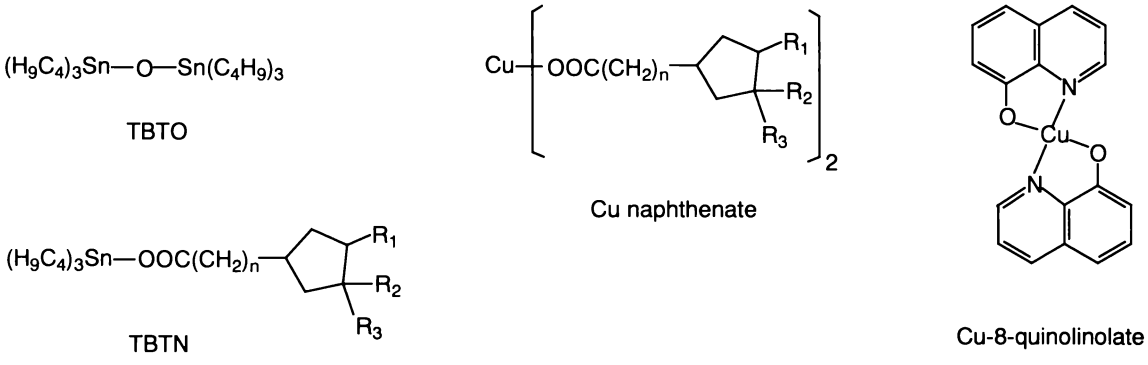


Figure 1.8: Traditional organometallic wood preservatives.

Copper and zinc naphthenates have found application as fungicides in wood preservative formulations. Copper naphthenate (Figure 1.8) is more widely used due to its greater fungitoxicity. Naphthenic acid, which is a mixture of several cyclopentane carboxylic acids derived from oil-refining, chelates the Cu or Zn ions facilitating the transport of the metal ions across the phospholipid membrane into the fungal cell where they can exert their inhibitory effects. The rising cost of producing naphthenic acids and concerns over environmental persistence have led to alternative copper and zinc chelates being developed and tested (see Section 1.3.4). Copper-8-quinolinolate (Figure 1.8), a copper chelate of 8-hydroxyquinoline, is an effective fungicide of low mammalian toxicity and is the only wood preservative

approved for the treatment of wood products in contact with foodstuff (49). Insecticides used in organic solvent wood preservatives have been recently reviewed by Robinson (57).

1.3.4 New Generation Wood Preservatives

Concerns over the health and safety, and the environmental impact of chromium and arsenic incorporated into traditional wood preservatives have stimulated worldwide research into novel copper-based formulations. Promising examples include N-cyclohexyldiazeniumdioxy chelates of copper (Cu-HDO) and aluminium (Al-HDO) (Figure 1.9) which have shown good potential for fixing these metals in wood (19, 49). Cuprammonium wood preservative formulations are being investigation in USA; these include ammoniacal copper carbonate, ammoniacal copper quaternary ammonium compounds, ammoniacal copper carboxylates, ammoniacal copper citrate and ammoniacal copper dithiocarbamates (49). Copper and zinc 'soaps' of synthetic aliphatic carboxylic acids has been a recent development in the LOSP field. Mixtures of linear and branched saturated C_9 and C_{10} iso- and neoacids when combined with Cu or Zn have shown potential against wood destroying organisms (49). These compounds, known as acypetacs-copper and acypetacs-zinc, have found application in the remedial treatment of joinery and in do-it-yourself preservatives (49, 56).

New organic compounds that have shown good potential to protect timber in service and have recently been commercialised as active components of wood preservative formulations include triazoles, isothiazolones, benzimidazoles and 3-iodo-2-propynylbutyl carbamate (IPBC). The triazoles, azaconazole, propiconazole and tebuconazole (Figure 1.9) are agricultural fungicides that have been developed for wood protection. Azaconazole is primarily an antistain fungicide while propiconazole and tebuconazole have been developed for use against decay fungi in water-borne and LOSP formulations (58, 59, 60). The triazoles show a good spectrum of activity, have low mammalian toxicities and are readily broken down in the environment (19). They inhibit the biosynthesis of ergosterol, a sterol that plays an important role in structure and function of cell membranes (19).

N-substituted isothiazolones, such as 4,5-dichloro-2-*n*-octyl-4-isothiazolin-3-one (Figure 1.9) have been shown to be highly active against brown, white and soft rot fungi (61). The isothiazolone ring is believed to react with enzymes, proteins and amino acids, initiating

biocidal activity (19). Carbendazim, methylbenzimidazole-2-yl carbamate (Figure 1.9), a plant protection fungicide, is an active ingredient in LOSP formulations for timbers used in above ground applications. Carbendazim is also active against blue stain fungi; it exerts its fungicidal activity through the inhibition of cell division (46). 3-Iodo-2-propynylbutyl carbamate (Figure 1.9) is an antistaining chemical, which also offers protection against decay fungi and is described as suitable for the treatment of timber used in above ground applications (62).

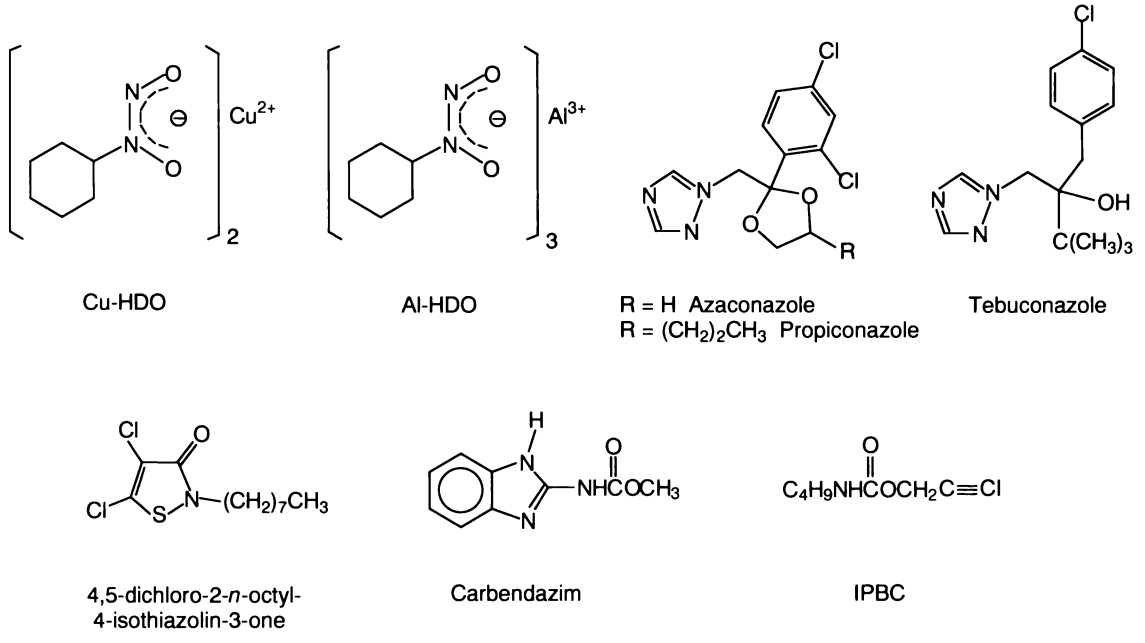


Figure 1.9: New generation wood preservatives.

1.3.5 Antistaining Chemicals

Attempts to prevent the discolouration of timber by sapstain fungi and moulds date back to the late 1800's when CaO and $Ca(OH)_2$ was spread on and under lumber piles. In the early 1900's dipping of green timber in 5% $NaHCO_3$ solution was initiated, this was surpassed by the practice of dipping or spraying with hot solutions of Na_2CO_3 and $NaHCO_3$. However, it was not until the introduction of organomercury compounds in the 1930's and chlorinated phenols shortly afterwards that effective means of controlling sapstain and mould growth were achieved.

Treatment with sodium pentachlorophenate (NaPCP) and to a lesser extent ethyl and phenyl mercury compounds became widely accepted practice. The high mammalian toxicities of

organomercurics lead to a ban on their use in the 1960's. Despite its high toxicity and irritant nature NaPCP remained in use. Attempts to reduce toxicity and irritancy involved co-formulation with other agents, including borax. Formulations containing NaPCP and borax in a 1:3 mixture became widely used (63). Growing concerns over worker health and safety, and the environmental persistance of trace amounts of dioxins in NaPCP formulations in the 1980's lead to restrictions and outright bans on the use of NaPCP and PCP in many countries. The impending loss of NaPCP led to substantial screening programmes to identify alternatives to NaPCP. This research has led to the emergence of numerous second-generation antisapstain and antimould chemicals.

The benzimidazole, benomyl (Figure 1.10), a highly successful plant protection fungicide was introduced as an antisapstain/antimould agent in the late 1970's. Solubility problems resulted in poor timber penetration and ineffectual control of internal stain (63). Another benzimidazole, carbenzadim (Figure 1.9), proved more successful and is currently used in a number of antistain formulations alone or in combination with chlorothalonil and triazoles. Chlorothalonil (Figure 1.10) is an important agricultural fungicide with very low mammalian toxicity. Formulations alone and in combination with carbenzadim have been shown to be as active as NaPCP against sapstain and mould fungi (64).

The phthalimide derivatives, captafol and folpet (Figure 1.10) prevent deterioration of timber by sapstain and mould fungi, although effectiveness appears to be dependent on the timber species and varies with geographical location (65). Captafol and folpet have been used commercially in New Zealand on *Pinus radiata*, in combination with chlorothalonil (65). Although both agents have low mammalian toxicities, solubility problems have led to their replacement by alternatives in more recent times. The sulphamides, dichlofluanide and tolylfluanide (Figure 1.10) are plant protection fungicides, which have been developed for wood protection. They are used primarily as agents for preventing blue-stain on service timbers, although dichlofluanide has found application as a mouldicide in LOSP formulations (49). The sulphamides also show activity against wood-destroying basidiomycetes (19).

Cu-8-quinolinolate (Figure 1.8) is used in sapstain and mould control as well as wood preservation. Developed for sapstain use in the 1980's Cu-8-quinolinolate provides protection equal to that of NaPCP and is one of the most cost-effective treatments (66). Problems

associated with its corrosiveness and instability in the presence of iron have resulted in a lack of market success (65). The benzothiazole, 2-(thiocyanomethylthio)benzothiazole (TCMTB) (Figure 1.10) is used to prevent development of sapstain fungi on freshly sawn timber. The effectiveness of TCMTB can be improved by combining it with methylene bis-thiocyanate (MBT) (Figure 1.10) (19). MBT is a highly mobile fungicide that readily diffuses into the timber giving protection against penetrating fungi. This diffusibility, however, leads to surface depletion. TCMTB, on the other hand, is immobile and gives good surface toxicity. MBT has relatively high mammalian toxicity and is not approved for use in some countries (67).

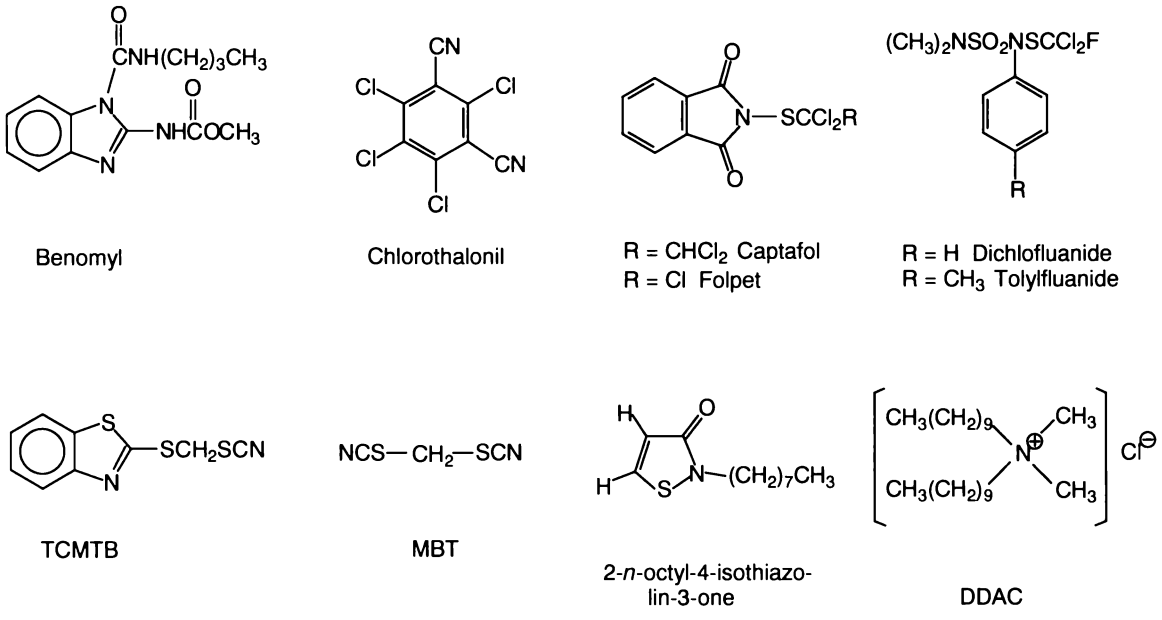


Figure 1.10: Some second-generation antistaining chemicals.

The isothiazolones are a group of compounds that have shown good effectiveness against sapstain and mould fungi (19, 65). 2-*n*-Octyl-4-isothiazolin-3-one (Figure 1.10) in formulation with MBT is used commercially in New Zealand for sapstain and mould control. 2-methyl-4-isothiazolin-3-one and its 5-chloro derivative are used as antimoulding agents in CCA solutions (49). Alkyl ammonium compounds (AACs), which are widely used as active ingredients in household and industrial disinfectants, have more recently been developed for use, as antistaining agents. Of a large number of AACs that have been tested, didecyldimethyl ammonium chloride (DDAC) (Figure 1.10) has proved the most effective (19, 65). DDAC is used as the fungicide in a number of antisapstain products, alone and in combination with borax, IPBC or triazoles (17, 68). Borax, $Na_2B_4O_7.10H_2O$, is often added to AACs and other

antistain formulations primarily as a pH buffer. The fungicidal activity of boron offers potential for synergistic effects (17).

3-Iodo-2-propynylbutyl carbamate (IPBC) (Figure 1.9) which was originally developed as a microbiocide for paint protection is currently used as a sapstain control agent alone or in combination with DDAC or azaconazole (17). IPBC has relatively low mammalian toxicity and can be formulated in both water and solvent based carriers (49). The most recent entrants into the field of timber stain prevention are the triazoles. Azaconazole, propiconazole and tebuconazole (Figure 1.9) have proved effective against sapstain and mould fungi and are used in antistain formulations alone and in combination with carbendazim, DDAC or chlorothalonil (17, 68).

Second generation antistain chemicals are, in general, relatively expensive compared to NaPCP and do not have the same broad spectrum of activity. Most modern antistain products are mixtures of active ingredients, as improved effectiveness against the range of stain fungi can be achieved in this way. The track record of many of the second-generation antistain agents is yet to be well established and in some cases effectiveness appears to vary considerably with wood species, geographical location and protection times. A number of the agents have application problems, the result of poor solubility, stability or 'solution stripping' (where strong adsorption to the wood gradually depletes the fungicide concentration of the dipping solution (69)). Other problems include microbial detoxification after application (recorded for TCMTB, MBT, Cu-8-quinolinolate and sulphamides (69, 70)), migration of the active compound in green timber and losses due to leaching. The rapid application of the fungicide after harvesting is critical to the success of several of the chemicals described above, as they are less effective at arresting pre-infection, than preventing initial colonisation. However, these compounds are too environmentally unsafe to allow widespread spraying at logging sites within the forest. In light of these shortcomings a totally satisfactory replacement for NaPCP has not yet been found. Further research is required to identify and develop more novel compounds, with biocidal potential and low mammalian toxicities.

1.4 Novel Wood Protection

Current technology for the protection of timber against biological deterioration is based exclusively on the approach of killing the invading organism by treating the timber with biocidal agents. Research into novel methods of wood protection is somewhat removed from conventional technologies and involves a number of innovative methods of protecting wood against biological attack. Only those strategies relevant to fungal deteriorators are considered in this thesis due to their importance to the wood durability industry in New Zealand. Novel protection methods are based on one or more of the following strategies; killing the fungus, rendering the food source unusable, preventing the timber from becoming wet, interfering with the chemistry of decay or stain development and limiting the availability of nutrients and space through competition from other organisms. Novel wood protection methods potentially offer enhancements in timber durability without the environmental and safety problems associated with some current wood protection biocides (71).

1.4.1 Chemical Modification of Wood

Chemical modification of wood involves reaction of small chemical reagents with the hydroxy groups of polysaccharide and lignin polymers to introduce a covalently bound group into the structure. A wide range of chemicals have been investigated as wood modifiers. These include anhydrides, isocyanates, epoxides, alkyl halides, acrylonitrile and formaldehyde (Figure 1.11). The technique has recently been reviewed by Rowell (72). Chemical modification can enhance a number of the timber's properties, of which biological durability is one. Studies have shown chemically modified wood to be more resistant to decay fungi, termites and marine borers (72, 73). In the case of decay fungi, the reason for enhanced durability is not entirely clear and is possibly a combination of two factors. Firstly, it is proposed that changes in the chemical structure of the wood components prevents the fungal enzymes from recognising the modified cellulose, hemicelluloses and lignin as substrates and subsequently degrading them (71). Secondly, Rowell (73) has proposed that chemical modification prevents water from gaining access to the glycosidic bonds of cellulose and in the absence of water the fungal cellulases are unable to hydrolyse these bonds.

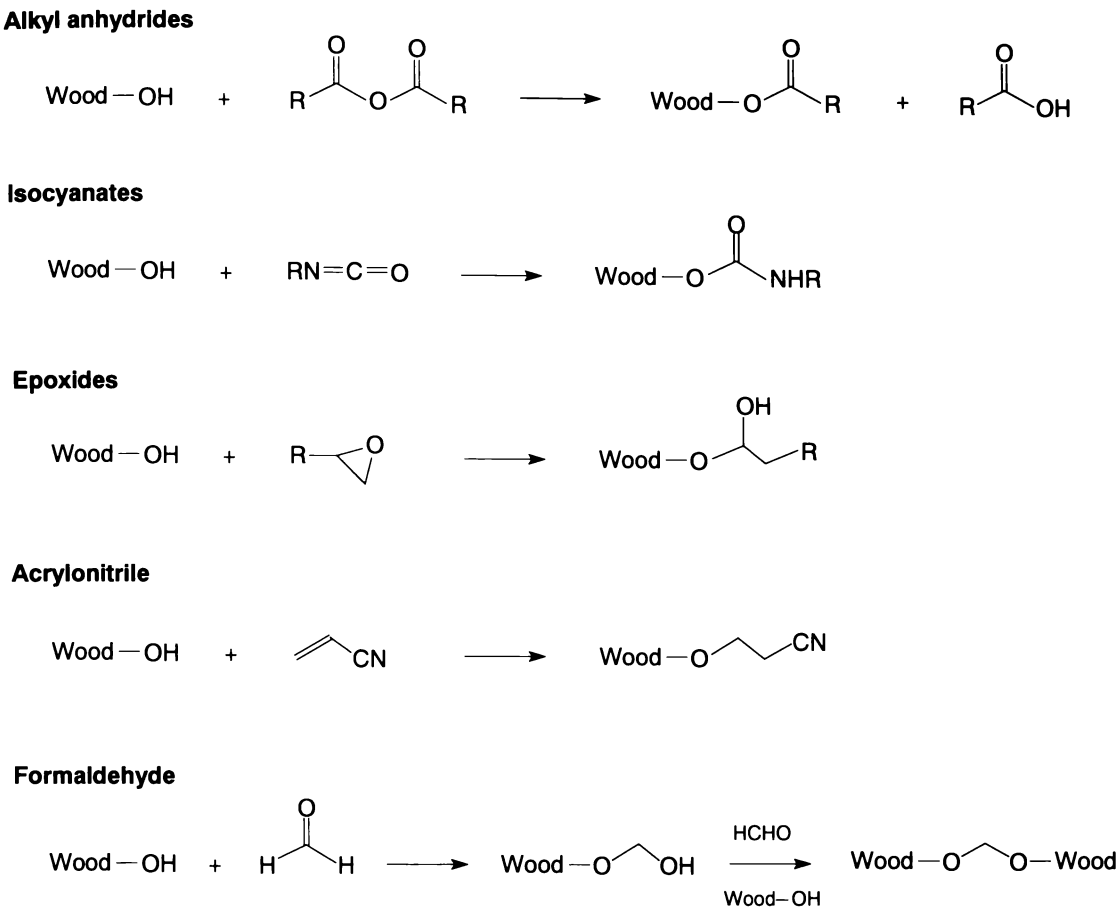


Figure 1.11: Reagents used for the chemical modification of wood.

The viability of chemical modification as a novel preservation technique for solid wood is severely limited by two factors. These are the need for very dry wood, in order to minimise reagent hydrolysis and the poor penetration of chemical reagents into wood greater than 1-2 cm thick (72). As a result, applications of the technology have mainly focused on composite products where uniform modification of wood chips, fibres or flakes is easier to achieve (71, 72).

1.4.2 Water Barriers

Water is an essential requirement of decay. Restricting the ingress of moisture into wood in service provides a mechanism for preventing fungal attack. Although conventional surface coatings can extend the service life of joinery timbers used above ground (74), they do not provide adequate protection against fungal attack. As well as not being truly hydrophobic, these polymeric coatings are susceptible to cracking and rupture under natural weathering conditions. Paraffin waxes or silicones have been incorporated into surface coating with little success due to poor affinity between components (74). Combining conventional preservatives

with water repellent agents has proved successful in protecting timber against deterioration due to exposure to weathering and decay. CSI Laporte's CCA/wax emulsion treatment for decking timbers is an example of such an approach (75).

Another method employed for restricting moisture ingress is the use of physical plastic barriers, such as heat-shrunk polyethylene, on timbers used below ground (71, 74). Impregnation of wood with monomers followed by polymerisation *in situ* to form wood plastic composites is an alternative method of combining wood and plastics to enhance durability. Impregnation with phenol-formaldehyde, melamine-formaldehyde resins followed by heat curing, produces wood polymers with greater decay resistance due to reduced moisture absorption capabilities (71, 76, 77). The high cost of these last two technologies severely limits their use.

1.4.3 Biochemical Methods

A greater understanding of the biochemical mechanisms by which decay fungi degrade wood carbohydrate and lignin polymers has stimulated research into specific methods of disrupting these degradative pathways. Research in this area is in its infancy. Suttie (71) has suggested possible avenues of control include interfering with the use of fungal metabolites and the inhibition of extracellular enzymes by anti-oxidants. According to Franich (78), the powerful anti-oxidant, BHT (2,6-di-t-butyl-4-methylphenol) was ineffective in inhibiting growth of the fungus, *Coniophora puteana*. Conversely, Schultz's work on hydroxystilbenes has led him to suggest that their anti-oxidant properties may contribute to their inhibitory activity against brown rot fungi (79). It has been proposed that brown rot fungi degrade cellulose using a mechanism involving Fenton's reagent, Fe^{2+}/H_2O_2, to produce hydroxyl radicals (see Section 1.2.3.4). Suttie *et al.* (80) have suggested that chelating agents which specifically form stable complexes with iron could remove Fe^{2+} from the reaction system, providing a mechanism of inhibiting brown rot attack. In investigating this hypothesis they found that wood blocks impregnated with iron chelators showed reduced mass losses compared to controls when exposed to brown rot attack (80). Kersten (81) has proposed that potential targets for disruption of wood decay by white rot fungi are the enzymes lignin peroxidase and glyoxal oxidase and has discussed potential strategies for inhibiting these enzymes.

Methods targeted towards inhibition of specific biochemical pathways are also under investigation for the control of biological stain. Staining fungi discolour wood due to the production of melanin, a brown-pigmented polymer. Inhibiting melanisation offers a potential targeted approach to stain prevention (17). Wolkow and colleagues (82) have reported the chemical inhibition of pigmentation in the stain fungus, *Colletotrichum lindemuthianum.*

1.4.4 Biological Protection

Biological protection of wood involves using one species of fungus to prevent colonisation by or to control the growth of, another wood deteriorating species. Biological control has been used successfully in the agricultural sector for many years but is a relatively new concept in wood protection. Biological control is believed to work by several mechanisms including competition for the ecological niche, antibiosis and parasitism (83, 84). For parasitism to work the wood damaging fungus must already be established in the wood which is undesirable. Antibiosis, the production of mycotoxins or antibiotic compounds which are inhibitory or toxic to the target fungus, has been studied for the control of decay fungi and staining fungi (84, 85). Competition for niche is the primary mode of action employed by most bioprotectants used to inhibit fungal stain. The organisms used as competitive bioprotectants are primary colonisers which have the ability to germinate and grow rapidly. They quickly utilise available nutrients and make the wood less suitable for other organisms.

Early studies on biological control on wood deterioration focused on decay (84, 86, 87). Preventing the decay of timbers in service by biological control represents an extreme challenge, as complete control against a range of organisms is required for the expected lifetime of the product, which can be up to 40-50 years. A more achievable goal, which has received considerable attention in more recent years, is the control of stain fungi with antagonistic organisms. The protection period is rarely greater than 6 months. The protecting organisms do not necessarily have to penetrate deeply into the timber to provide protection and complete control is not critical (84).

Laboratory studies have shown that prior colonisation by a number of fungi and bacteria can provide protection against disfigurement caused by a variety of staining fungi (18, 88, 89, 90,). Albino (non-pigmented) strains of staining fungi developed by genetic engineering methods from wild strains are currently the focus of many studies. These organisms have

similar ecological and biological attributes to wild fungi which cause staining and can become established in freshly-felled sapwood without causing aesthetic damage. Although biological protection has found success in the laboratory the technique has been plagued with poor performance under field conditions (84). Recent strategies have been targeted at integrating biological protection with other methods for control, such as chemical pretreatments (91).

1.4.5 Wood Extractives

Constitutive compounds present in the heartwoods of naturally-durable timbers can protect these timbers against biological attack (Section 1.5). The use of wood extractives in wood protection is a concept which has been proposed and investigated by a number of researchers (71, 79, 92-99). While initial research focused on using the wood extractives themselves (94-99), an alternative approach is to use these biotoxic heartwood extractives as synthetic leads to novel chemical analogues with antifungal properties. This concept and the literature pertinent to this approach are explored in the proceeding sub-chapters.

1.5 Natural Wood Protection

1.5.1 Natural Durability

Timber species vary markedly in their ability to resist degradation by decay fungi. In situations of high decay risk the heartwood timbers of some species, *e.g.* teak and totara, would last for decades without deteriorating, while other species, such as birch, would be destroyed in a matter of years. The ability of the heartwood of a particular species to resist decay is termed 'natural durability'. Natural durability is used only in reference to heartwood as all sapwood is inherently vulnerable to fungal decay, although many species have little sapwood (100, 101). Differences in durability between the heartwood and sapwood of a particular species and between heartwoods of different species are mainly due to differences in the chemical nature and concentration of extractives which are deposited during heartwood formation.

Natural durability is determined by the life expectancy of standard-sized (50 x 50 mm) heartwood stakes subjected to high hazard natural exposure in ground contact (51, 100). In New Zealand timbers are classified into five durability categories ranging from perishable to

very durable (Table 1.1). These categories are only applicable to exposure conditions in temperate climates. In the tropics the decay hazard is generally more severe and life expectancies may be shorter (100). The durability rating for a particular species can also vary with geographical location. This is primarily due to differences in the local species of decay fungi and their biodeteriogenic abilities. Natural durability ratings for a selection of important native and exotic species grown in New Zealand are given in Table 1.1. Some very durable timbers, referred to in the following discussion, have been included. Natural durability can vary considerably between individual trees of the same species, possibly due to genetic factors (100). Durability ratings, as a result, are usually the average of a wide range of results and should be viewed comparatively rather than as absolutes (51).

Table 1.1: Natural durability of a selection of New Zealand native and exotic timbers and some very durable timbers from around the world (adapted from (51)).

Perishable	**Non-durable**	**Moderately Durable**	**Durable**	**Very Durable**
< 5 years	5-10 years	10-15 years	15-25 years	>25 years
Softwoods				
Ponderosa pine	Radiata pine	Rimu		Totara
Corsican pine	Douglas fir	Macrocarpa		Silver pine
	Kauri	Lusitanica		Incense cedar
	Matai	Californian redwood		Western red cedar
Hardwoods				
Silver birch	*E. regnans*	Silver beech	Hard beech	Robinia
Tawa	*E. viminalis*	Black beech	Red beech	Osage orange
Alder	Oak	*E. globulus*	*E. saligna*	Tallowwood
	Hinau			Teak

Natural durability can also vary within a tree, in many species, particularly those that are very durable. The inner heartwood shows lower durability than the outer heartwood. The durability classification given in Table 1.1 applies only to situations of high decay risk from basidiomycetes (brown and white rot fungi) and is not applicable to soft rot fungi and insect deteriorators. Durability to insect attack is dependent on the insect type and species. Timbers are rated either as resistant or non-resistant as the rate the timber is destroyed is dependent on the size and age of the attacking insect population (11). As with decay resistance it is only the heartwood that shows resistance.

The process of heartwood formation involves loss of water and stored food substances from the central zone of the tree and their replacement with oils, resins, tannins and aromatic compounds (102). These extractives accumulate in the lumen, become impregnated in the cell walls and form incrustations on cell wall pits (102). Extractives can enhance the natural durability of the heartwood by virtue of their toxicity to wood damaging organisms and by lowering the permeability of the heartwood to water and oxygen.

A direct relationship between the natural durability of a timber and its heartwood extractives has been demonstrated on numerous occasions by investigators over the past 75+ years (93, 97, 101, 103-112). The methods adopted by these researchers have included:

- extracting durable timbers and testing the extracts for fungal toxicity;

- testing durable species before and after successive extraction with a variety of solvents;

- testing non-durable timbers impregnated with extracts from durable timbers.

The importance of heartwood extractives in determining not only decay resistance but also resistance to insects and marine borers is well established. Bioactive heartwood extractives encompass a diverse array of compounds, including members of the following classes: terpenoids, tropolones, stilbenes, flavonoids, quinones, lignans and condensed tannins (Section 1.5.2). Commonly different toxic principles are responsible for resistance to fungi and resistance to insects (termite resistance is most commonly studied), although some extractives, *e.g.* *l*-citronellic acid, possess both antifungal and antitermite properties (103, 107). Antifungal properties can be either fungistatic (the fungus is prevented from growing, but is not killed) or fungicidal (the fungus is killed). Anti-insect properties can be due to toxicity, repellency or unpalatablility.

Individual heartwood extractives vary in their effective concentrations and in their activity against different fungi (106, 108). A common feature of natural durability is that complex mixtures of extractives are often responsible for durability. Durability is the result of multiple components, each with some biocidal activity, working synergistically rather than one or two components with potent activities. Alternatively durability can be the result of high concentrations of moderately toxic compounds. Another common observation for durable timbers is that exhaustive extraction with solvents reduces but does not completely remove

decay resistance. Furthermore, introduction of extractives from a durable timber into a non-durable timber improves its durability but often not to the same level as the original durable timber. Covalent bonding between extractives and wood cell wall polymers has been proposed to explain these results (93, 109, 112).

1.5.2 Heartwood Extractives

Bioactive heartwood extractives are part of a tree's natural defence mechanism against attack by micro-organisms and insects. Heartwood extractives are classed as constitutive defensive agents as they are pre-formed in the heartwood prior to biological attack. Phytoalexins, induced defensive agents, are briefly considered in Sections 1.6.3 and 1.7.1. Heartwood extractives toxic to fungi are generally alcoholic, phenolic, polyphenolic, acidic or quinoidal and include terpenoid alcohols and acids, tropolones, hydroxystilbenes, flavonoids, tannins, and various quinones.

1.5.2.1 Terpenoids

The terpenes and their derivatives comprise a large group of natural products that are widespread throughout the plant kingdom. They possess carbon skeletons composed of isoprene units. A number of heartwood terpenoids with phenolic, hydroxyl, carboxylic acid and quinoidal functional groups have been found to contribute to the natural durability of certain softwoods. These antifungal terpenoids are discussed separately in Section 1.6 as members from this class were used as lead compounds in the synthetic studies that follow.

1.5.2.2 Tropolones

Tropolones is a generic term used to describe a group of compounds which contain the unsaturated seven-membered carbon ring, 2-hydroxy-2,4,6-cycloheptatriene-1-one. Tropolones, found in the heartwood of certain species of the Cupressaceae family, are responsible for the durability of these timbers. Tropolones are among the most potent of the antifungal extractives found in heartwood. Tropolones are terpenoidal in origin, however because of their unique structure and properties they have been discussed separately from the terpenoids (Section 1.7).

1.5.2.3 Stilbenes

Stilbenes are found as heartwood extractives in both hardwoods and softwoods and are characterised by an α,β-diphenylethylene skeleton. The B ring is commonly substituted with hydroxyl or methoxyl groups in the 3' and 5' positions (Figure 1.12). The A ring can have hydroxyl, methoxyl or no substituents (79, 113). Hydroxystilbenes are believed to contribute to the durability of those timbers in which they are found. The heartwood of *Pinus* species, although non-durable, is more durable than the sapwood due to pinosylvin and pinosylvin monomethyl ether (109, 114) (Figure 1.12). Oxyresveratrol in osage orange (*Maclura pomifera*) and pterostilbene in *Pterocarpus* species (Figure 1.12) are believed to contribute to the high natural durability of these timbers (4, 101, 112).

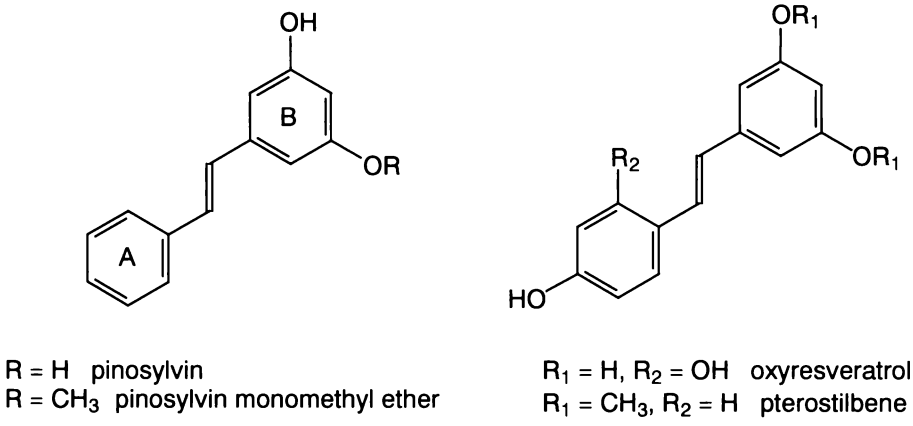


Figure 1.12: Antifungal heartwood stilbenes.

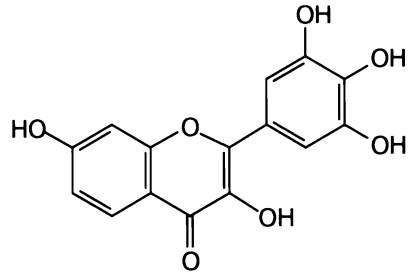
Heartwood hydroxystilbenes have shown high fungistatic or fungitoxic properties in nutrient agar based assays (94, 114, 115). Hart and Shrimpton (109), however, have questioned the role of hydroxystilbenes in natural durability based on their observations of 90-99% reductions in stilbene fungitoxicity when assays were performed in a woody substrate compared to nutrient agar. They have attributed these reductions in activity to binding between the stilbenes and wood components, which results in deactivation (109). Rudman (107) using a wood sawdust assay reported the antifungal activity of oxyresveratrol and pterostilbene against basidiomycetes as surprisingly low compared to earlier agar-based toxicity tests. A recent study of the efficacy of pinosylvin and its monomethyl ether against brown and white rot fungi in agar and wood blocks showed these stilbenes to be only weakly active (116). Significant differences were observed between the two test methods. These and

other studies have highlighted the importance of the assay medium in determining the fungitoxicity of extractives and suggest results obtained on nutrient agar are only broadly indicative of activities in wood.

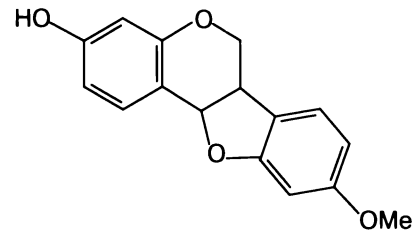
Studies in wood media suggest that hydrostilbenes are only slightly toxic to basidiomycetes (107, 109, 113). The high durabilities of the North American timbers osage orange (*Maclura pomifera*) and red mulberry (*Morus rubra*) are due in part to the high concentrations of the weakly active oxyresveratrol, 2.5% and 7.3% respectively (112). Synergism with other stilbenes and flavonoids may play a role but is yet to be demonstrated (112). It has been proposed that the fungitoxicity properties of hydroxystilbenes are based on the inactivation of enzymes containing -SH groups in their active sites (108, 113). Schultz *et al.* (79) have proposed that hydroxystilbenes, as powerful antioxidants, inhibit decay through scavenging of fungal-generated free radicals. Hydroxyl radicals have been implicated as part of the decay mechanism of brown rot fungi (Section 1.2.3).

1.5.2.4 Flavonoids

Flavonoids encompass a large group of compounds with the diphenylpropane (C_6-C_3-C_6) skeletal structure. Flavonoids are widespread throughout the plant kingdom and can be subdivided into various subgroups that include flavones, flavanes, flavanones, isoflavones and neoflavones. Flavonoids of various types are found in the heartwoods of a number of softwoods and hardwoods. In general, flavonoids are considered to be of relatively low toxicity to fungi (101, 107), although there are some exceptions. The flavone, robinetin (Figure 1.13) was found to be toxic to *Lenzites lepideus* at the 1% level in wood sawdust although inactive against three other brown rot fungi (107). The heartwood of *Robinia psuedoacacia*, a very durable North America timber, contains 2% robinetin and 5.3% hydrorobinetin (112). Its durability has been attributed to the high concentrations of these flavonoids, possibly in combination with synergism with other flavonoid components or condensed tannins (111, 112). The pterocarpans are a class of isoflavonoids, which have been implicated in the durability of certain *Pterocarpus* hardwoods (101). The heartwood of *Platymiscium yucatanum* contains, among other flavonoids, the pterocarpan (+)-medicarpin (Figure 1.13) which is active against the brown rot fungus, *Lenzities trabea* and the white rot fungus, *Trametes versicolor* (117).



robinetin

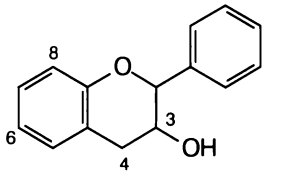


medicarpin

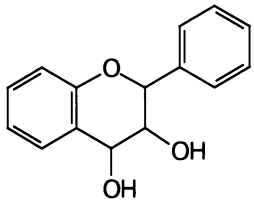
Figure 1.13: Antifungal heartwood flavonoids.

1.5.2.5 Condensed Tannins

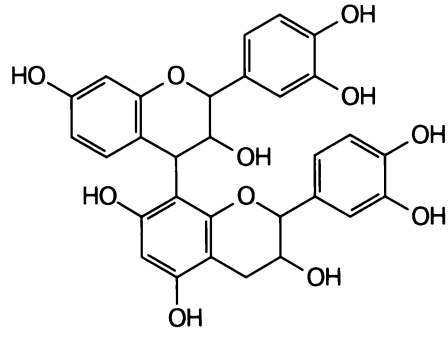
Condensed tannins are polymers based on the flavonoid units of flavan-3-ols (catechins) and flavane-3,4-diols (proanthocyanidins) (Figure 1.14) (114). Condensed tannins consist of 3 to 8 flavonoid units with the most common linkages between the 6- and 8-positions of the A ring of one unit and the 4-position of the heterocyclic ring of the other (Figure 1.14).



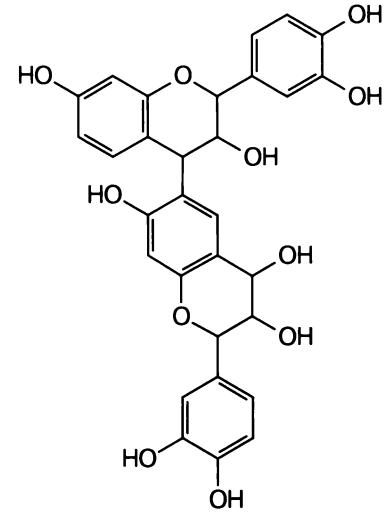
flavan-3-ol unit



flavane-3,4-diol unit



4,8-linked dimer



4,6-linked dimer

Figure 1.14: Common flavonoid units and inter-flavonoid linkages found in condensed tannins.

Condensed tannins can occur in large quantities in certain tropical and subtropical hardwoods. The heartwood of the very durable species *Eucalyptus microcory* (tallowwood) can contain up to 15% condensed tannins (118). According to Rudman (118) these polyphenolics are responsible for the durability of tallowwood. Anderson (119) has shown that the condensed tannins contribute to the durability of *Sequoia sempervirens* (californian redwood). Redwood

condensed tannins completely inhibited the growth of the fungus *Fomes annosus* at the 2.5% level (119).

Condensed tannins are fungistatic rather than fungicidal (101). The principal mechanism of toxicity is believed to be through their ability to precipitate proteins (93, 120). Extra-cellular enzymes excreted by wood decay fungi form complexes with condensed tannins and are rendered insoluble and inactive. Alternative mechanisms of activity including deprivation of substrates, inhibition of oxidative phosphorylation and iron deprivation have been proposed (120). Condensed tannins are water soluble and hence leachable; extraction of Californian redwood with water drastically reduces its decay resistance (119). To overcome this problem Laks and co-workers (93) have investigated covalent bonding of tannin residues to wood cell wall components. Pine wafers covalently modified with condensed tannins to a retention of 1% suffered a weight loss of only 1% after leaching and exposure to the brown rot fungus, *Gleophyllum trabeum*, compared to 24% for untreated controls. The capability of condensed tannins to complex metal ions has been utilised by Laks and colleagues (121) in investigating condensed tannin/cupric complexes as wood preservatives.

1.5.2.6 Lignans

Lignans are extractives that consist of two phenylpropane units linked in different ways. Some of these compounds are representative of dimeric structures found in lignin (114). Lignans are found in the heartwood of both softwoods and hardwoods, in some cases in considerable quantities (114, 122). Examples with selective antifungal character include matairesinol (Figure 1.15), which is found in the heartwood of matai (*Prumnopitys spicatus*) (101, 123) and pinoresinol (Figure 1.15) from the heartwood of *Pinus* species (106). The hardwood lignan, syringaresinol (Figure 1.15), is also reported to have antifungal activity (124). The contribution of heartwood lignans to natural durability has not been studied extensively.

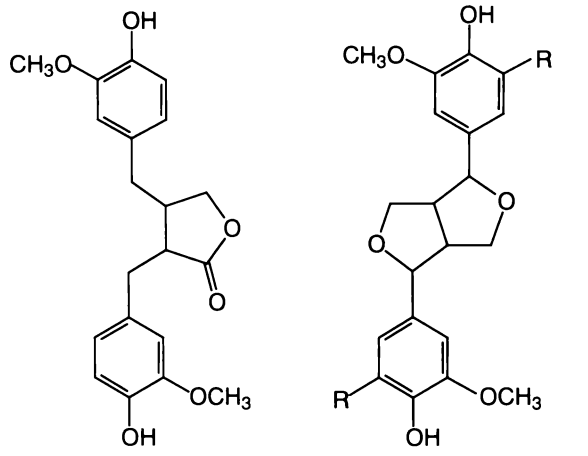


Figure 1.15: Antifungal heartwood lignans.

1.5.2.7 Quinones

Quinones of various types have been proposed as important durability principles in a number of naturally durable timbers. Terpenoidal quinones are discussed in Section 1.6.3. Neoflavonoid quinones from *Dalbergia* species, such as 4-methoxydalbergione (Figure 1.16), possibly contribute to the natural durability of these timbers (101, 125). The heartwood of *Dalbergia retusa* contains the *p*-quinone methide, obtusaquinone (Figure 1.16), which is moderately active against brown and white rot fungi (96) and a very effective deterrent to marine boring organisms (95).

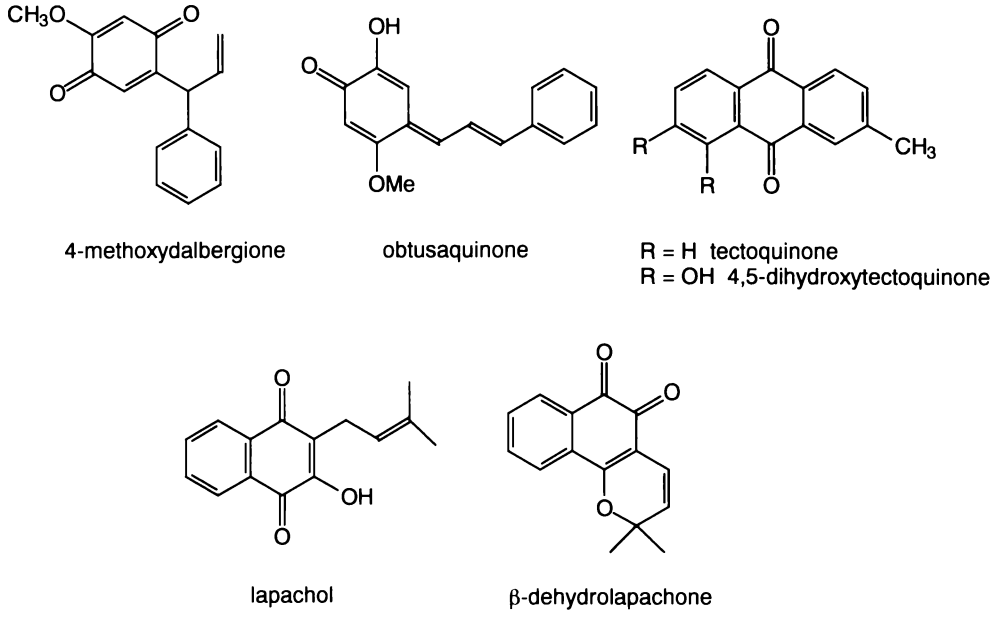
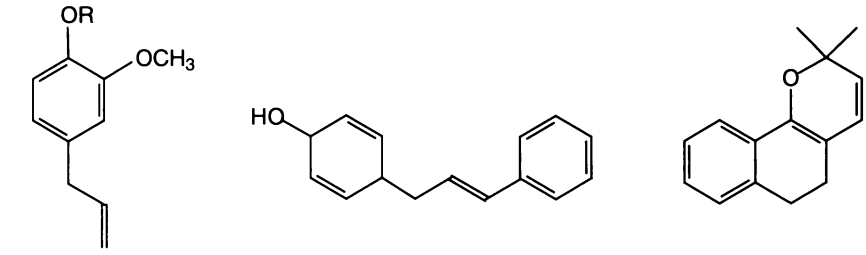


Figure 1.16: Antifungal heartwood quinones.

The natural durability of the heartwood of *Tectona grandis* (teak) is thought to be due to the anthraquinones, tectoquinone and 4,5-dihydroxytectoquinone (101) (Figure 1.16). The naphthoquinones, lapachol and β-dehydrolapachone (Figure 1.16), found in the heartwood of *Tabebuia* species, contribute to the durability of these timbers (101, 125). Lapachol is weakly antifungal (106) yet possesses good termiticidal properties (101); β-dehydrolapachone has antifungal and antibacterial properties (126).

1.5.2.8 Miscellaneous Extractives

The heartwood of *Lagarostrobos franklinii* (huon pine) which is particularly resistant to soft rot, contains 6.6% methyl eugenol (127). Methyl eugenol and eugenol (Figure 1.17) have been found to completely inhibit the growth of six soft rot fungi at concentrations ranging from 0.025% to 0.1% in nutrient agar (127). Obtusastyrene (Figure 1.17) found in the heartwood of *Dalbergia obtusa*, is reported to be a highly effective microbiocide (125, 128). The naphthopyran, lapachonone (Figure 1.17), occurs in the heartwood of decay resistant *Tabebuia* and *Paratacoma* species (101, 106). Rudman (106) found lapachonone to be highly toxic to four basidiomycetes in wood sawdust based assays. The toxicity of lapachonone was comparable with that of the thujaplicins, heartwood tropolones (Section 1.7).



R = H eugenol
R = CH_3 methyl eugenol

obtusastyrene

lapachonone

Figure 1.17: Miscellaneous antifungal heartwood extractives.

1.5.2.9 Anti-insect Extractives

Heartwood extractives which impart insect resistance include terpenoid acids and alcohols, terpenoid aldehydes and ketones, quinones, flavonoids, anthrones, xanthones and naphthopyrans and have been reviewed by Rao (101).

1.5.2.10 Concluding Comments

Natural durability is a complex phenomenon. Durability has been attributed to highly bioactive extractives, to synergism between extractive components and to high concentrations of weakly bioactive extractives. Fungistatic and/or fungicidal properties are not the sole mechanisms by which extractives can impart durability. Many heartwood extractives are water insoluble. If present in sufficiently high concentrations the water repellency properties of the extractives can contribute to the protection of the wood against fungal colonisation. Although heartwood extractives play a major role in determining natural durability other factors such as lignin type and quantity (Section 1.2.3), cellulose crystallinity and nitrogen and starch content (100, 108) can contribute to the susceptibility of different timbers to fungal deterioration.

On reviewing the literature in this field the impression gained was that oxygenated terpenoids and tropolones offered the greatest potential as models to novel chemical analogues with antifungal activity. Representatives from these classes show good to potent activities against wood decay and staining fungi, have low molecular weights and structural analogues would be less complicated to synthesise compared to several of the other classes. Antifungal terpenoids and tropolones are not exclusive to the heartwood of durable timbers. Fungitoxic terpenoid and tropolone phytoalexins, which are produced by trees in response to fungal infection, are known in the literature. Essential oils from the wood, leaves and bark of certain plants are rich sources of oxygenated terpenoids that show antifungal properties. Examples from such sources have been included in the following review of the antifungal oxygenated terpenoids and tropolones from higher plants (Sections 1.6 and 1.7).

1.6 Antifungal Terpenoids

1.6.1 Terpenoid Chemistry and Biosynthesis

Terpenes are composed of C_5 isoprene units which as a general rule (the isoprene rule) are linked from the head of one isoprene unit to the tail of the next (Figure 1.18). Monoterpenes are assembled from two isopropene units, the sesquiterpenes from three, the diterpenes from four (Figure 1.18), the sesterterpenes and triterpenes from five and six isoprene units

respectively. This discussion focuses only on the lower isoprenoids, the monoterpenes, sesquiterpenes and diterpenes.

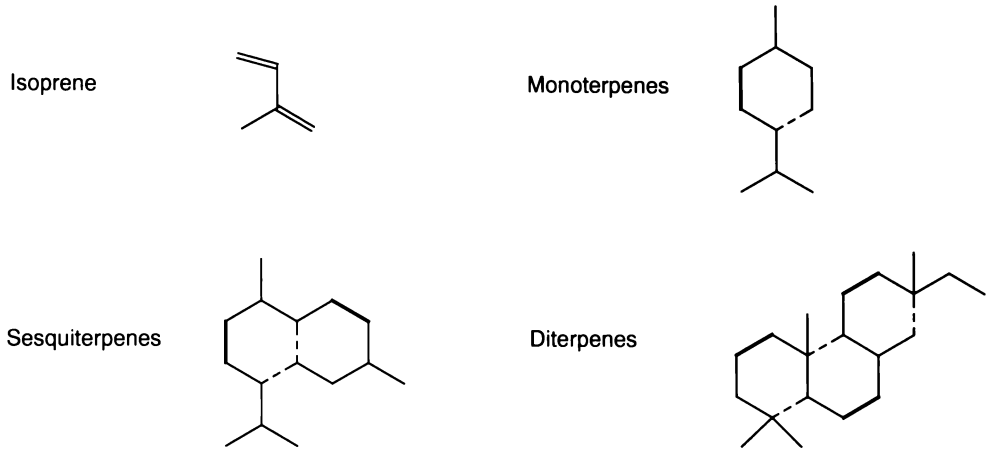


Figure 1.18: Isoprene and the lower molecular weight terpenes (bonds between isoprene units are shown as bolded lines, bonds formed by internal cyclization are shown as dashed lines).

The terpenes are produced biochemically from isopentenyl pyrophosphate (IPP) (129, 130); two pathways to IPP are known, the classic mevalonate pathway and the more recently proposed glyceraldehyde-3-phosphate (GAP)/pyruvate pathway (130, 131). IPP isomerase converts IPP into dimethylallyl pyrophosphate (DMAPP), which combines with IPP, with the elimination of pyrophosphate, to give geranyl pyrophosphate (GPP), the precursor to monoterpenoids (Figure 1.19). Nucleophilic attack of an olefinic π electron on the electron deficient carbon bearing the pyrophosphate group is common in terpene biosynthesis (Figure 1.19). The condensation of IPP with GPP gives *trans*-farnesyl pyrophosphate (FPP) which leads to the sesquiterpenoids. The diterpenoid skeleton is formed through the condensation of IPP with FPP to give geranyl geranyl pyrophosphate (GGPP) (Figure 1.19).

Mono and sesquiterpenoids may retain an acyclic skeleton or undergo cyclization *via* an internal version of the nucleophilic attack described above, for example the cyclization of GPP to limonene (129) (Figure 1.20). Enzymatic cyclizations, rearrangements and hydride shifts governed by steric and electronic considerations lead to a multitude of monoterpenoid and sesquiterpenoid structures (130). The diterpenoids are for the most part cyclic compounds, although a few exceptions exist. The diterpenes are typified by internal cyclization of double bonds as represented in the cyclization and allylic rearrangement of geranylgeraniol to manool (129) (Figure 1.20).

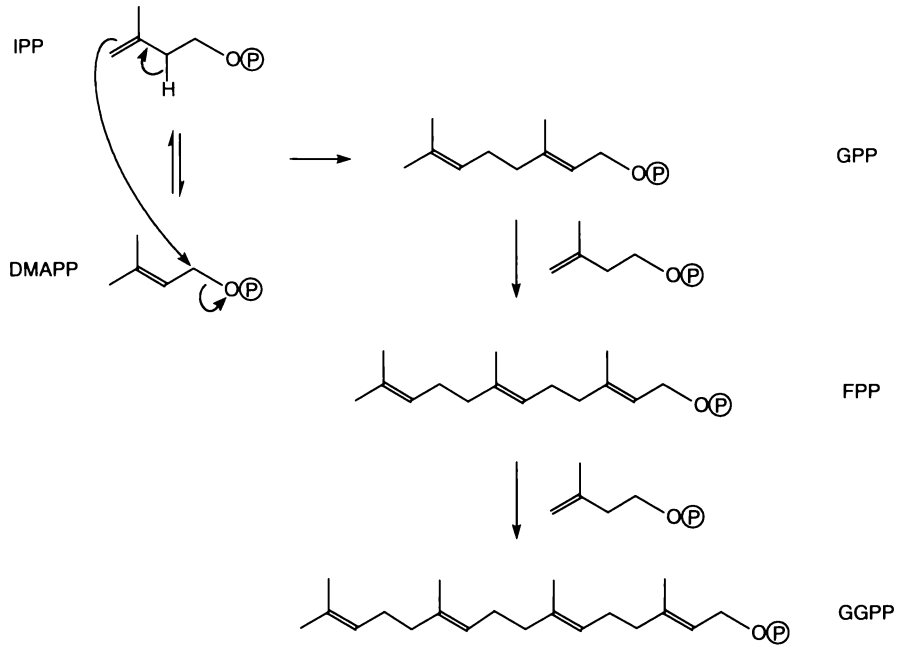


Figure 1.19: Biosynthesis of GPP, FPP and GGPP.

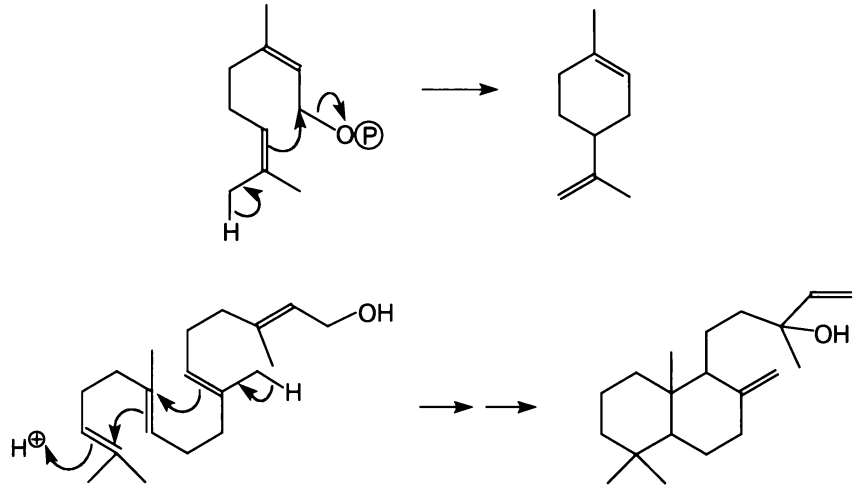


Figure 1.20: Cyclization of GGP to limonene (top) and geranylgeraniol to manool (bottom).

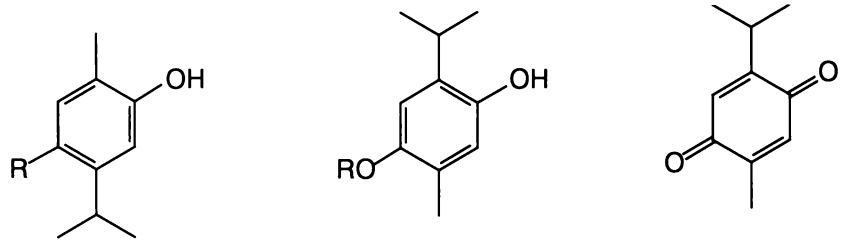
Oxidations and reductions of the parent terpene skeleton by various oxidases and reductases lead to a wide range of oxygenated terpenoids. These biochemical oxidations and reductions form terpenoids with hydroxyl, phenolic, carboxylic acid, carbonyl and quinoidal functional groups. A number of oxygenated terpenoids have been shown to exhibit fungicidal and fungistatic properties and are known to be responsible for the natural durability of several decay resistant softwoods.

1.6.2 Heartwood Terpenoids

1.6.2.1 Monoterpenoids

Several oxygenated monoterpenes have been reported as active principles in decay resistance. The heartwood of *Libocedrus decurrens* (incense cedar), which is highly prized for its durability, contains the monoterpenoids; carvacrol, *p*-methoxycarvacrol, *p*-methoxythymol, hydroxythymoquinone, thymoquinone (Figure 1.21) and the tropolone, γ-thujaplicin (132) (Figure 1.33). Andersson *et al.* (132) tested these extractives against the white rot fungus *Trametes versicolor* and the brown rot fungi, *Gloeophyllum trabeum*, *Lentinus lepideus* and *Oligoporus placenta* in wood blocks at 1.2% loadings. The tropolone was the most potent followed by hydroxythymoquinone and *p*-methoxythymol which showed good to moderate activity across all four fungi. Carvacrol and *p*-methoxycarvacrol were partly active against two of the brown rot species. Thymoquinone was largely ineffective, suggesting a phenolic hydroxyl group was essential for antifungal activity. The natural mixture of the phenols completely inhibited the growth of *Gloeophyllum trabeum* and *Lentinus lepideus* indicating synergism between the monoterpenoids is an important feature of incense cedar durability.

Erdtman and Rennerfelt (133) reported carvacrol and thymoquinone, from the heartwood of *Tetraclinis articulata*, as active fungicides, having determined their activities on nutrient agar. Conversely Rudman (106) tested the activity of carvacrol and thymol against the aforementioned brown rot fungi in a wood sawdust substrate at concentrations of 0.1-1.0% and found them to be largely non-toxic.



R = H carvacrol
R = CH_3 *p*-methoxycarvacrol

R = CH_3 *p*-methoxythymol
R = H hydroxythymoquinone

thymoquinone

Figure 1.21: Antifungal monoterpenoids from the heartwood of *Libocedrus decurrens.*

Anderson (105) has proposed that oxidative coupling of the aforementioned phenols can result in detoxification of these monoterpenoids. The monoterpenoid dimers libocedrol and heyderiol (Figure 1.22) occur in the heartwood of mature incense cedar trees and appear to be formed *in situ* by enzymatic coupling of *p*-methoxythymol and *p*-methoxycarvacrol units. Unlike their monoterpenoid precursors libocedrol and heyderiol have no antifungal properties (105, 132).

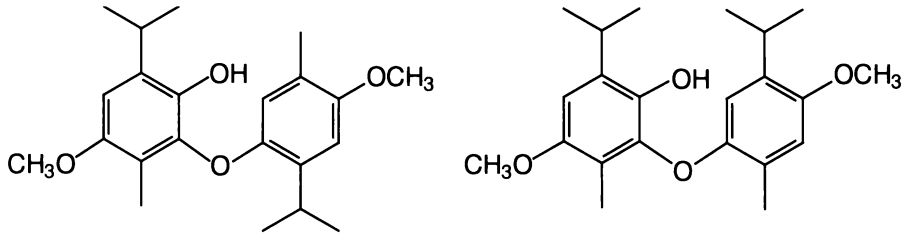


Figure 1.22: Monoterpenoid dimers libocedrol (left) and heyderiol (right).

The durability of *Chamaecyparis nootkatensis* (yellow cedar) heartwood has been attributed to the tropolone, nootkatin (Figure 1.33) and the terpenoid acids, chamic and isochamic acid (134) (Figure 1.23). Rennerfelt and Nacht (115) reported that chamic acid was toxic to a range of decay and staining fungi at concentrations of 0.01-0.02% on nutrient agar. Isochamic acid was also fungicidal yet less active. Both acids are reported to be strong termite repellents (101). The acyclic terpenoid acid, *l*-citronellic acid (Figure 1.23) is found in the heartwood of *Callitris columellaris* (inland form) which is noted for its resistance to decay and termites (107). Rudman (107) reported *l*-citronellic acid effectively inhibited weight loss of *Pinus radiata* sapwood by *Coniophora olivacea* at a loading of 2.0% with partial inhibition at concentrations of 0.5% and 1.0%.

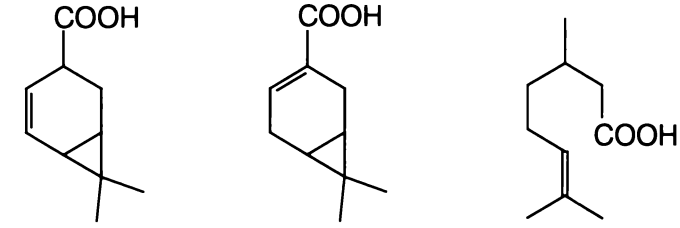


Figure 1.23: Antifungal heartwood monoterpenoid acids.

1.6.2.2 Sesquiterpenoids

In addition to *l*-citronellic acid, the sesquiterpene alcohols, α-eudesmol and β-eudesmol (Figure 1.24) contribute to the natural durability of *Callitris columellaris*. A mixture of the two eudesmols was moderately effective at inhibiting decay by *Coniophora olivacea* at 1.0-2.0% in wood block tests (107). The eudesmols (α, β and γ) have also been implicated in the durability of *Pterocarpus santalines* heartwood (101). Antifungal eudesmanoids have been isolated from the resin of *Parthenium argentatum* (135, 136).

Extracts from the heartwood of *Chamaecyparis obtusa* were found to be strongly antifungal against *Tyromyces palustris* and *Trametes versicolor* in wood block trials (137). Sesquiterpene alcohols identified in the active fractions included α-cardinol, *t*-cardinol and *t*-muurolol (Figure 1.24). Chang *et al.* (138) isolated α-cadinol and cedrol (Figure 1.24) from extracts of the *Taiwania cyptomerioides*, a Taiwanese softwood renowned for its excellent durability. Both sesquiterpene alcohols showed good activity against the basidomycetes *Trametes versicolor* and *Laetiporus sulphureus* on nutrient agar. Cedrol isolated from the wood of *Cunninghamia lanceolota* inhibited mycelial growth of the brown rot fungus, *Lentinus edodes* (139). Cedrol is a major component of the essential oil of the durable timbers *Juniperus communis* and *Juniperus virginiana* (101).

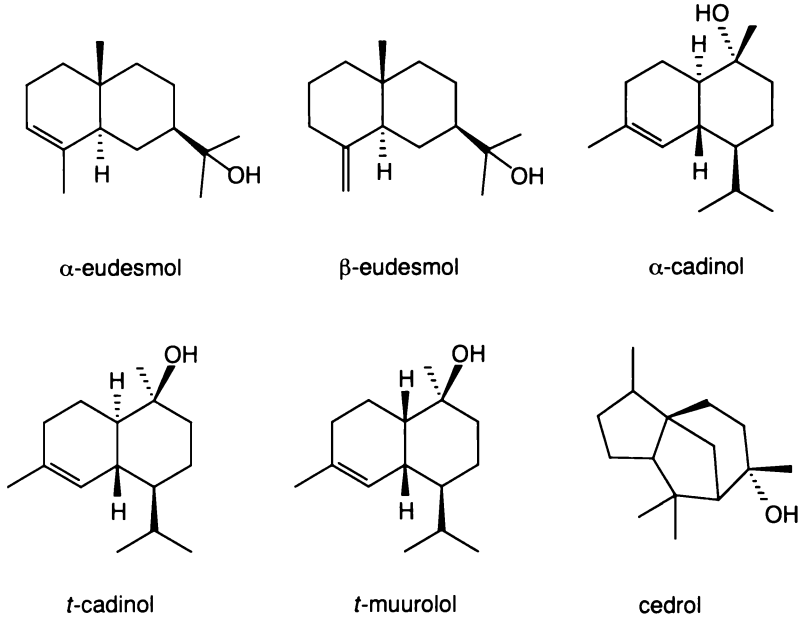


Figure 1.24: Antifungal heartwood sesquiterpenoid alcohols.

Richardson *et al.* (140) investigated the resin from *Dipterocarpus kerrii* on account of its antifungal and termiticidal properties. Three sesquiterpene alcohols and an enone with the gurjunene skeleton, including spathulenol (Figure 1.25), were isolated from the active fractions of the resin. Himachalol (Figure 1.25) isolated from the durable timber *Cedrus deodara* exhibits antifungal activity against the mould *Aspergillus fumigatus* (141). 7-Hydroxy-3-methoxycadalene (Figure 1.25), isolated from the heartwood of *Zelkova serrata*, had recently been reported to have strong antifungal and anti-oxidative properties (142).

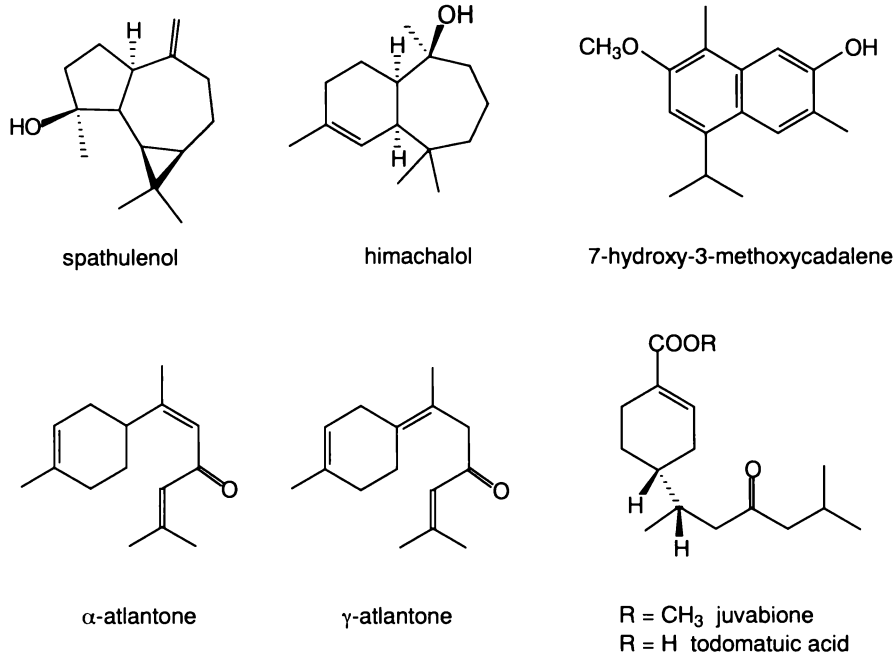


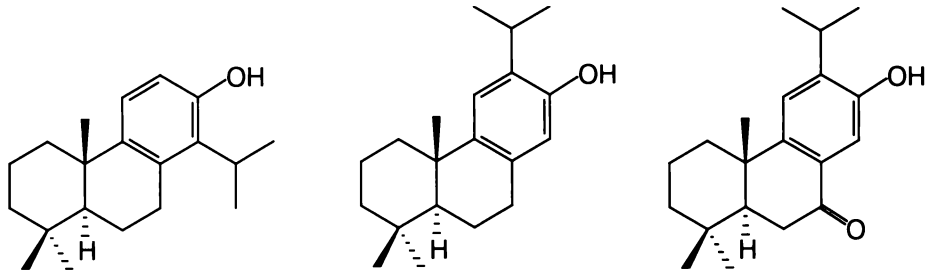
Figure 1.25: Antifungal heartwood sesquiterpenoid alcohols, ketones and acids.

The α,β-unsaturated sesquiterpenoid ketones, α- and γ-atlantone (Figure 1.25) are the main components of the heartwood essential oil of decay-resistant species *Cedrus atlantica* and *Cedrus deodara* (101). The volatile oils, which contain many other oxygenated sesquiterpenoids, including himachalol (Figure 1.25), exhibit good antifungal properties (101, 143). At present it is unclear whether natural durability is due primarily to the atlantones or the result of synergism with other oxygenated sesquiterpenoids. The wood of *Abies sachalinensis* (Japanese fir) contains the antifungal sesquiterpene ester, (+)-juvabione (144, 145) (Figure 1.25). Aoyama *et al.* (145) studied the activity of juvabione and its saponified product, (+)-todomatuic acid (Figure 1.25) against 9 species of decay fungi in nutrient agar. Although activities were species dependent, strong inhibition of the growth of most of the fungi was observed for both compounds at 500 mg/ml. (+)-Todomatuic acid was generally

more fungitoxic. The quinoidal sesquiterpenes, the mansonones and cadinane-type sesquiterpene alcohols found in the heartwood of *Mansonia* and *Ulmus* species (146), have been reported as fungitoxic. These compounds are also produced as phytoalexins by *Ulmus* species in response to fungal infection and are discussed in Section 1.6.3.

1.6.2.3 Diterpenoids

The heartwood of *Podocarpus totara* (totara) is very durable, primarily due to the presence of the phenolic diterpenoid, totarol (Figure 1.26). Totarol is found in numerous timbers but is most abundant in *P. totara* (147). Cambie and Mander (148) obtained totarol in 5% yield from the dry timber. The related diterpenoid, ferruginol (Figure 1.26), has been implicated in the durability of *Lagarostrobus colensoi* (silver pine) (149) and *Cryptomeria japonica* (4). Rudman (106) found totarol and ferruginol inhibited the growth of *Lentinus lepideus* at 1% in wood sawdust but were inactive against *Coniophora olivacea*. Sugiol (Figure 1.26) was found to be non-toxic to both fungi indicating antifungal activity is destroyed by the C_9 keto group. Chang *et al.* (150) found ferruginol to be moderately active against *Trametes versicolor* and *Laetiporus sulphureus* at 100 ppm in nutrient agar.



totarol ferruginol sugiol

Figure 1.26: Heartwood diterpenoid alcohols.

1.6.3 Terpenoid Phytoalexins

Antifungal phytoalexins are part of a tree's natural defence mechanism against microbial attack. They can accumulate in sapwood in response to infection by fungal pathogens and are synthesised by the tree to impede the progress of the invading pathogen. These low molecular weight secondary metabolities are chemically very diverse. Compounds with terpenoid,

stilbenoid, flavonoid, phenylpropanoid, biphenyl, dibenzofuran, lignan, and alkaloid structures are known. Kemp and Burden (146) and Gottstein and Gross (151) have reviewed phytoalexins from woody plants. Antifungal terpenoid phytoalexins are potential sources of lead compounds to new wood protection agents, some examples are discussed below.

Oxygenated sesquiterpenoids are important phytoalexins in several tree families, while monoterpenoid and diterpenoid phytoalexins are relatively rare (146, 151). The sesquiterpenoid 1,2-naphthoquinones, mansonone C, E, and F (Figure 1.27) have been isolated from the sapwood of *Ulmus hollandica* (dutch elm) and *Ulmus glabra* (wych elm) infected with the plant pathogen fungus *Ceratocystis ulmi* and basidiomycetes *Trametes versicolor* and *Chondrostereum pupureum* (151, 152, 153,). 7-Hydroxycalamenene and 7-hydroxycadalenal (Figure 1.27) are also known phytoalexins of *U. glabra* (152, 153). In nutrient agar assays mansonone E was found to be the most active against *Ceratocystis ulmi*, while 7-hydroxycadalenal was the most effective against the two above basidiomycetes (152). 7-Hydroxycalamenene was strongly inhibitory against all three fungi (152). Mansonone E and F have been shown to be fungitoxic to several fungi including the moulds *Aspergillus niger*, *Cladosporium cumerium* and *Penicillium italicum* (153). 7-Hydroxycalamenene has been found to accumulate in *Tilia europea* (european lime) colonized by the white rot fungus *Ganoderma applanatum* (154) and is a heartwood extractive in several elm species (146).

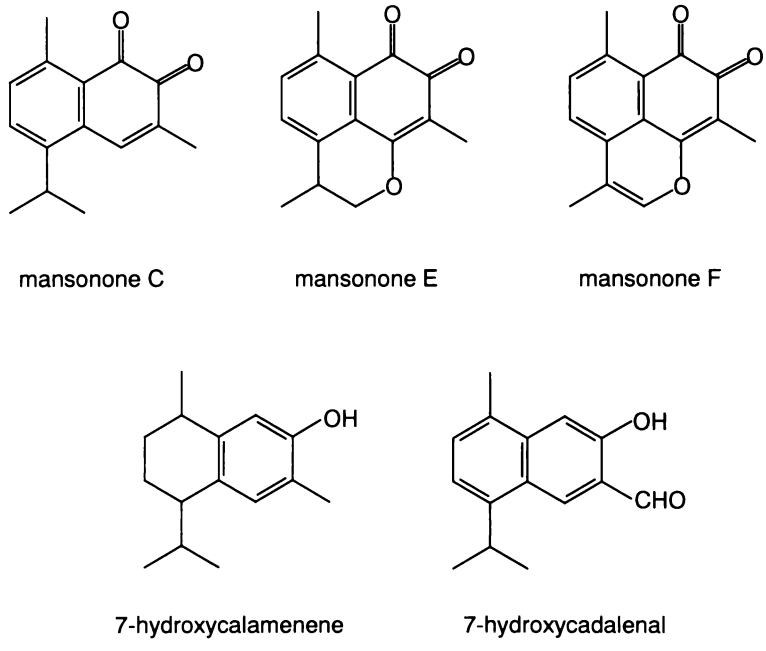


Figure 1.27: Antifungal sesquiterpenoid phytoalexins.

1.6.4 Terpenoids from Essential Oils

The literature contains numerous examples of plant essential oils that have antifungal properties on account of their oxygenated terpenoid contents (155). The following discussion focuses on studies in which individual terpenoid components have been tested and shown to possess antifungal properties, in particular activities against wood damaging fungi. In the majority of these studies antifungal properties have been measured in nutrient agar media rather than woody substrates. These assays enable rapid assessment with small amounts of material, although these assays are not representative of the circumstances faced in wood protection, they can provide useful indicative information on structure/activity relationships.

In many essential oils monoterpenoid phenols, alcohols and/or aldehydes are the principal antifungal components. Kurita *et al.* (156) studied a range of essential oil components including aliphatic and aromatic aldehydes and ketones, 1° and 2° alcohols, phenols, ethers and hydrocarbons against seven fungi in nutrient agar. Monoterpenoids that exhibited strong antifungal activity across the spectrum of fungi included thymol, geraniol, citronellol, and cinnamaldehyde (Figure 1.28). Citral, perillalcohol and perillaldehyde (Figure 1.28) were also strongly active but more selectively toxic. Carvacrol was not tested.

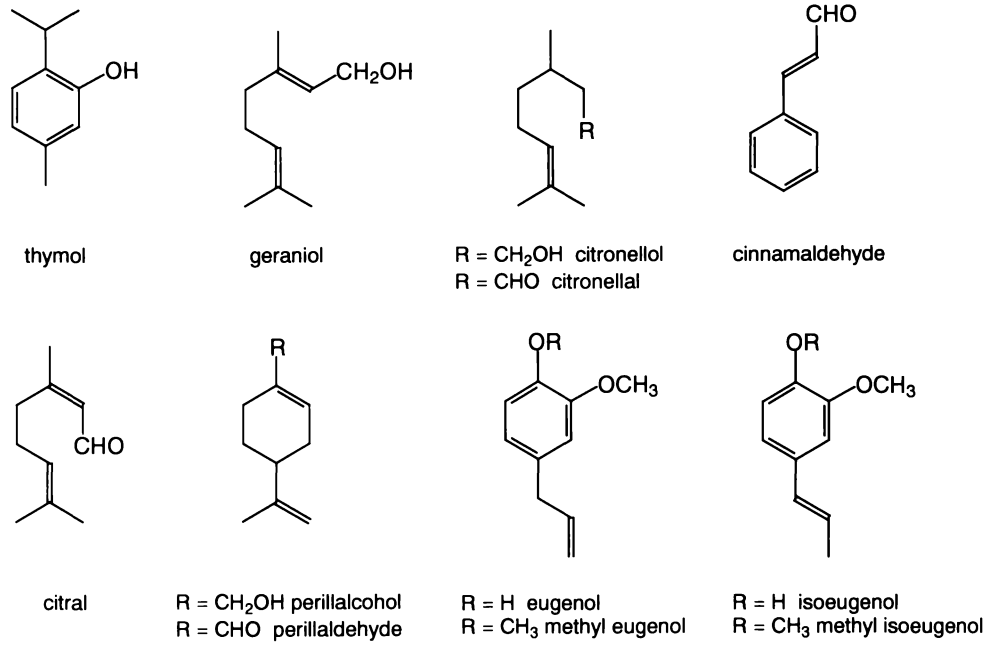


Figure 1.28: Antifungal monoterpenoid and phenolic essential oil components.

The phenols *p*-*n*-propylphenol, *p*-ethylphenol, eugenol and isoeugenol and the ethers methyl eugenol and methyl isoeugenol (Figure 1.28) were also strongly active. In the case of the phenolic compounds alkylation was found to enhance activity. Orders of antifungal activity were as follows: thymol ≅ *p*-*n*-propylphenol > *p*-ethylphenol > cresol > phenol and isoeugenol > creosol > guaiacol. Thymol and isoeugenol were approximately ten times as active as phenol and guaiacol respectively. A free phenolic group was not necessary for fungitoxicity as methyl isoeugenol and methyl eugenol were of comparable activity to isoeugenol and eugenol.

The antifungal properties of thymol and carvacrol are well-known. Essential oils from various aromatic plants rich in these phenolic monoterpenes have been reported to have antifungal, antibacterial and insecticidal properties (157-161). Thymol and carvacrol are reported to be active against various wood decay, staining and food storage fungi. (108, 132, 157, 162, 163) The literature on the monoterpenoid alcohols and aldehydes is less extensive. Hill *et al.* (164) screened 51 plant essential oils for antifungal effect against the sapstain fungus *Ophiostoma piceae* on agar plates and freshly cut pine wood blocks. The most active oils contained high levels of phenolic and alcoholic monoterpenoids. Pine oil, which contains 40-60% α-terpineol, was reported to show good activity against sapstain fungi in timber (164). The use of commercial pine oil for the control of sapstain has been evaluated (165). Holland *et al.* (166, 164) explored the relationship between chemical structure and activity against staining fungi for monoterpenoid alcohols found in pine oil. The activities of the monocyclic isomeric alcohols, α-terpineol, γ-terpineol, (+)*trans*-β-terpineol, (-)*trans*-β-terpineol and terpinen-4-ol (Figure 1.29) were assessed against the staining fungus *Ophiostoma piceae* and *Cladosporium* and *Diplodia* species. (+)*trans*-β-Terpineol was the most active, with the others only slightly less active. The bicyclic monoterpene alcohol, borneol (Figure 1.29), was reported to have low activity.

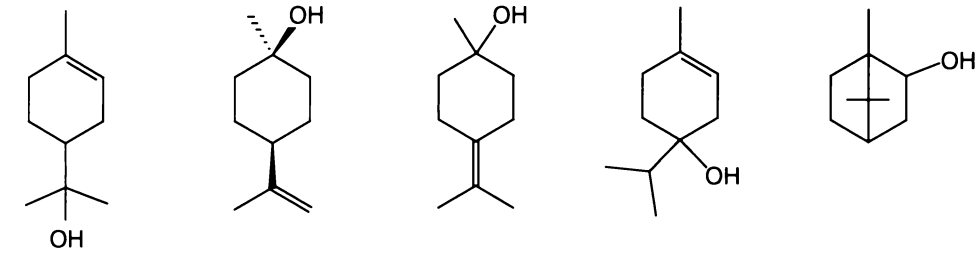
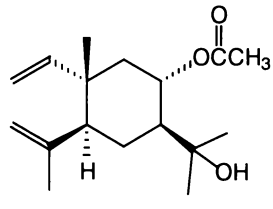


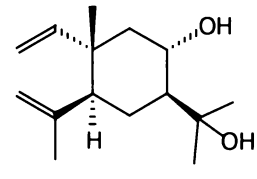
Figure 1.29: Antifungal monoterpenoid alcohols and borneol.

Tripathi *et al.* (167) have reported geraniol and to a lesser extent citronellol (Figure 1.28) were strongly inhibitory to the three plant pathogenic fungi. Saito (168) *et al.* tested a range of monoterpenoid alcohols and aldehydes against seven plant pathogenic fungi and reported perillaldehyde (Figure 1.28) and α-terpineol (Figure 1.29) exhibited good antifungal activities. Kurita *et al.* (169) compared the efficacy of cinnamaldehyde, perillaldehyde, citral and citronellal (Figure 1.28) against 18 fungi and found cinnamaldehyde exhibited the greatest antifungal effect. They proposed a relationship between the molecular orbital energies of monoterpenoid aldehydes and antifungal activity. The above studies provide some insights into the relationships between chemical structure and antifungal activity in the monoterpenoids. Active compounds generally have phenolic, alcohol or aldehyde functional groups, yet the presence of such groups does not guarantee activity. The situation is understandably more complex with possible electronic, steric and stereochemical factors influencing the level of toxicity.

Oxygenated sesquiterpenes undoubtedly play a role in the antifungal activity of essential oils of higher plants from sources other than heartwood. Sesquiterpene phenols, alcohols, ketones, dialdehyde and lactones with antifungal properties have been isolated from a variety of plant sources (101, 146, 155, 170-176). Few studies, however, have assessed the activity of individual sesquiterpenoid components against wood damaging fungi. β-Eudesmol (Figure 1.24), isolated from *Magnolia obovata* bark, is reported to be strongly antifungal against basidiomycetes in nutrient agar (170). Ohasi *et al.* (171) isolated 8-acetoxyelemol and 8-hydroxyelemol (Figure 1.30) from *Juniperus chinensis* leaves and found these alcohols to be toxic to three water eczema fungi but inactive against to the sapstain fungus *Ceratocystis piceae*. The antifungal sesquiterpene alcohols, α-cardinol, *t*-cardinol and *t*-muurolol (Figure 1.24) were isolated by Chalchat *et al.* (172) from the essential oil obtained from *Pinus syvestris* twigs.



8-acetoxyelemol



8-hydroxyelemol

Figure 1.30: 8-Acetoxyelemol and 8-hydroxyelemol

The foliage oil of *Podocarpus totara* is rich in oxygenated terpenoids (177). A preliminary bioassay on a sesquiterpenoid rich fraction of the oil indicated activity against the decay fungus, *Coniophora puteana* at a treatment concentration of *ca.* 2% (178). The anti-basidiomycetes activity of oxygenated sesquiterpenoids from the foliage oils of *P. totara* and other species native to New Zealand is an area worthy of further investigation (see Chapter 3).

1.7 Antifungal Tropolones

Dewar, in 1945, proposed that the perplexing properties of the mould metabolite, stipitatic acid, could be explained by considering the acid a derivative of 2-hydroxy-2,4,6-cycloheptatrien-1-one (179) (Figure 1.31). He named this novel seven-membered ring structure, tropolone. Tropolone cannot be correctly represented by one structural formula. The physical evidence (180, 181) indicates that tropolone exists as two readily interconvertible tautomers (Figure 1.31), each of which is stabilised by hydrogen bonding and resonance with the corresponding ionic forms shown in Figure 1.31.

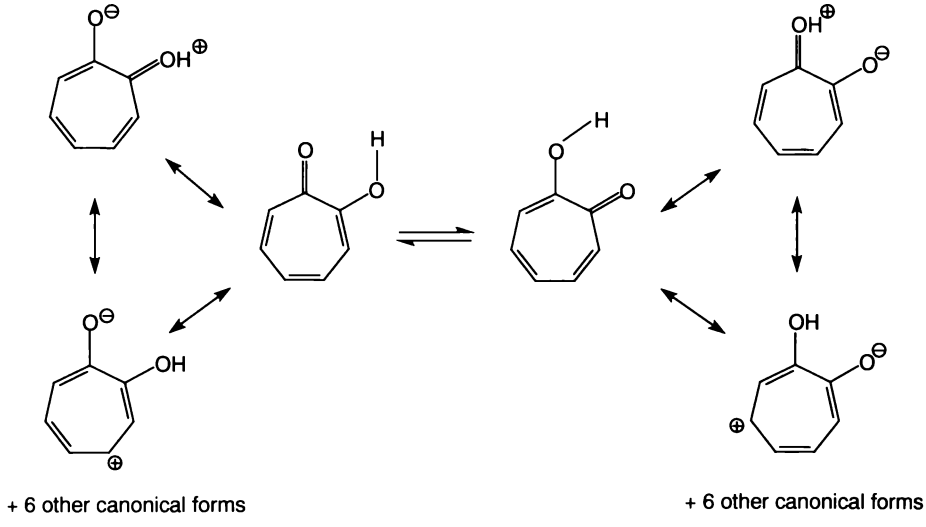
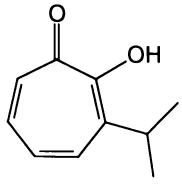


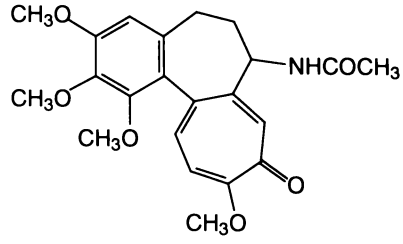
Figure 1.31: Structural representation of tropolone.

Since Dewar's inspired deduction a number of natural products have been shown to be tropolone derivatives. These include alkylated tropolones (e.g. α-thujaplicins from the heartwood of species of the *Cupressaceae* family), alkaloidal tropolones (e.g. colchicine, isolated from plants of the *Liliacae* family) and acid tropolones (e.g. stipitatic acid from *Penicillium* species) (180, 182) (Figure 1.32). Buchanan and Raphael (180), Pietra (181),

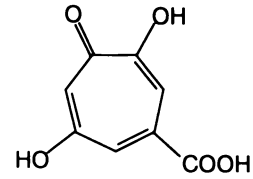
Pauson (182) and Nozoe (183) have published reviews on the synthesis, structure and properties of natural and synthetic tropolones. Gardener (184) has reviewed tropolones from wood. The following discussion covers aspects of the chemistry and biological properties of tropolones found in wood.



α-thujaplicin



colchicine



stipitatic acid

Figure 1.32: Examples of naturally occurring tropolones.

1.7.1 Tropolones in Wood

1.7.1.1 Chemistry, Biosynthesis and Distribution

The thujaplicins were the first tropolones to be found in wood; all three isomers α-, β- and γ-thujaplicin (Figures 1.32 and 1.33) are known. They have been isolated from the heartwood of a number of decay-resistant Cupressaceae species, including *Thuja plicata*, *Thujopsis dolobrata*, *Cupressus macrocarpa*, *Chamaecyparis lawsoniana* and *Juniperus conferta* by steam distillation, solvent extraction and supercritical CO_2 extraction (184-189). β-Thujaplicin is also known as hinokitiol due to its independent isolation and characterisation from taiwan hinoki (*Chamaecyparis taiwanensis*) by Nozoe (190). The alkenyltropolones, β-dolabrin and nootkatin (Figure 1.33) were first isolated by steam distillation from the heartwood of *Thujopsis dolabrata* and *Chamaecyparis nootkatensis* respectively (191, 192). They occur in varying amounts in many other species (184, 187). The hydroxytropolone, α-thujaplicinol and the methoxytropolone, pygmaein (Figure 1.33) are found in the heartwood of *Cupressus pygmaea* (193). β-Thujaplicinol (Figure 1.33) has been isolated from *Thuja plicata* (194) and detected in several *Cupressus* species (187). Other tropolones found in wood include; 5-ethyl tropolone, dolabrinol, isopygmaein, procerin, hydroxynootkatinol and chanootin (181, 184, 187) (Figure 1.33).

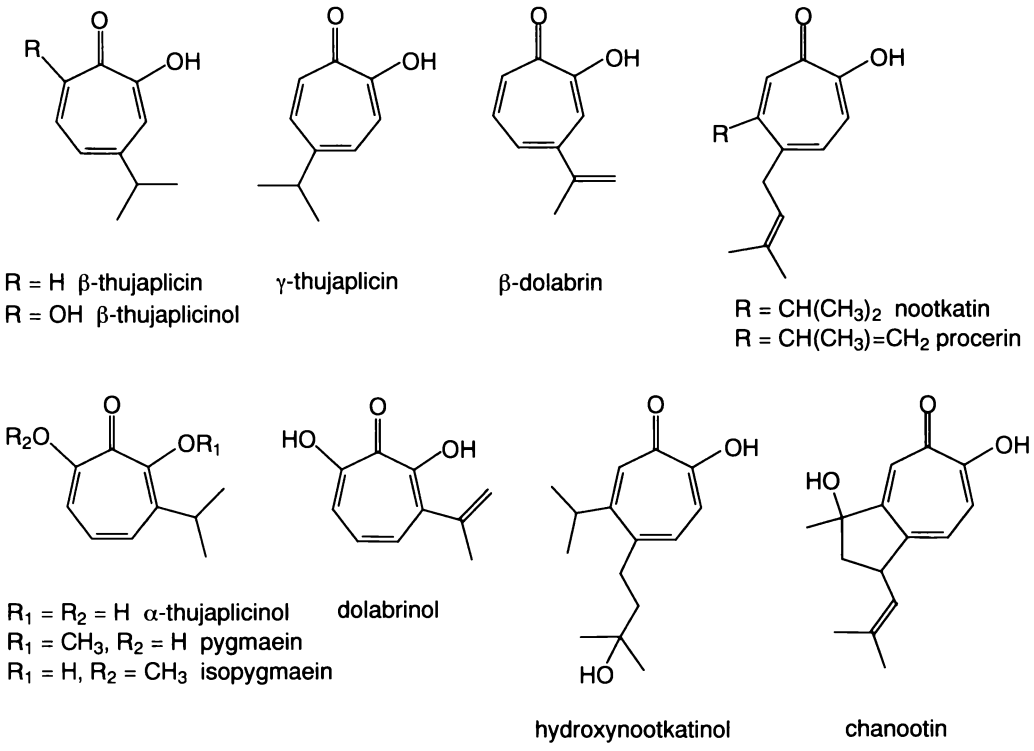


Figure 1.33: Heartwood tropolones from species of the Cupressaceae family.

The biosynthesis of wood tropolones has not been explored in detail. Tropolones are believed to be modified terpenoids formed by ring enlargement. Support for this hypothesis can be found in the frequent co-occurrence of terpenoids with tropolones and isolation of sesquiterpenoid tropolones, such as nootkatin (181). Sakai and colleagues, studying the biosynthesis of β-thujaplicin in cell cultures of *Cupressus lustanica* (195, 196), recently showed that ^{14}C-labelled geraniol was incorporated into β-thujaplicin (197). This revealed that the tropolone is biosynthesised from geranylpyrophosphate (GPP) and hence is a monoterpenoid. They proposed that GPP is transformed to β-thujaplicin *via* a limonane type skeleton (197).

The aforementioned tropolones occur only in heartwood. No evidence of their occurrence in sapwood, bark or leaves has been reported (184). Studies on the within-tree variation of tropolone content of *Thuja plicata* have shown a pattern of increasing content with increasing distance from the pith within the heartwood (198, 199). Variations in tropolone content correlate with observed variations in decay resistance. The distribution of tropolones within the tree can impact on the timber's end-use. Consequently methods of measuring total tropolone content from core samples taken from standing trees have been developed (200).

In the Cupressaceae family tropolones have been detected in species of the following genera; *Austrocedrus*, *Athroxtaxis*, *Biota*, *Cupressus*, *Chamaecyparis*, *Juniperus*, *Libocedrus*, *Thuja* and *Thujopsis*. Researchers have summarized available information on the tropolone content of species of these genera (184, 187). In some cases tropolone content has been used for taxonomic classification.

1.7.1.2 Antifungal Properties

In terms of the tropolones the most thoroughly studied species of the Cupressaceae family is *Thuja plicata* (western red cedar), an important commercial species in Northern USA and Canada. Research in the early to mid 1900s proved the heartwood to be very resistant to decay (201, 202, 203). Anderson and Sherrard (201) reported that $C_{10}H_{12}O_2$ 'phenol-like' substances, from the steam distillate of the wood, were extremely toxic to the decay fungi, *Heterobasidion annosum* and *Gloeophyllum trabeum*. Toxicities ten-fold that of creosote which was the wood preservative at the time were reported. These compounds were later identified as β-thujaplicin and γ-thujaplicin (Figure 1.33) and the connection between the presence of tropolones in wood and natural durability was established. Rennerfelt (204) showed the thujaplicins to have a strong fungicidal effect upon wood decay and staining fungi in nutrient agar. γ-Thujaplicin was reported as being a hundred-fold more effective than phenol against decay fungi and ten-fold more effective against staining fungi. Activities were comparable with sodium pentachlorophenate. Of the isomeric thujaplicins, according to the agar diffusion method, β-thujaplicin was the most toxic followed by γ-thujaplicin and α-thujaplicin (204).

Roff and Whittaker (205) compared the toxicity of γ-thujaplicin and β-thujaplicinol (Figure 1.33), isolated from *Thuja plicata*, in nutrient agar and found them similar in activity against brown rot fungi. β-Thujaplicinol was less effective than γ-thujaplicin against white rot fungi. Rennerfelt and Nacht (114), studying the fungicidal activity of some conifer heartwood extractives against a range of decay and staining fungi, showed nootkatin and γ-thujaplicin (Figure 1.33) to be ten-fold more potent than the stilbenes, pinosylvin and pinosylvin monomethyl ether (Figure 1.12) and the terpene acids, chamic and isochamic acid (Figure 1.23). Fungal growth was inhibited at concentrations of 0.001-0.002%. Although agar based assays provide useful information on relative antifungal activities of extractives, as previously mentioned (Section 1.5.2), absolute activities are a poor indicator of activity in wood.

Minimum inhibitory concentrations (MICs) in agar cannot be related to the natural concentrations of the wood extractive in the heartwood.

Rennerfelt and Nacht (114) reported total inhibition of *Lentinus lepideus* by γ-thujaplicin at 0.002% in nutrient agar, whilst 1.2% just succeeded in inhibiting its growth in wood blocks (105). γ-Thujaplicin was effective against *Trametes versicolor* and *Gloephyllum trabeum*, but largely ineffective against *Oligoporus placenta* at 1.2% in wood (105). Anderson *et al.* (193) studied the effectiveness of γ-thujaplicin, α-thujaplicinol and pygmaein (Figure 1.33) against the same fungi (with the exception of *Trametes versicolor*) at 1.2% in wood. α-Thujaplicinol was the most potent, controlling the growth of all three fungi. γ-Thujaplicin was completely effective against two of the three (partially controlling *Oligoporus placenta*). Pygmaein was selectively toxic to *Lentinus lepideus* and only partially controlled the growth of the other two fungi. The tropolone hydroxyl group was required for fungitoxicity, as methylation destroyed activity (193).

The need for relatively large amounts of the wood extractive has meant that only a limited number of fungitoxicity studies have been carried out on tropolones in wood media. To overcome this problem, Da Costa and Rudman (104) developed a sawdust dish technique for testing antifungal activity that required only 60 mg of extractive. Using this method, Rudman (206) reported partial to complete control of the brown rot fungi *Lentinus lepideus*, *Gloephyllum trabeum* and *Coniophora olivacea* and the white rot fungus *Trametes versicolor* by β-thujaplicin, γ-thujaplicin and β-thujaplicinol (Figure 1.33) at concentrations of 0.33-1.0%. In an extensive study of the antifungal activity of various wood extractives, including 62 compounds from 10 different classes, Rudman (106) concluded that the tropolones were the only group of extractives that could be classed as highly toxic to basidiomycetes. The only other extractive to show the same level of toxicity across the range of fungi tested was the naphthopyran, lapachonone (106) (Section 1.5.2.8).

Although tropolones are toxic to many common decay and staining fungi (106, 114, 193, 204, 207), as well as food spoilage fungi (208), fungitoxicity is by no means universal. Detoxification of thujaplicins in *Thuja plicata* by non-decay heartwood inhibiting fungi has been demonstrated (209, 210). In addition to fungicidal properties the antibacterial properties of tropolones are well documented (211, 212, 213). Becker (214) has reported β-thujaplicin as

toxic to the wood boring larvae of *Hylotropes bajules*, the house longhorn beetle. Inamori *et al.* (215) found that β-thujaplicin, β-dolabrin and γ-thujaplicin (Figure 1.33) exhibited strong insecticidal activities against the subterranean termite, *Coptotermes formosanus* and mould mite *Tyrophagus putrescentiae*. Arndt (216) reported β-thujaplicin and γ-thujaplicin as inactive against the subterranean termites, *Reticulitermes lucifugus* and *Reticulitermes flavipes*.

The hydroxyl group of tropolones is keto-enolic in nature and as such possesses properties of both phenols and enols (184). The acidity of the hydroxy group is enhanced by the neighbouring carbonyl group and is midway between that of carboxylic acids and phenols. The keto-enolic structure of tropolones enables them to rapidly form chelate complexes with metal ions (217, 218). The fungicidal effect of tropolones is believed to be due to this chelating capacity (92, 219). Methylation of the hydroxyl group results in loss of antifungal activity (193), as does biochemical oxidative dimerisation (210), both of which destroy the keto-enolic character of the tropolone. Formation of metal chelates with metal ions such as Fe^{2+}, Fe^{3+}, Mg^{2+}, Ca^{2+}, deprives the fungi of metals essential for survival or growth. As discussed earlier the involvement of Fe^{2+} is postulated in a mechanism of cellulose degradation by brown rot fungi (Section 1.2.3).

There is evidence to suggest that tropolones, like other phenolic compounds, are inhibitors of oxidative phosphorylation (the main source of energy in decay fungi) (108, 220, 221). Lyr (220) proposed the hypothesis that the thujaplicins act as uncouplers of phosphorylation allowing electron transport down the respiratory chain but preventing the formation of ATP. However, the results of Raa and Goksøyr (221) disagreed with this hypothesis, they found that phosphorylation rates closely followed oxygen uptake at all levels of inhibition. They suggested β-thujaplicin inhibited both glycolysis and the tricitric acid cycle thus inhibiting phosphorylation at the substrate level and through the electron transport system. The mechanism of inhibition of glycolysis was not speculated on. Succinic dehydrogenase, however, was proposed as a possible site of inhibition of the citric acid cycle (221). Tropolones have been shown to inhibit metalloenzymes through their chelating ability (213, 222, 223). Tropolone is a strong inhibitor of the fungal enzyme tyrosinase, a copper-containing enzyme that catalyses the oxidation of mono and diphenols (222, 223).

1.7.1.3 Tropolone Phytoalexins

While constitutive antifungal tropolones have been known for over 50 years, few tropolone phytoalexins have been reported. Sakai *et al.* (224) have reported the formation of β-thujaplicin in cell culture of *Cupressus lustanica* treated with fungal elicitors. Madar *et al.* (225) isolated two fungistatic tropolone glycosides, cupressotropolone A and B (Figure 1.34) from the bark of *Cupressus sempervirens* in response to infection by the staining fungus, *Sphaeropsis sapinea*.

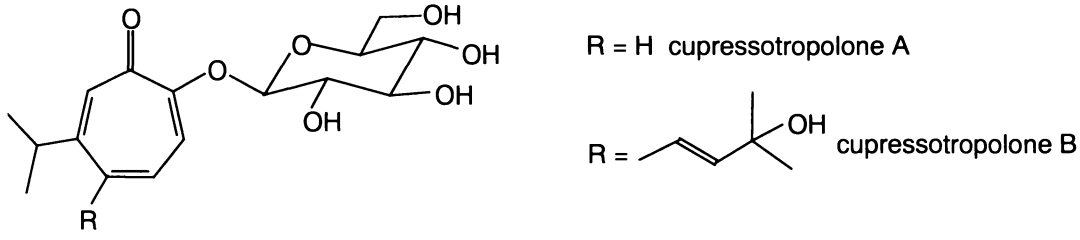


Figure 1.34: Tropolone phytoalexins from *Cupressus sempervirens* bark.

1.8 Research Objectives

The research undertaken and described in this thesis follows the approach of using antifungal wood extractives as lead compounds in the development of novel synthetic analogues with antifungal properties. The overall aim was to investigate this concept by synthesising novel analogues of natural terpenoids and tropolones that potentially could be useful as wood preservatives or antisapstain agents. By gaining insights into those terpenoid and tropolone structures that are important in toxicity to wood decay and staining fungi it was envisaged that the research would contribute to the body of knowledge in this field. This accumulated knowledge ultimately may lead to novel wood bioprotectants being developed from natural product origins.

Specifically, the objectives were three-fold. Firstly, the literature contains numerous examples of monoterpenoid (and some diterpenoid) compounds from heartwood and other plant tissues that possess good antifungal properties. Three of these natural products, namely thymol (**1**), β-terpineol (**2**) and totarol (**3**) were selected as suitable lead compounds to synthetic analogues with wood protection potential. The objective was to develop synthetic pathways to simple

analogues of these terpenoids compounds from *p*-nonylanisole and naphthol starting materials. The justification behind the selection of the lead compounds and the synthetic starting materials is discussed in Chapter 2. In additional to engineering antifungal activity, improving suitability for wood protection applications was a necessary consideration in the case of the monoterpenoid analogues. Assays against wood decay and staining fungi provided a suitable method of assessing wood protection potential.

Secondly, a number oxygenated sesquiterpenes from heartwood and other plant tissues have been observed to have activity against fungal deteriorators of wood. Few antifungal oxygenated sesquiterpenoids have been studied in detail and little is known about the relationships between their chemical structure and antifungal activity. The potential of oxygenated sesquiterpenes to provide leads to novel compounds with wood protection potential is an unexplored concept. The objective was to explore this concept through a bio-activity led investigation of antifungal oxygenated sesquiterpenes from the foliage essential oils of the some native New Zealand timber species. Assuming antifungal oxygenated sesquiterpenoids were discovered and identified the goal was then to synthesise simple synthetic compounds based on these sesquiterpenoids and test their activity against wood damaging fungi. Conservation of certain structure features, while sacrificing others, was an important consideration in achieving antifungal activity, while keeping the chemistry practicable.

The third objective was the synthesis and antifungal testing of tropolone analogues based on the natural thujaplicins. These natural tropolones have known potent anti-basidiomycetes properties, but are not ideally suited to wood preservation on account of their leachability from timber. The primary objective was to synthesise tropolone analogues with varying hydrophilic and hydrophobic character and evaluate differences in activity against decay and staining fungi. Maintaining the tropolone functionality was critical to antifungal activity and manipulations of structure were largely centred around secondary structural features. Gaining insights in structure/antifungal activity relationships was central to this objective. A secondary objective within this area of research was to explore the concept of combining tropolone and oxygenated stilbene structures within the single compound with the aim for improving hydrophobic character and enhancing antifungal activity through possible synergistic effects.

Give me a place to stand and I will move the Earth

Achimedes

Chapter 2: Synthetic Analogues of Antifungal Terpenoids

2.1 Introduction

In Section 1.6 antifungal terpenoids from higher plants were discussed from the point of view of identifying candidates which potentially could be used as structural leads to novel wood protection agents. Three synthetic compounds (**4**, **5**, **6**)[1], analogues of the two monoterpenoids thymol (**1**) and β-terpineol (**2**) and the diterpenoid, totarol (**3**) were selected for synthesis and activity testing against decay and staining fungi. The results of syntheses towards these analogues are discussed in this Chapter while the bioassay results are discussed in Chapter 5.

Thymol (**1**) is among the most potent of the antifungal monoterpenoids. The simplicity of its structure makes it suitable as a synthetic lead. The partial steric crowding of the phenolic group may be an important feature in antifungal activity. Alkylation was shown by Kurita *et al.* (156) to enhance the antifungal activity of phenols. Phenolic terpenoids, such as thymol, are believed to assert their antifungal activity through inhibition of oxidative phosphorylation, possibly as uncouplers of electron transport, preventing the synthesis of ATP (108). A number of monoterpenoid tertiary alcohols exhibit antifungal character. The terpineols have proved effective against sapstain fungi (164, 166). The tertiary alcohol structure of β-terpineol (**2**) is synthetically achievable from a phenolic starting material. The mode of antifungal action of monoterpenoid alcohols has not been studied. Having identified structural elements that will potentially result in antifungal activity other features of the prospective wood protection agents need to be considered.

[1] Only those compounds that were used as synthetic leads and those that were synthesised and/or tested for antifungal activity were assigned numbers and included in the chemical structure schematic provided at the end of this thesis.

Monoterpenoids, in general, are relatively volatile on account of their low molecular weight. The major factor inhibiting the commercial use of pine oil (40-60% α-terpineol) as a sapstain control agent is the loss of active compounds from the wood due to evaporation. The need for high treatment loadings to overcome this problem makes the process uneconomical (165). Using analogues with a larger carbon skeleton can alleviate problems of volatility. The additional hydrocarbon could at the same time provide enhanced lipophillic character, which is desirable from the point of view of resistance to leaching and persistence in timber. Lipophilic character is also desirable from the point of view of enhancing the water repellency characteristics of the treated timber. With these characteristics in mind, the synthesis of the proposed terpenoid analogues from a readily available inexpensive starting material is desirable. Nonylphenol provides a cheap source of a fifteen carbon skeleton which is amenable to synthetic conversion into the proposed monoterpenoid analogues. Thymol and β-terpineol analogues (**4**, **5**) (Figure 2.1), prepared from the methyl ether of nonylphenol, were selected for synthesis and assessment of wood protection potential.

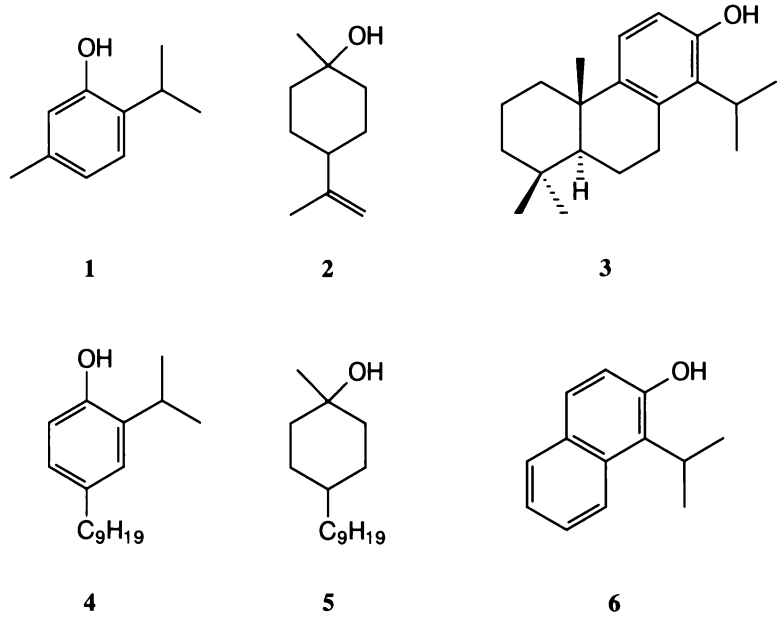


Figure 2.1: Synthetic analogues of thymol, β-terpineol and totarol; potential wood protection agents.

The diterpenoid phenol, totarol (**3**) is well-known as a durability principle in the heartwood of *Podocarpus totara*. Totarol (**3**) is structurally complex having three stereochemical centres (Figure 2.1). Such chemical complexity may not be necessary for antifungal activity. It is proposed that activity can be maintained or even enhanced using a simplified carbon skeleton. As with thymol the partial steric crowding of the phenol group was considered an important

functionality to maintain in the structure. The sesquiterpenoid-like phenol, 1-isopropyl-2-naphthol (**6**) (Figure 1.30), was selected as a simplified synthetic analogue of totarol (**3**) and assessed for activity against wood damaging fungi.

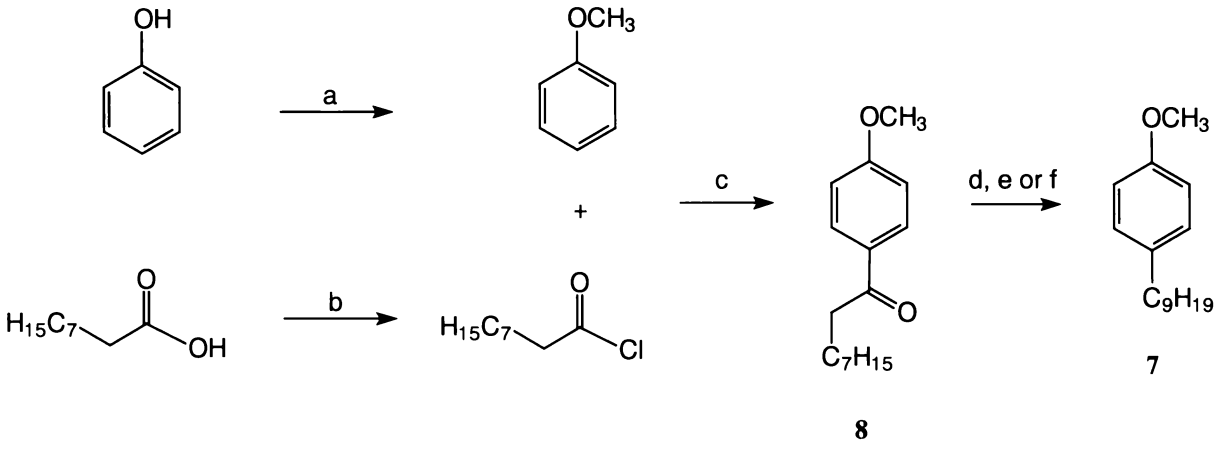
The mammalian toxicities for thymol (**1**), terpineol (mixture of α, β and γ-isomers) and 2-naphthol, in terms of oral LD_{50} (rat), are 980, 4300 and 1960 mg kg^{-1} respectively (226). In the case of phenols alkylation leads to a reduction in mammalian toxicity. As the length of the alkyl chain increases mammalian toxicity decreases. The oral LD_{50} (rat) values for phenol, isopropylphenol and nonylphenol are 370, 875 (mouse) and 1600 mg kg^{-1} respectively (226). Therefore the proposed synthetic terpenoid analogues would be expected to have lower mammalian toxicities than those mentioned above. The β-terpineol analogue (**5**) could be expected to be the least toxic to humans. By comparison the traditional wood preservatives PCP, TBTO and TBTN have oral LD_{50} (rat) of 50, 127 and 224 mg kg^{-1} respectively (19, 226). Components of CCA, copper sulphate, potassium dichromate and arsenic pentoxide have oral LD_{50} (rat) of 300, 190 and 8 mg kg^{-1} respectively (226). Borax, which is considered to have low mammalian toxicity, has an oral LD_{50} (rat) of 4500 mg kg^{-1} (226). Therefore the proposed terpenoid analogues compare favourable in their mammalian toxicities to current wood preservatives.

2.2 Nonylphenol Based Monoterpenoid Analogues

Analogues (**4**, **5**) of thymol (**1**) and β-terpineol (**2**) based on the nonylphenol carbon skeleton were selected as potential wood protection candidates. Commercial nonylphenol is an inexpensive source of a fifteen carbon skeleton, however, it contains approximately nine structural isomers. These isomers differ in the branching of the alkyl chain. A complex mixture of isomers is unsuitable for multi-step synthetic investigations. *p-n*-Nonylanisole (**7**) was used as a model compound for the development of synthetic pathways to the analogues (**4**) and (**5**). Consequently, the first step towards the proposed analogues was the synthesis of *p-n*-nonylanisole (**7**).

2.2.1 Synthesis of *p-n*-Nonylanisole (7)

The four-step synthesis of *p-n*-nonylanisole (**7**) is outlined in Scheme 2.1. In the first step, phenol was methylated with dimethyl sulphate in aqueous sodium hydroxide to give anisole in 83% yield. The base abstracts the phenolic proton to form sodium phenoxide, which in turn reacts with dimethyl sulphate to give anisole and sodium methyl sulphate. In the second step nonanoic acid was converted to nonanoyl chloride in quantitative yield with thionyl chloride. This halo-dehydroxylation involves nucleophilic substitution of the acid hydroxyl group by a chloride from $SOCl_2$, resulting in the formation of the acid chloride and the evolution of SO_2 and HCl.



Scheme 2.1: Synthetic procedure used for the preparation of *p-n*-nonylanisole (**7**) from phenol and nonanoic acid, (a = $(CH_3)_2SO_4$, NaOH; b = $SOCl_2$; c = $AlCl_3$, dry CS_2; d = H_2, Pd/C, AcOH; e = $N_2H_4.H_2O$, KOH, triethyl glycol; f = Zn-Hg amalgam, HCl/H_2O, toluene).

The third step involved a Friedel-Crafts acylation. An acylium ion formed by reaction of the nonanoyl chloride with $AlCl_3$ initiates an electrophilic attack on the aromatic ring of anisole. Steric hindrance from the methoxy group (which is both activating and *ortho-para* directing) results in exclusive formation of the *para* product. The Friedel-Crafts acylation proceeded smoothly giving *p*-nonanoylanisole (**8**) in 87% yield. Spectroscopic evidence and elemental analysis confirmed the purity and structural identity of *p*-nonanoylanisole (**8**) (Section 7.2.2). A single peak, observed in the GC/MS chromatogram of the product, displayed a molecular ion of *m/z* 248, a base peak of *m/z* 135 and a prominent fragment of *m/z* 150 (82%). The *m/z* 135 ion represents loss of a $CH_3(CH_2)_7$ radical due to benzylic cleavage; the *m/z* 150 ion is possibly generated by a McLafferty rearrangement involving β-γ cleavage of the alkyl chain

and abstraction of an α–H by the keto group. The ^{1}H and ^{13}C NMR spectra of the product were assignable to *p*-nonanoylanisole (**8**) (Section 7.2.2). The presence of two aromatic ^{1}H signals (2 × d) and four aromatic ^{13}C signals verified the *para* rather than *ortho* configuration. *p*-Nonanoylanisole (**8**) has been synthesised before by alternative methods including Pd-catalysed coupling of a nonanoylzirconocene chloride with a *p*-methoxyphenoliodonium salt (227) and Ni-catalysed electosynthesis using 1-methoxy-4-chlorophenol, 1-chlorooctane and iron pentacarbonyl (228).

Catalytic hydrogenation of *p*-nonanoylanisole (**8**) was initially attempted using Pd/C and PtO_2 catalysts in acetic acid and acetic acid/HCl (1:1) and 1 and 2 atmospheres of H_2. These hydrogenations were unsuccessful, resulting in the recovery of only starting material. According to Hudlicky (229), catalytic hydrogenation of carbonyl groups adjoining aromatic rings occurs readily and in high yield under these conditions. The failure of the reduction was possibly due to poisoning of the catalysts by residual sulphur compounds carried over from the CS_2 solvent used in the previous step. When *p*-nonanoylanisole (**8**) was recrystallised from aqueous ethanol and the hydrogenation repeated at 2 atmospheres of H_2, using a fresh batch of Pd/C, *p-n*-nonylanisole (**7**) was produced in 99% purity and 44% yield. The poor yield was possibly due to losses sustained on workup. Poor recoveries on recrystallisation meant that any large-scale recrystallisation would be tedious, consequently alternative methods of reduction were investigated.

A Huang-Minlon modification (230) of a Wolff-Kishner reduction using hydrazine hydrate and KOH in triethylene glycol was undertaken. The reaction gave a crude product in 68% yield that consisted of *p-n*-nonylanisole (**7**) (*ca.* 37%) and *p-n*-nonylphenol (**9**) (*ca.* 63%) as determined by GC/MS analysis. Demethylation to form *p-n*-nonylphenol (**9**) was unexpected, as aryl ethers are generally stable to nucleophilic substitution by hydroxyl ions (231). However, the presence of the electron withdrawing *p*-keto group markedly modifies this stability (231). The demethylation most likely proceeds *via* an S_NAr mechanism with the first step involving slow addition of OH^- to form a stabilised Meisenheimer-type complex (**A**), which on loss of a methoxyl ion and reduction forms *p-n*-nonylphenol (**9**) (Figure 2.2). *p-n*-Nonylanisole (**7**) was prepared from the crude Wolff-Kishner product by methylating the *p-n*-nonylphenol (**9**) by-product with $(CH_3)_2SO_4$ and aqueous NaOH. The overall yield was poor (37%). Therefore as an alternative, the Clemmensen reduction was investigated.

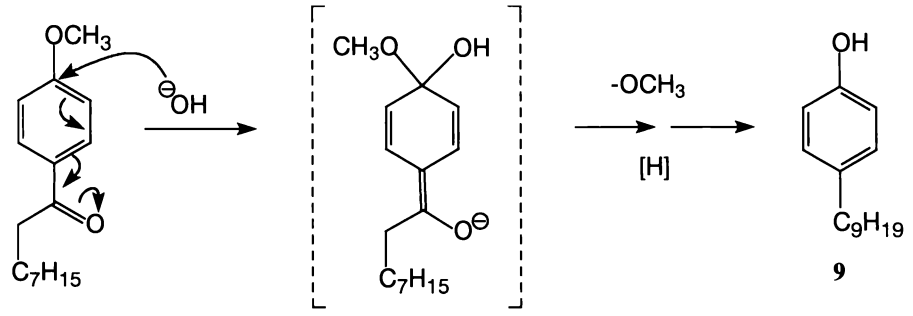


Figure 2.2: Nucleophilic substitution of the methoxy group of *p*-nonanoylanisole (**8**) by a hydroxyl ion in a Wolff-Kishner reduction.

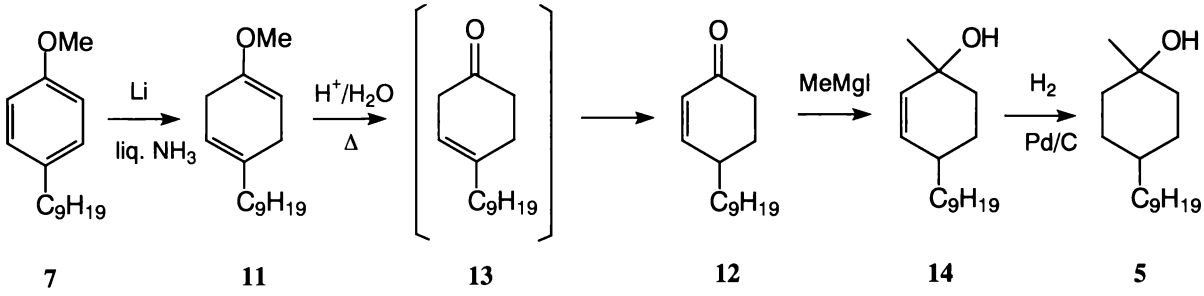
A Clemmensen reduction involves heating the ketone with zinc amalgam and aqueous HCl. Reaction for 20 hrs produced a crude product in 84% yield, which consisted of 95% *p-n*-nonylanisole (**7**) as determined by GC/MS and NMR spectroscopy. The minor by-product obtained in *ca.* 5% was believed to be *p*-nonenylanisole (**10**), based on its GC/MS spectrum. The mechanism of the Clemmensen reaction is not fully understood, although Horner and Schmidt (232) have proposed a complex mechanism. Alcohols fail to undergo reduction under Clemmensen reaction conditions, demonstrating that the reaction does not proceed through an alcohol intermediate (233). The mechanism is believed to involve protonation of the oxygen by the acid and ultimately its elimination as water after a transient unstable organozinc species is formed (232, 233). It is possible for a carbene-like intermediate to be formed (233) and this would give rise to the alkene (**10**) (234). Risinger *et al.* (234) found that for some ketones the proportion of olefin produced compared to saturated hydrocarbon increased with decreasing concentration of the acid. *p*-Methoxyacetophenone, however, gave 100% *p*-ethylanisole regardless of acid concentration (234).

Martin (235) introduced a general improvement to the Clemmensen reduction, which involved adding a layer of toluene. The benefits of the Martin improvement are thought to be two fold; undissolved reactant is kept out of contact with the metal and secondly, polymolecular reactions are inhibited as the two-phase system ensures that the reduction occurs at a high dilution (236). Considering the trend observed by Risinger *et al.* (234) (*i.e.* an increase in the ratio of acid to reactant improves the yield of saturated hydrocarbon compared to the olefin) it seemed logical that addition of toluene to the reaction mixture would reduce the formation of the alkene as the aqueous phase will contain a higher ratio of acid to reactant. This hypothesis proved correct. The Martin modification improved the crude yield to 91% and eliminated the

alkene by-product (**10**). The crude product consisted of *ca.* 96% *p-n*-nonylanisole (**7**) and *ca.* 4% unreacted *p*-nonanoylanisole (**8**) as indicated by GC/MS analysis. Purification by column chromatography on Al_2O_3 gave *p-n*-nonylanisole (**7**) in 85% yield. The mass spectrum of the product was dominated by an ion of *m/z* 121. This fragment is representative of a highly favoured benzylic cleavage with loss of a $CH_3(CH_2)_7$ radical. The disappearance of carbonyl signals in the IR and ^{13}C NMR spectra confirmed the success of the reduction. The ^{1}H and ^{13}C NMR spectra were assignable to the *p-n*-nonylanisole (**7**) structure (Section 7.2.2). Elemental analysis confirmed the purity and completed the structural characterisation of *p-n*-nonylanisole (**7**). Wightman and Malaiyandi (237) have reported the syntheses of *p-n*-nonylanisole (**7**) by the reaction of *p*-anisaldehyde with an *n*-octyl Grignard followed by dehydration and catalytic hydrogenation.

2.2.2 Synthesis of the β-Terpineol Analogue, 1-Methyl-4-nonylcyclohexan-1-ol (5)

The strategy initially devised for the synthesis of 1-methyl-4-nonylcyclohexan-1-ol (**5**) from *p-n*-nonylanisole (**7**) involved four steps and is outlined in Scheme 2.2. The first step involved a Birch reduction of the aromatic ring of *p-n*-nonylanisole (**7**) to give 1-methoxy-4-nonylcyclohexa-1,4-diene (**11**). Birch and Chamberlain (238) reduced anisole with Li in liquid NH_3/THF, with 2-methyl-2-propanol as a proton source, to give 1-methoxycyclohexa-1,4-diene in 75% yield. Hence the proposed reaction appeared feasible.



Scheme 2.2: Pathway to the β-terpineol analogue (**5**) from *p-n*-nonylanisole (**7**).

By treatment with acid, hydrolysis and rearrangement of 1-methoxy-4-nonylcyclohexa-1,4-diene (**11**) to 4-nonylcyclohex-2-enone (**12**), which is thermodynamically more stable than 4-nonylcyclohex-3-enone (**13**), was predicted (Scheme 2.2). Birch obtained 4-methylcyclo-

hex-2-enone from 4-methylanisole in 33% yield by Birch reduction and treatment with hot aqueous sulphuric acid (239). Reaction of the ketone group of 4-nonylcyclohex-2-enone (**12**) with a methyl Grignard reagent would yield the tertiary alcohol functionality, the product (**14**) being an analogue of menth-2-en-1-ol. Both the *cis* and *trans* diastereoisomers would be produced. Reduction of the double bond by catalytic hydrogenation would afford the β-terpineol analogue, 1-methyl-4-nonylcyclohexan-1-ol (**5**) (Scheme 2.2).

In the first synthetic step a Birch reduction of *p-n*-nonylanisole (**7**) is proposed. A Birch reduction of an aromatic ring employs Na or Li metal dissolved in liquid ammonia, which behaves as a solution of metal cations and solvated electrons in equilibrium with metal atoms (239). The reduction is usually carried out in the presence of an alcohol (hydrogen source) and hydrogen is added 1,4 to the aromatic ring to give nonconjugated cyclohexadienes (239, 240) (Figure 2.3). Electron donating groups, such as OMe and C_9H_{19} decrease the rate of reaction and are generally found in the non-reduced positions of the product (239, 240). The mechanism of the Birch reduction is well understood (240). The transfer of an electron to the aromatic ring directly from the Li metal oxidises it to Li^+ and creates a radical ion (**i,** Figure 2.3). This radical then abstracts a proton from the alcohol to give a radical (**ii**).

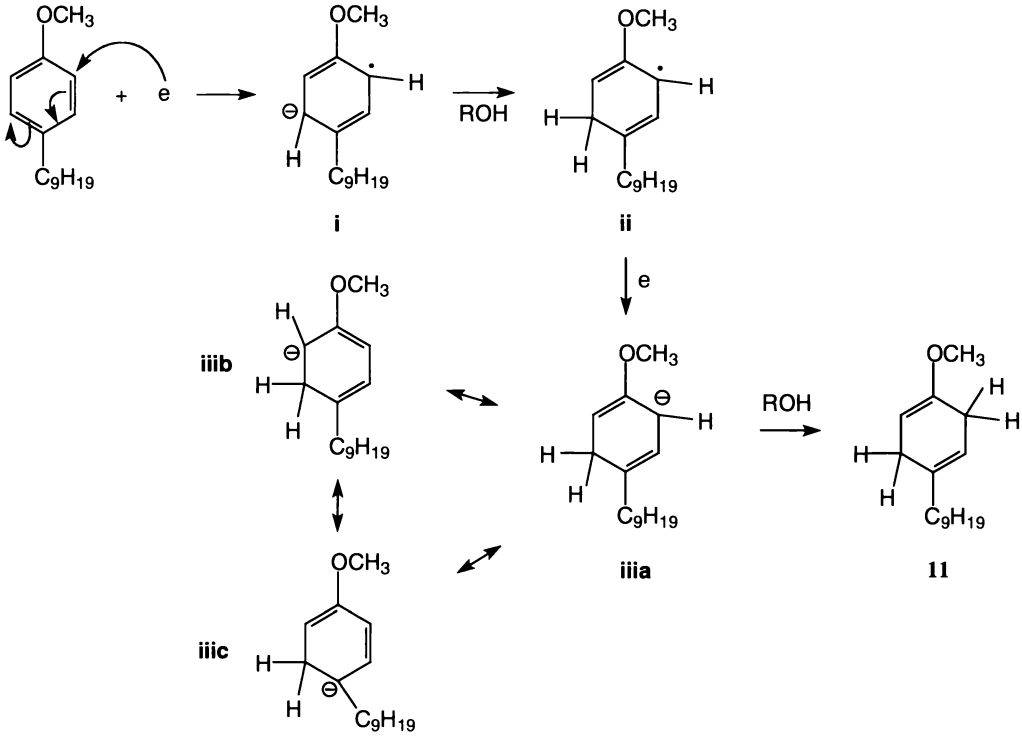


Figure 2.3: Mechanism for the Birch reduction of *p-n*-nonylanisole (**7**).

Transfer of a second electron from the metal generates a carbanion (**iii**), which accepts a proton to form the cyclohexadiene (**11**) (Figure 2.3). The carbanion exists as a resonance hybrid represented by the three canonical forms (**iii (a, b, c)**). Hine (241) has proposed an explanation as to why the carbanion accepts a proton at the 6 position (to form the 1,4-diene) and not the 2 position (to form the 1,3 diene). Based on the literature, the expected product of the Birch reduction of *p-n*-nonylanisole (**7**) is 1-methoxy-4-nonylcyclohexa-1,4-diene (**11**).

A Birch reduction of *p-n*-nonylanisole (**7**) was initially attempted using a 4 mol excess of Na in liquid NH_3 and *t*-BuOH (added as a proton source). The blue colour of the dissolved metal solution discharged during refluxing and the crude product obtained on workup was predominantly starting material (*ca.* 0.5% diene). Reduction with Li was initially reported in the literature to be superior to Na (242), however, it was later discovered that this was because of iron salt impurities in commercial NH_3. These salts reduced the yield of the reduction with Na to a greater extent than with Li (242). Dryden *et al.* (243) was able to reduce phenolic ethers in good yields with Na and *t*-BuOH in iron-free NH_3. As care was taken to distil the commercial NH_3 prior to use, the lack of reaction with Na metal could not be attributed to iron impurities in the NH_3.

Nevertheless, the reaction was repeated using an 8 mol excess of Li. After refluxing for 1.5 hours the reaction mixture no longer retained its blue colouration. The worked up product contained *ca.* 55% unreacted *p-n*-nonylanisole (**7**) as estimated by GC/MS. Incomplete reaction was thought to be due to poor solubility of the starting material in liquid NH_3. The use of 10% dry THF as a co-solvent improved the extent of the reduction although *ca.* 36% *p-n*-nonylanisole (**7**) still remained unreacted. The reaction was repeated using a 25 mol excess of Li in 10% THF/liquid NH_3. On this occasion the blue colour of the reaction mixture was retained throughout the reaction and was only discharged on workup. The crude product contained *ca.* 6% unreacted *p-n*-nonylanisole (**7**) and *ca.* 2% *p-n*-nonylphenol (**9**). Birch (239) reduced a number of methoxyalkylbenzenes with Na in liquid NH_3 in the presence of MeOH and found that the phenol was formed consistently in about 10% yield.

The GC/MS chromatogram of the crude product indicated the presence of four reduction products. The mass spectra of these products indicated they were two methoxynonylcyclohexadienes (*ca.* 47% and 29%, *m/z* 236 ($M^{\cdot+}$)), a nonylcyclohexene (possibly **15** or **16**, *ca.*

10%, *m/z* 208 ($M^{\bullet+}$)) and a methoxynonylcyclohexene (possibly **17**, *ca.* 4%, *m/z* 238 ($M^{\bullet+}$)). The two over-reduced products were a consequence of the large excess of Li used to carry the reduction to near completion (their formation is explained below). The GC/MS evidence suggested that the two products of molecular weight 236 Da were 1-methoxy-4-nonylcyclohexa-1,4-diene (**11**) (*ca.* 29%) and 1-methoxy-4-nonylcyclohexa-1,5-diene (**18**) (*ca.* 47%) (Figure 2.4). While the formation of the 1,4-diene (**11**) was consistent with the known mechanism (Figure 2.3) and major signals observed in the ^{1}H and ^{13}C NMR spectra of the crude product, the formation of the 1,5-diene (**18**) was not.

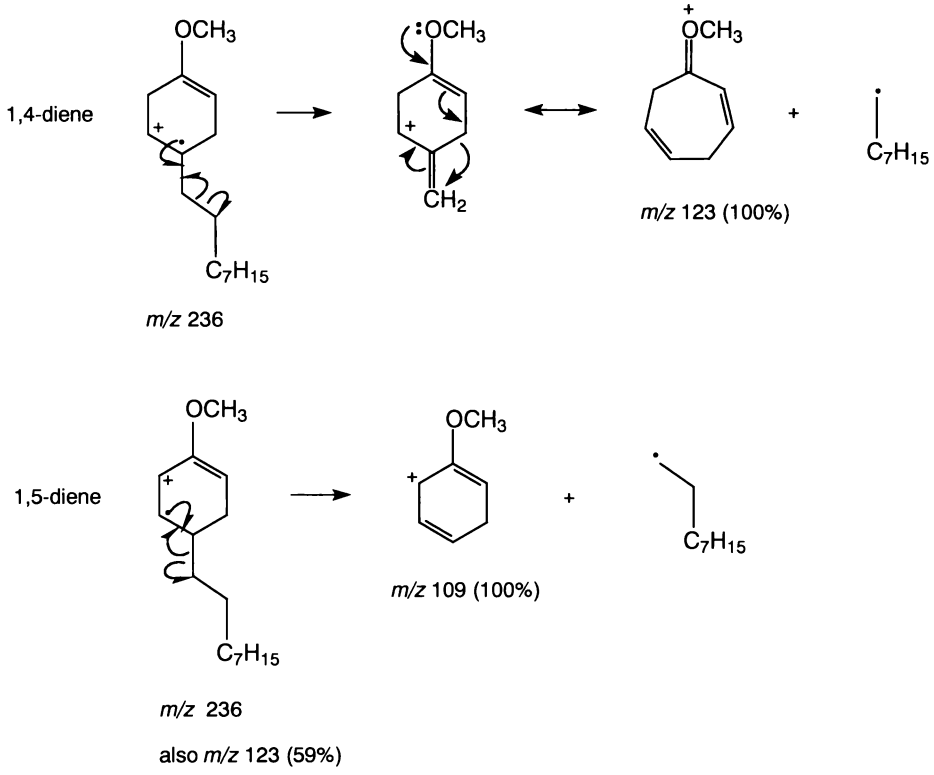


Figure 2.4: Major MS fragmentations of 1,4-diene (**11**) and 1,5-diene (**18**) isomers of 1-methoxy-4-nonylcyclohexadiene; highly favoured allylic cleavage.

The ^{1}H NMR spectrum of the crude product exhibited two olefinic proton signals at δ 4.63 and 5.36 ppm (unresolved triplets). These resonances are assignable to the olefinic protons of the unconjugated 1-methoxy-4-nonylcyclohexa-1,4-diene (**11**). No signals representative of protons in conjugated double bonds were observed. Additionally only four major olefinic carbon signals were observed in the ^{13}C NMR spectrum of the crude product. The chemical shifts for C4 and C5 of the 1,4-diene (**11**) would differ significantly from those of C5 and C6 of the 1,5-diene (**18**). Considering the relative proportions of the two isomers observed in the GC/MS chromatogram (29% (**11**) and 47% (**18**)) both sets of signals would be expected,

which was not the case. The most plausible explanation for this disparity is that the 1,4-diene (**11**) has thermally rearranged to the 1,5-diene (**18**) in the GC/MS injection port (set at 200°C). Rearrangement of the 1,5-diene back to the 1,4-diene may also have occurred after GC separation and ionisation as the spectrum of the 1,5-diene exhibits an intense *m/z* 123 ion (59%) (Figure 2.4). This fragmentation is not favoured in the case of the 1,5-diene (**18**), but is the base peak of the 1,4-diene (**11**). Thermal rearrangements of 1,4-dienes have been observed by other researchers (244). Varo and Heinz (244) have reported that 1,4-*p*-menthadien-7-al isomerises to 1,3-*p*-menthadien-7-al on GC injection.

Methoxycyclohexa-1,4-dienes can be isomerised to 1,3-dienes by basic reagents (*i.e.* potassamide) (245). In the reaction in question, *t*-butyloxy anions are produced by proton abstraction from *t*-BuOH. These alkoxide ions appear to be sufficiently basic to isomerise the nonconjugated 1-methoxy-4-nonylcyclohexa-1,4-diene (**11**) to the conjugated 1-methoxy-4-nonylcyclohexa-1,3-diene (**19**). The reason for this assertion is that isomerisation to the 1,3-diene (**19**) is required before further reduction can occur. Small peaks assignable to over-reduced products were observed in the GC/MS spectrum of the crude product. According to the literature these are most likely 1-nonylcyclohex-1-ene (**15**) or 3-nonylcyclohex-1-ene (**16**) and 1-methoxy-4-nonylcyclohex-1-ene (**17**) (245, 246). As these minor by-products were only analysed by GC/MS (low resolution) these identifications are tentative.

Methoxyalkylcyclohexadienes can be converted to α,β-unsaturated ketones by treatment with hot dilute mineral acid (239). Birch (239) produced 4-methylcyclohex-2-enone in 33% yield by Birch reduction and acid treatment of 4-methylanisole. The evidence suggests that hydrolysis of the enol ether by aqueous H^+ initially yields the nonconjugated cyclohex-3-enone which then isomerises to the more stable conjugated cyclohex-2-enone (239, 247). Treatment of the crude Birch reduction mixture in ethanol with 12% aqueous HCl at 65°C for 1 hour gave a 2:1 mixture of two 4-nonylcyclohexenones in 47% yield after chromatographic purification from the aforementioned Birch reduction by-products. The two peaks that were observed in the GC/MS chromatogram showed molecular ions of *m/z* 222. The IR spectrum of the mixture exhibited both conjugated and nonconjugated carbonyl stretching vibrations. The two GC/MS peaks were accordingly assigned to 4-nonylcyclohex-2-enone (**12**) and 4-nonylcyclohex-3-enone (**13**). Further validation for these assignments was found in the ^1H and ^{13}C NMR spectroscopic evidence.

The major olefinic signals in the ^{1}H and ^{13}C NMR spectra of the above product mixture were assigned to 4-nonylcyclohex-2-enone (**12**). The ^{1}H NMR spectrum showed two olefinic proton signals at δ 5.97 (dd) and 6.87 (ddd) ppm assignable to H2 and H3 in (**12**) respectively. The chemical shifts of the olefinic carbons in the ^{13}C NMR spectrum (δ 128.6 (C2) and 155.3 ppm (C3)) were consistent with an environment in which the double bond was conjugated to the carbonyl. Hence 4-nonylcyclohex-2-enone (**12**) was identified as the major product (*ca.* 64%) and 4-nonylcyclohex-3-enone (**13**) was assumed to be the minor product. When a subsequent acid hydrolysis of the Birch reduction mixture was undertaken at 20°C a 6:1 mixture of the two 4-nonylcyclohexenones was obtained. 4-Nonylcyclohex-3-enone (**13**) was identified as the major product of the mixture from the IR, GC/MS and ^{1}H and ^{13}C NMR spectroscopic evidence (Section 7.2.3). Indicative signals in the NMR spectra included a single olefinic proton at δ 5.45 ppm (m, H3) and the olefinic carbons signals at δ 117.3 ppm (C3) and 138.8 ppm (C4). While 4-nonylcyclohex-3-enone (**13**) is a new compound, 4-nonylcyclohex-2-enone (**12**) has been reported (248).

A variety of reaction conditions, catalysts and solvents were investigated in an attempt to form 4-nonylcyclohex-2-enone (**12**) exclusively. In all the experiments investigated mixtures of the 2-enone (**12**) and 3-enone (**13**) were obtained in varying proportions. 4-Nonylcyclohex-2-enone (**12**) and 4-nonylcyclohex-3-enone (**13**) were postulated to exist in equilibrium. Such an equilibrium has been described by other researchers with α,β- and β,γ-unsaturated ketones (249, 250, 251, 252) and is due to the small difference in stability between the conjugated and unconjugated isomers in cases where the double bond in the β,γ-position has more alkyl substituents that in the α,β-position (252). As a consequence, the preparation of 4-nonylcyclohex-2-enone (**12**) was less straight-forward than initially anticipated.

Due to the difficulties encountered in the attempted preparation of 4-nonylcyclohex-2-enone (**12**) the preparation of the menth-2-en-1-ol analogue (**14**) was abandoned in favour of concentrating on the preparation of the primary synthetic target, 1-methyl-4-nonylcyclohexan-1-ol (**5**) (the β-terpineol analogue). The mixture of 4-nonylcyclohex-2-enone (**12**) and 4-nonylcyclohex-3-enone (**13**) was reduced by catalytic hydrogenation using 10% Pd/C and 1 atmosphere of H_2 in ethanol at room temperature. Under these conditions the ketone was resistant to reduction. Column chromatography of the reduced product on neutral alumina gave 4-nonylcyclohexanone (**20**) in 67% yield. The structure was confirmed from the IR,

GC/MS and ^{1}H and ^{13}C NMR spectroscopic evidence. The IR spectrum displayed a carbonyl stretching band at 1719 cm^{-1}. The mass spectrum showed a molecular ion of *m/z* 224 ($C_{15}H_{28}O^{\bullet+}$), a base peak of *m/z* 97 ($M^{\bullet+}$-C_9H_{19} radical) and an ion of 59% abundance of *m/z* 55. The fragmentation pathway depicted in Figure 2.5 explains the formation of this ion. The absence of olefinic proton and carbon signals in the ^{1}H and ^{13}C NMR spectra confirmed the reduction was successful. The assignment of ^{1}H and ^{13}C NMR spectra (Section 7.2.3) was facilitated by interpretation of gCOSY, HMQC and HMBC spectra (Appendix D). 4-Nonylcyclohexanone (**20**) has been prepared before from *p*-nonylphenol by catalytic hydrogenation and CrO_3 oxidation (253). This is the first reported synthesis of 4-nonylcyclohexanone (**20**) from *p-n*-nonylanisole (**7**) by Birch reduction, enol ether hydrolysis and catalytic hydrogenation.

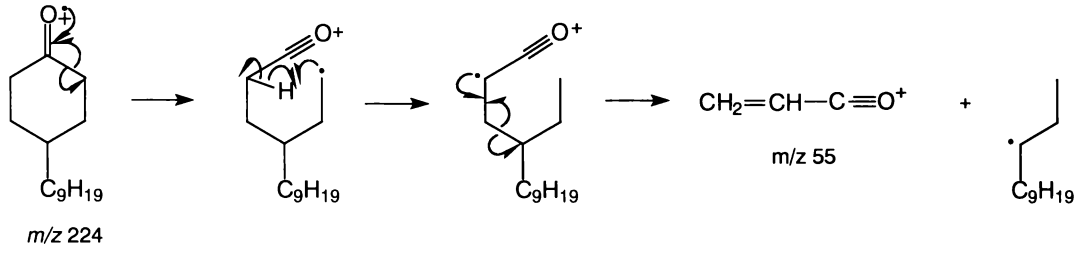


Figure 2.5: MS fragmentation of 4-nonylcyclohexanone (**20**).

The final step in the preparation of the β-terpineol analogue, 1-methyl-4-nonylcyclohexan-1-ol (**5**) was reaction of the ketone group of 4-nonylcyclohexanone (**20**) with a methyl Grignard reagent to give the tertiary alcohol. Reaction of 4-nonylcyclohexanone with MeMgI in ether gave the *cis* (**21**) and *trans* (**22**) isomers of 1-methyl-4-nonylcyclohexan-1-ol (**5**) in a crude yield of 93%. The isomers were separated from each other and from unreacted starting material by column chromatography on silica gel. The *cis* isomer (**21**) eluted first (where *cis* refers to the two highest order groups OH and C_9H_{19} (Cahn-Ingold-Prelog system (254)) giving white crystals in 28% yield. The *trans* isomer (**22**) gave a colourless liquid in 36% yield. The purity of the two isomers was confirmed by elemental analysis (Section 7.2.3).

The two products were confirmed as isomers of 1-methyl-4-nonylcyclohexanol (**5**) from their mass spectra, ^{1}H and ^{13}C NMR spectra and microanalytical data. The mass spectra of the two isomers were essentially identical, both dominated by a *m/z* 71 ion ($CH_3(CH_2)_3CH_2$+). Molecular ions (*m/z* 240), *m/z* 225 ions ($M^{\bullet+}$-CH_3 radical) and *m/z* 222 ions ($M^{\bullet+}$-H_2O) were

also evident. The two isomers gave similar ^{1}H and ^{13}C NMR spectra, the most significant difference was a δ 5.3 ppm variance between the chemical shifts of the methyl carbons in axial and equatorial environments. Due to a high degree of overlapping of proton signals and several similar carbon environments signals were assigned with assistance from the 2D ^{1}H-^{1}H and ^{13}C-^{1}H correlation spectra (Table 2.1).

Table 2.1: Selected gCOSY, HMQC and HMBC correlations observed for the *cis* (**21**) and *trans* (**22**) isomers of 1-methyl-4-nonylcyclohexan-1-ol.

***cis* isomer (21)**			***trans* isomer (22)**		
Signal	**δ (ppm)**	**Correlated ^{1}H signals, δ (ppm)**	**Signal**	**δ (ppm)**	**Correlated ^{1}H signals, δ (ppm)**
H2/H6	1.34	H3'/H5'	H3/H5	1.02	H2/H6, H2'/H6'
H2'/H6'	1.61	H2/H6, H3/H5, H7	H7	1.13	H2/H6
			H2/H6	1.41	H2'/H6', H3'/H5'
			H2'/ H6'	1.58	H3'/H5'
C3/C5	28.5	H3/H5, 1.16; H3'/H5' 1.54	C7	25.9	H7, 1.13
C7	31.2	H7, 1.18	C3/C5	30.3	H3/H5, 1.02; H3'/H5', 1.67
C4	36.9	H4, 1.15-1.25	C4	36.7	H4, 1.24
C2/C6	38.6	H2/H6, 1.34; H2'/H6', 1.61	C2/C6	39.8	H2/H6, 1.41; H2'/H6', 1.58
C3/C5	28.5	H2/H6, H2'/H6', H3/H5	C7	25.9	H2/H6, H2'/H6'
C7	31.2	H2/H6	C3/C5	30.3	H2/H6, H2'/H6'
C4	36.9	H2'/H6', H3'/H5'	C4	36.7	H2/H6, H3'/H5'
C2/C6	38.6	H3'/H5', H7	C2/C6	39.8	H6/H2, H7
C1	69.3	H2'/H6', H3'/H5', H7	C1	71.0	H2/H6, H2'/H6', H7

The relative stereochemistry of the two diastereomers was determined from their retention indices (RIs) by analogy with the reported retention indices of *cis*- and *trans*-β-terpineol and *cis*- and *trans*-dihydro-β-terpineol. The retention indices of *cis*- (**21**) and *trans*-1-methyl-4-nonylcyclohexan-1-ol (**22**) were 1800 and 1818 respectively. The order in which the two isomers eluted is consistent with that of *cis*- and *trans*-β-terpineol (RIs of 1144 and 1163 respectively (255)) and *cis*- and *trans*-dihydro-β-terpineol (RIs of 1136 and 1158 (255)). This synthesis is the first reported preparation of *cis*-1-methyl-4-nonylcyclohexan-1-ol (**21**) and *trans*-1-methyl-4-nonylcyclohexan-1-ol (**22**).

2.2.3 Side-reactions Encountered During the Synthesis of 1-Methyl-4-nonylcyclohexan-1-ol (5)

2.2.3.1 Autoxidation of 1-Methoxy-4-nonylcyclohexa-1,4-diene (11)

The crude 1-methoxy-4-nonylcyclohexa-1,4-diene (**11**) appeared to undergo a slow oxidation reaction upon standing in the presence of air at room temperature. The freshly worked up crude product of the Birch reaction of *p-n*-nonylanisole (**7**) contained *ca.* 77% 1-methoxy-4-nonylcyclohexa-1,4-diene (**11**) and *ca.* 5% unreacted *p-n*-nonylanisole (**7**) (*ca.* 18% over-reduced products) as determined by GC/MS analysis (Section 2.2.2). After 4 months standing in the presence of air *ca.* 17% 1-methoxy-4-nonylcyclohexa-1,4-diene (**11**) remained. The composition of the aged Birch reduction product is shown in Table 2.2.

Table 2.2: Composition of aged Birch reduction product.

Compound(s)	**Approximate % Composition (based on GC/MS peak areas)**
1-methoxy-4-nonylcyclohexa-1,4-diene (**11**)	17%
4-nonylcyclohex-2-enone (**12**) and 3-enone (**13**)	13%
p-n-nonylanisole (**7**)	26%
p-n-nonylphenol (**9**)	4%
unknown (MW = 268 Da)	14%
4-nonyl-4-hydroxycyclohex-2-enone (**23**)	5%
over-reduced products (possibly **15** or **16** and **17**)	*ca.* 20%

A possible explanation of these observed changes can be offered in terms of the autoxidation of the 1,4-diene (**11**) at the allylic carbon. In the absence of photosensitisers, an allylic C-H bond in the presence of oxygen can be autoxidised by a free-radical process (Figure 2.6) (256, 257). The incompletely characterised product, found in 14% of the mixture, is possibly the stable hydroperoxide intermediate (**24**). The mass spectrum of this component showed a molecular ion of *m/z* 268 and base peak of *m/z* 88. The postulated pathway to this ion is shown in Figure 2.7. Acid catalysed heterolysis of the hydroperoxide could lead to *p-n*-nonylanisole (**7**) (Figure 2.6). The overall outcome was a reversal of the Birch reduction! A pathway to the formation of 4-nonyl-4-hydroxycyclohex-2-enone (**23**) is postulated in Section 2.2.3.2. The chemistry proposed is purely speculative as a detailed examination of the autoxidation was deemed outside the scope of this thesis.

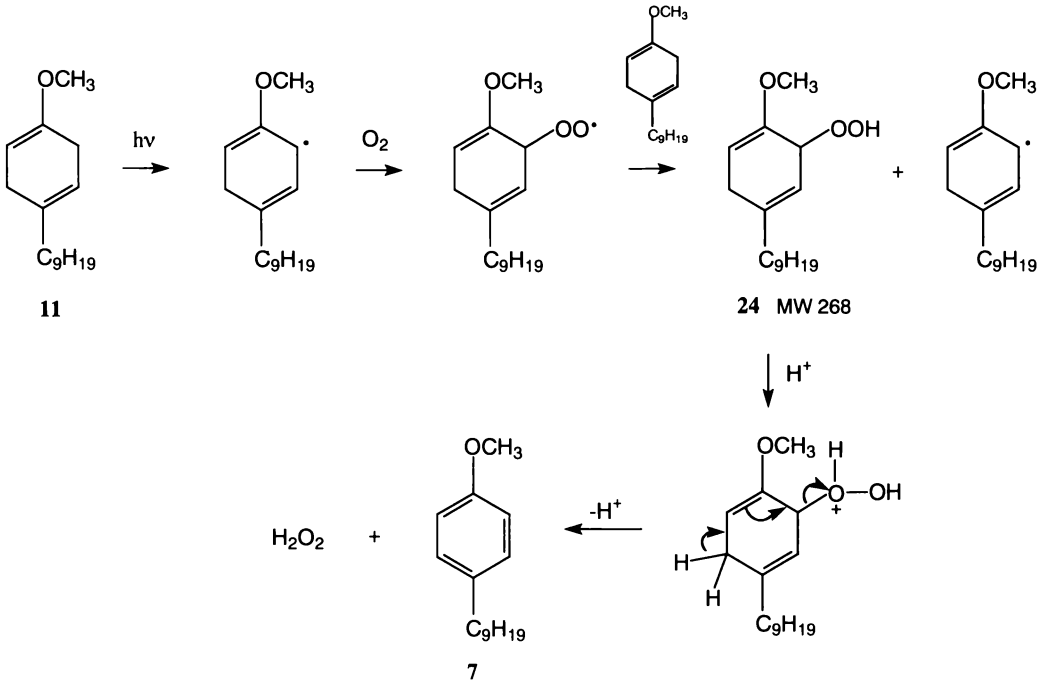


Figure 2.6: Proposed pathway to autoxidation of 1-methoxy-4-nonylcyclohexa-1,4-diene (**11**) leading to *p-n*-nonylanisole (**7**).

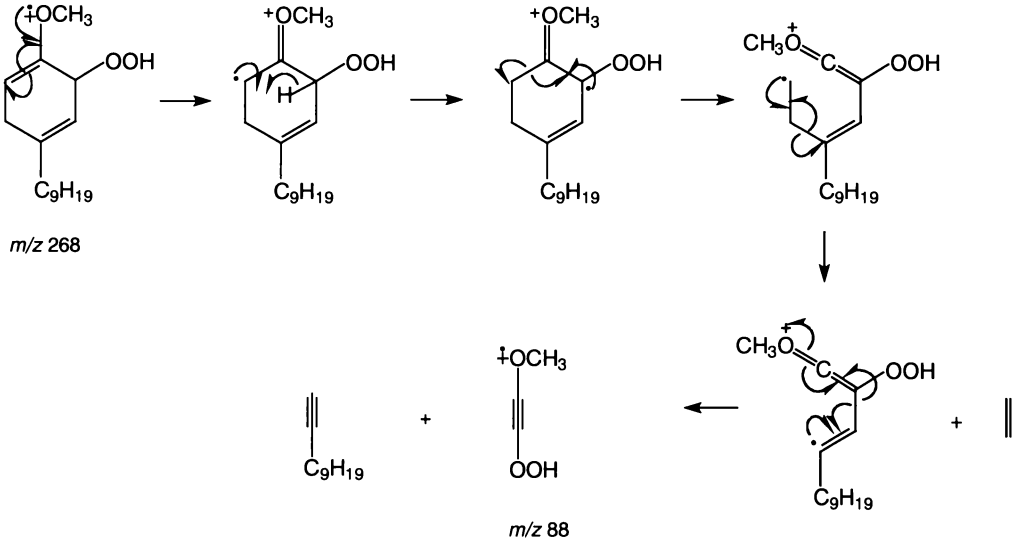
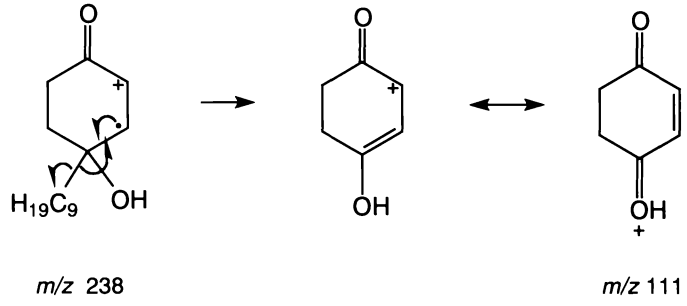


Figure 2.7: Proposed fragmentation pathway to major MS ion of proposed hydroperoxide intermediate (**24**) in autoxidation of 1-methoxy-4-nonylcyclohexa-1,4-diene (**11**).

2.2.3.2 Autoxidation of 4-Nonylcyclohexenones

A 6:1 mixture of 4-nonylcyclohex-3-enone (**13**) and 4-nonylcyclohex-2-enone (**12**) underwent autoxidation when left to stand in the presence of air at room temperature under laboratory lighting. After 3 days *ca.* 17% of the hexenones had become oxidised as indicated by the presence of an addition peak in the GC/MS spectrum. Separation of the 4-nonylcyclohexenones (**13**) and (**12**) from the autoxidation product by column chromatography on neutral alumina gave a 6:1 mixture of two compounds. The major autoxidation product (*ca.* 86%) was tentatively identified from the spectroscopic evidence as 4-nonyl-4-hydroxycyclohex-2-enone (**23**). The IR spectrum showed absorption maxima at 3308 cm^{-1} (broad, (ν O-H)) and 1668 cm^{-1} (ν C=O, conjugated enone) indicating the presence of hydroxyl and carbonyl groups. The mass spectrum exhibited a molecular ion of *m/z* 238 and a base peak of *m/z* 111. The base peak ion is likely to be formed by highly favoured allylic cleavage of a C_9H_{19} radical (Figure 2.8).



m/z 238 *m/z* 111

Figure 2.8: Proposed base peak MS fragmentation of the autoxidation product (**23**) of 4-nonylcyclohex-3-enone (**13**) and 4-nonylcyclohex-2-enone (**12**).

The ^1H NMR spectrum of the autoxidation product showed 2 olefinic proton signals at δ 6.05 (d, *J* = 10.4 Hz, H2) and 6.85 (dd, *J* = 10.4, 0.9 Hz, H3) ppm. The spectrum of 4-nonylcyclohex-2-enone (**12**) displayed 2 olefinic proton signals at 5.97 (dd, *J* = 10.2, 2.1 Hz, H2) and 6.87 (ddd, *J* = 10.2, 2.7, 1.3 Hz, H3). It is evident from this comparison that the oxidised product must contain the hydroxyl group at C4 as no coupling between H2 and H3 and a hypothetical H4 is observed. Further evidence for the 4-nonyl-4-hydroxycyclohex-2-enone (**23**) structure is evident in the ^{13}C NMR spectrum. A signal at δ 81.6 ppm is assignable to the tertiary alcohol carbon at C4. Signals at δ 130.5 and 151.5 ppm for C2 and C3 respectively are similar to the olefinic signals for the 2-enone (**12**) (δ 128.6 and 155.3 ppm) rather than the 3-enone (**13**) (δ 117.4 and 138.8 ppm).

A possible pathway to 4-nonyl-4-hydroxycyclohex-2-enone (**23**) from 4-nonylcyclohex-3-enone (**13**) and 4-nonylcyclohex-2-enone (**12**) is proposed in Figure 2.9. The pathway involves the isomerisation of the 3-enone (**13**) to the 2-enone (**12**) followed by its autoxidation at the tertiary allylic carbon to form a hydroperoxide intermediate (**25**) as described in Section 2.2.3.1. As mentioned above the major autoxidation product (possibly 4-nonyl-4-hydroxycyclohex-2-enone (**23**)) contained a minor impurity (*ca.* 14%). The mass spectrum of this minor compound displayed a molecular ion of *m/z* 254 (3%), a base peak of *m/z* 99 and an ion of *m/z* 127 in 80% abundance. The postulated hydroperoxide intermediate (**25**) has a molecular weight of 254 Da. Fragmentations pathways to observed MS ions are proposed in Figure 2.10.

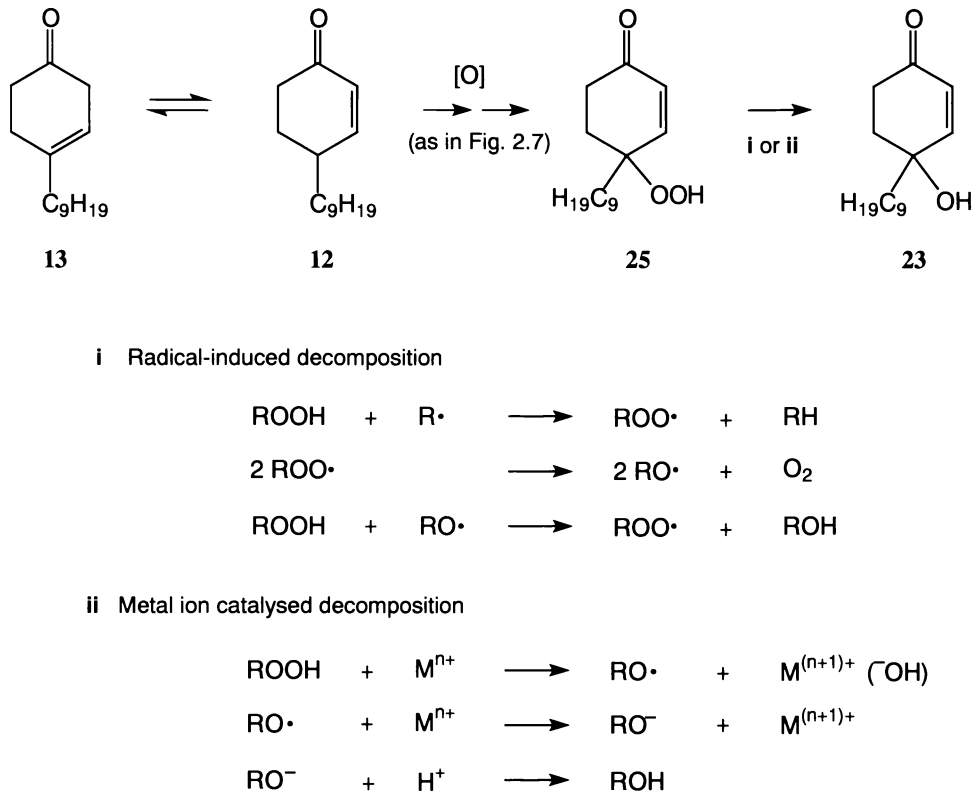


Figure 2.9: Possible pathways to 4-nonyl-4-hydroxycyclohex-2-enone (**23**) from 4-nonylcyclohex-3-enone (**13**) and 4-nonylcyclohex-2-enone (**12**) via autoxidation.

Two possible pathways for the decomposition of the hydroperoxide (**25**) to 4-nonyl-4-hydroxycyclohex-2-enone (**23**) are proposed based on the known mechanisms of homolytic cleavage of alkyl hydroperoxides (Figure 2.9) (256). Tertiary hydroperoxides can react with radicals to give alcohols as depicted (Figure 2.9). Alternatively decomposition of hydroperoxides at room temperature catalysed by minuscule quantities of transition metal ions

(ie. traces of Cr^{2+} on glassware for chromic acid washing) could result in the tertiary alcohol (**23**) by the reactions depicted (Figure 2.9). The pathways depicted in Figure 2.9 are largely speculative. An investigation of the actual pathway to the proposed oxidation product, 4-nonyl-4-hydroxycyclohex-2-enone (**23**) was deemed beyond the scope of this thesis as this autoxidation digresses from the core aims of the synthesis work.

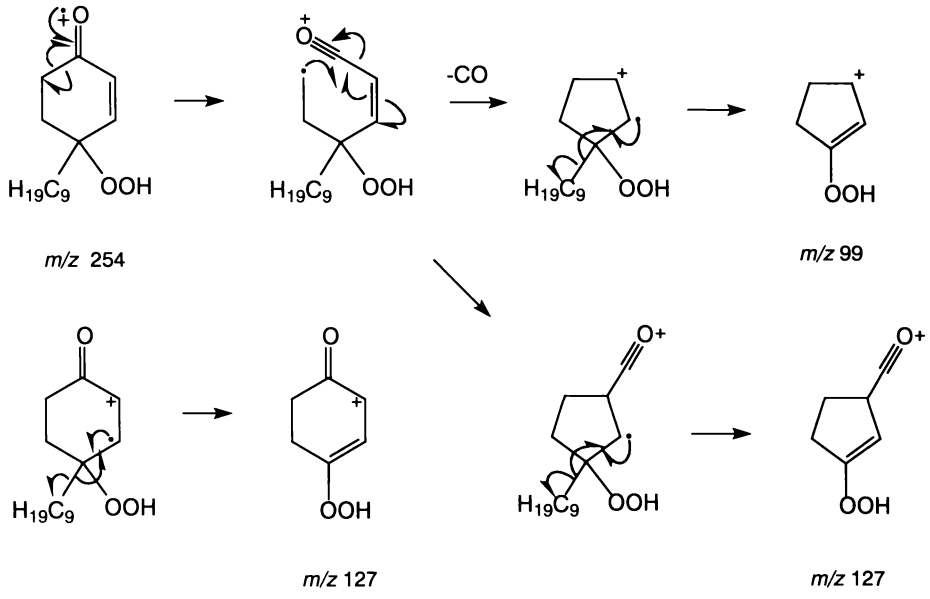


Figure 2.10: Major MS ions and proposed fragmentations pathways for the possible hydroperoxide intermediate (**25**) in autoxidation of 4-nonylcyclohex-3-enone (**13**) and 4-nonylcyclohex-2-enone (**12**).

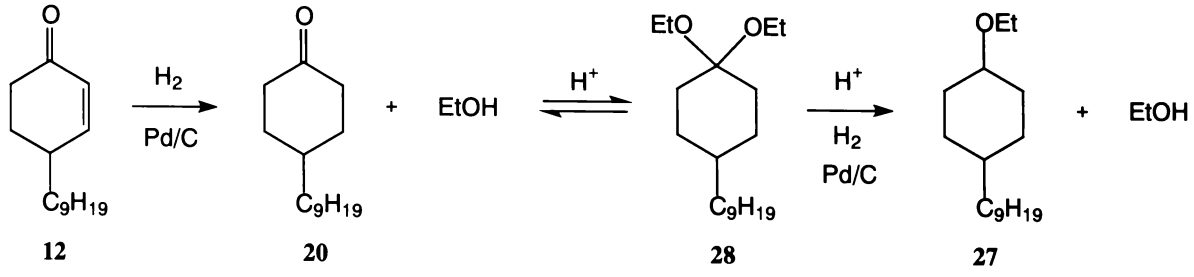
2.2.3.3 Preparation of 1-Ethoxy-4-nonylcyclohexane (27)

A mixture of 4-nonylcyclohex-2-enone (**12**) (*ca.* 36%) and 4-nonylcyclohex-3-enone (**13**) (*ca.* 51%) and a 4-nonylcyclohexene (possibly **15** or **16**) (*ca.* 14%) was obtained from a Birch reduction of *p-n*-nonylanisole (**7**) followed by acid rearrangement (Section 7.2.3). A decision was made to reduce the double bonds of the 4-nonylcyclohexenones (**12**, **13**) and the 4-nonylcyclohexene prior to column chromatography. An attempted catalytic hydrogenation of the mixture in ethanol with 10% Pd/C at 2 atmospheres of H_2 and 20°C gave a mixture of products, rather than the expected 4-nonylcyclohexanone (**20**) and 4-nonylcyclohexane (**26**). In addition to the expected products, 1-ethoxy-4-nonylcyclohexane (**27**) and possibly 1,1-diethoxy-4-nonylcyclohexane (**28**) were obtained. The diethyl ketal (**28**) was only characterised by GC/MS and hence was only tentatively identified. When the crude catalytic hydrogenation mixture was treated with aqueous acid additional 4-nonylcyclohexanone (**20**)

was recovered suggesting successful hydrolysis of the diethyl ketal (**28**). Column chromatography of the mixture gave a fraction containing pure 4-nonylcyclohexanone (**20**) and a fraction containing possibly 4-nonylcyclohexane (**26**) (*ca.* 12%) and 1-ethoxy-4-nonylcyclohexane (**27**) (*ca.* 88%) (*cis* and *trans* diastereomers). 4-Nonylcyclohexane (**26**) was only tentatively identified from the GC/MS evidence.

The two diastereomers of 1-ethoxy-4-nonylcyclohexane (**27**) were identified from their different GC retention times, identical mass spectra and the ^{1}H and ^{13}C NMR spectra of the *trans* isomer isolated by column chromatography (Section 7.2.3). Interpretation of the gCOSY, HMQC and HMBC NMR spectra enabled assignment of all the ^{1}H and ^{13}C signals and provided further evidence for the 1-ethoxy-4-nonylcyclohexane (**27**) structure. The isolated diastereomer of (**27**) was given the *trans* stereochemistry based on coupling constants observed for H1 to H2/H6 in the ^{1}H NMR spectrum. The H1 signal was observed as a triplet of a triplet (tt) with coupling constants of 11.0 and 4.2 Hz, which are attributable to couplings of axial H1 with axial H2/H6 and equatorial H2/H6 respectively. An equatorial H1 would not give coupling constants of this nature. It is reasonable to assume the nonyl group would occupy the equatorial position in the most stable conformation of both diastereomers hence the isolated diastereomer of (**27**) was assigned the *trans* configuration.

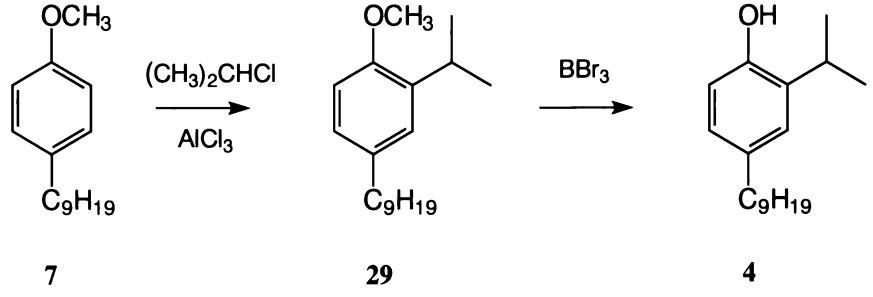
A pathway to 1-ethoxy-4-nonylcyclohexane (**27**) is proposed in Scheme 2.3. This pathway requires a catalytic amount of acid in order to generate the ketal (**28**) from the ketone (**20**). It is possible a catalytic amount of acid could have been carried over in the starting material from the acid treatment step used to generate the 4-nonylcyclohexenones (**12** and **13**). Hydro-de-alkoxylations of ketals of cyclohexanone by catalytic hydrogenation have been reported (258). The reduction is believed to proceed by acid-catalysed cleavage of the ketal to an alcohol and unsaturated ether, which are subsequently hydrogenated (258). In this way 1-ethoxy-4-nonylcyclohexane (**27**) could have been generated from 1,1-diethoxy-4-nonylcyclohexane (**28**).



Scheme 2.3: Preparation of 1-ethoxy-4-nonylcyclohexene (**27**) on catalytic hydrogenation of 4-nonylcyclohex-2-enone (**12**) and 4-nonylcyclohex-3-enone (**13**) in ethanol.

2.2.4 Synthesis of the Thymol Analogue, 2-Isopropyl-4-nonylphenol (4)

The strategy for the synthesis of the thymol analogue 2-isopropyl-4-nonylphenol (**4**) from *p-n*-nonylanisole (**7**) involved two steps, a Friedel-Crafts alkylation and a demethylation (Scheme 2.4). Friedel-Crafts alkylation was chosen as the method of introducing the *o*-isopropyl group as it provides the shortest and simplest pathway to the target compound. Multiple isopropyl substitution was anticipated, as alkylation will enhance the aromatic ring to further electrophilic substitution. Nevertheless, initial exploration of this option was deemed justified due to the simplicity of the Friedel-Crafts alkylation pathway. Demethylation of the proposed product, 2-isopropyl-4-nonylphenol (**29**) with BBr_3 should yield the target compound 2-isopropyl-4-nonylphenol (**4**) (Scheme 2.4).



Scheme 2.4: Pathway to the thymol analogue (**4**) from *p-n*-nonylanisole (**7**).

The synthesis of 2-isopropyl-4-nonylphenol (**4**) was initially attempted as outlined in Scheme 2.4 by firstly alkylating and then demethylating. In a second set of experiments the order of the two steps was reversed. As mentioned Friedel-Crafts alkylation provides the shortest pathway to the target compound. However, two important synthetic limitations need to be

considered. These are the tendency for di- and poly-alkylation to occur and a propensity for alkyl rearrangements. Both the methoxy and nonyl groups are *ortho* and *para* directing. The methoxy group is more strongly activating, hence isopropylation in the 2 or 6 positions is favoured. The new isopropyl substituent will also have an activating effect resulting in the alkylated product being more reactive than the starting material. Condon (259) reported that the $AlCl_3$ catalysed propylation of isopropyl benzene occurs 1.7 times faster than with benzene. Initially it was assumed that this activating effect was the reason for the observed accumulation of polyalkylated product in Friedel-Crafts alkylation reactions (260). Subsequently, however, it was found that the propensity for di- and poly-alkylation was due to preferential solubility of the alkylated products in a catalytic layer, which forms under heterogeneous reaction conditions. It is within this catalytic layer that the reaction takes place (260). According to Francis (260) this 'selective solvent effect' and hence multiple alkylation can be eliminated by the use of a mutual solvent for reactant and catalyst, high temperatures, high-speed stirring or vapour phase operation.

Rearrangements of alkylating agents are usually in the order of primary → secondary → tertiary (261). The proposed isopropylation proceeds through an isopropyl carbocation (or ion pair), which cannot rearrange to a tertiary carbocation. Hence rearrangement prior to attack on the aromatic ring is unlikely. The order of thermodynamic stability of alkyl benzenes is primary > secondary > tertiary (262). Friedel-Crafts alkylations show a degree of reversibility hence the rearrangement of the isopropylated product to a *n*-propylated product under the reaction conditions is possible from the point of view of thermodynamic control.

The Friedel-Crafts isopropylation of *p-n*-nonylanisole (**7**) was initially carried out using 2-chloropropane and $AlCl_3$ in a mole ratio of 1:1:1. The anisole and catalyst were both soluble in CH_3NO_2 giving a homogeneous reaction. The crude product consisted of a mixture of mono and multiple isopropylated products and starting material, of which 2-isopropyl-4-nonylanisole (**29**) (MW = 276 Da) constituted *ca.* 43%. Other products with molecular weights of 276, 318, 360 and 402 constituted approximately 5%, 11%, 9% and 8% of the crude product respectively. Starting material constituted *ca.* 20%. A minor amount of di-isopropylanisoles (*ca.* 3%) formed by denonylation was detected. The percentage compositions were approximations based on GC/MS peak areas. 2-Isopropyl-4-nonylanisole

(**29**) was confirmed as the major product after demethylation and purification by column chromatography (see below).

On first inspection, the other alkylated products with molecular weights of 276, 318, 360 and 402 could be assumed to be mono, di, tri and tetra-isopropylated nonylanisoles. However, considering the 1:1 anisole:alkyl halide starting stoichiometry and the fact that only *ca.* 20% of the *p-n*-nonylanisole (**7**) remained unreacted such levels of isopropylation are not possible (Table 2.3).

Table 2.3: Theoretical proportions of starting materials in observed products if only isopropylated products were formed in the Friedel-Crafts reaction.

	Percentage of original starting material in possible product					
No of possible Pr^i groups	0	1	2	3	4	Total
***p-n*-nonylanisole**	20	48	14	9	8	99
2-chloropropane	0	48	28	27	32	135

The presence of di-isopropylanisoles (*ca.* 3%) in the crude product when considered in conjugation with the above disparity indicates that a certain degree of nonyl group cleavage has occurred. The mass spectra of the two di-isopropylanisoles (MW = 192 Da) are characterised by a base peak of *m/z* 177 ($M^{\bullet+}$-CH_3). The mass spectra of the nonylated products, on the other hand, are characterised by $M^{\bullet+}$-113 base peaks (Section 7.2.4). The mass spectra of products of molecular weight 234, 276 and 318 Da indicate these species are nonylanisoles (non, mono- and di-isopropylated) rather than isopropylanisoles (tri, tetra and penta respectively) with the exception of one minor product of molecular weight 276 Da. The mass spectra for the products of molecular weight 360 and 402 Da contain fragments ions suggesting these compounds contained two nonyl groups (plus one isopropyl group in the case of product of MW = 402 Da) rather than a single nonyl group and 3 and 4 isopropyl groups respectively. Direct evidence of the di-nonylanisole products was obtained when a di-nonylphenol was isolated in 95% purity after demethylation and chromatographic purification (see below).

Dialkylation was a less significant complicating factor than alkyl rearrangement. The multiplicity of the mono and di-propylated products is possibly due to isomerisation of *o*-isopropyl products to *meta* (260) and/or the rearrangement of isopropyl groups to *n*-propyl groups. The formation of di-nonyl products *via* rearrangement occurred to a significant level

(*ca.* 17%) possibly due to the vigorous nature of the $AlCl_3$ catalyst. The use of less reactive $FeCl_3$ in place of $AlCl_3$, however, gave a similar level of rearranged products and approximately double the degree of dialkylation.

Lowering the temperature of the reaction (to 60°C) did not improve the yield of the target compound, neither did reducing the reaction time or amount of catalyst. Alcohols show less tendency to undergo rearrangements that alkyl halides (262). Isopropanol was used in place of 2-chloropropane in a series of reactions in CH_3NO_2 with varying stoichiometry and reaction temperatures. However, no conditions were found that gave a greater yield of 2-isopropyl-4-nonylanisole (**29**) than the reaction described above. In general, reaction with isopropanol gave a larger proportion of unreacted *p-n*-nonylanisole (**7**). An isopropanol:$AlCl_3$ stoichiometry of 1:2 and temperature of at least 60°C were required for adequate reaction.

The mixture of alkylated products was inseparable and therefore the ethers were converted to phenols prior to column chromatography. BBr_3 is an effective demethylating agent. McOmie and Watts (263) demethylated phenolic methyl ethers in high yield using a 1/3 mole equivalent of BBr_3 in CH_2Cl_2 at -80°C. Using a modification of this method, the crude Friedel-Crafts product mixture was demethylated in 99% crude yield. Column chromatography on neutral Al_2O_3 gave the target compound, 2-isopropyl-4-nonylphenol (**4**) in 12% yield and *ca.* 91% purity as estimated from GC/MS analysis. High resolution MS analysis confirmed the identity of 2-isopropyl-4-nonylphenol (**4**) although microanalysis gave unsatisfactory results due to the presence of the minor di-isopropylanisoles by-products (inseparable from (**4**) by column chromatography). The mass spectrum showed a molecular ion of *m/z* 262 ($C_{18}H_{30}O^{\bullet+}$) and base peak of *m/z* 149 which was formed by the benzylic cleavage of a C_8H_{17} radical. Interpretation of the 1D ^1H and ^{13}C and 2D gCOSY, HMQC and HMBC NMR spectra verified the product as 2-isopropyl-4-nonylphenol (**4**). Structurally informative ^1H-^1H and ^{13}C-^1H correlations are shown in Table 2.4. This is the first reported synthesis of 2-isopropyl-4-nonylphenol (**4**).

Other chromatographic fractions of the crude product consisted of mixtures of the mono- and di-isopropylated nonylphenol with di-nonylphenol and isopropyl-di-nonylphenol with one exception. A di-nonylphenol was isolated in *ca.* 95% purity in one of the column fractions.

The GC/MS and ^{1}H and ^{13}C NMR spectra indicated this product was 2,4-di-nonylphenol (**30**) (Section 7.2.4), although microanalytical data was not obtained to confirm this identification.

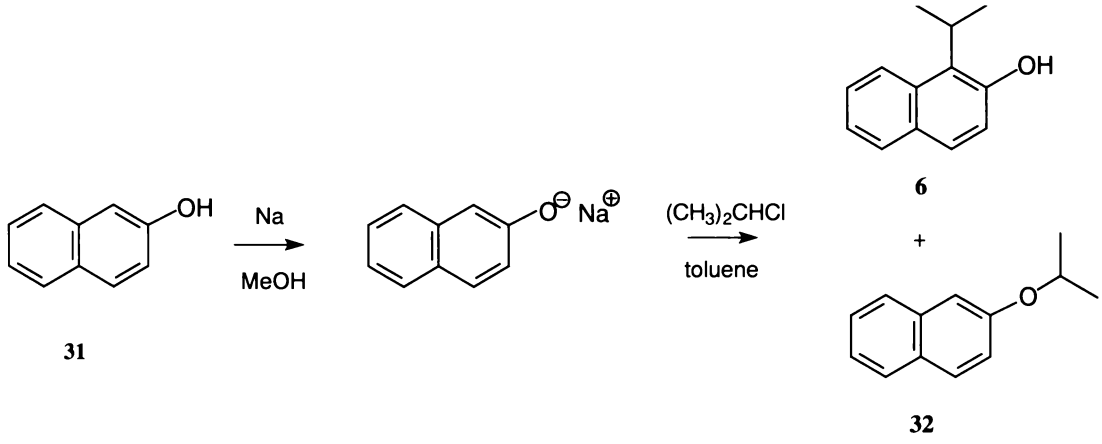
Table 2.4: Selected gCOSY, HMQC and HMBC correlations observed for 2-isopropyl-4-nonylphenol (**4**).

Signal	δ (ppm)	Correlated ^{1}H signals, δ (ppm)	^{13}C	δ (ppm)	Correlated ^{1}H signals
H8 & H9	1.16-1.36	H7	C8 & C9	22.7	H7
H5	6.85	H3, H6	C7	27.0	H3, H8, H9
			C10	35.4	H3, H5
C8 & C9	22.7	H8 & H9, 1.16-1.36	C6	115.1	H5
C7	27.0	H7, 3.16	C5	126.3	H3, H6, H10
C10	35.4	H10, 2.51	C3	126.3	H5, H7, H10
C6	115.1	H6, 6.64	C2	134.1	H6, H7, H8, H9
C5	126.3	H5, 6.85	C4	135.3	H6, H10
C3	126.3	H3, 6.97	C1	150.6	H3, H5, H6, H7

Demethylation followed by Friedel-Crafts alkylation was investigated to assess whether the reverse strategy would improve the yield of the target compound 2-isopropyl-4-nonylphenol (**4**). Phenolic groups are more strongly activating toward electrophilic aromatic substitution than methoxy groups. However, due to their basic nature they form coordination complexes with the catalyst which impede the reaction (261) (methoxy groups also form coordination complexes although they are less basic (261)). Demethylation of *p-n*-nonylanisole (**7**) with BBr_3 gave *p-n*-nonylphenol (**9**) in 92% yield. The demethylation product was confirmed as *p-n*-nonylphenol (**9**) from the melting point, IR, GC/MS and ^{1}H and ^{13}C NMR spectroscopic evidence (Section 7.2.4). Reaction in CH_3NO_2 with a 1:1:1 stoichiometry of *p-n*-nonylphenol:2-chloropropane:$AlCl_3$ at reflux gave *ca.* 20% of 2-isopropyl-4-nonylphenol (**4**), *ca.* 40% unreacted *p-n*-nonylphenol (**9**) and *ca.* 28% diisopropylphenols. The remainder consisted of a second isopropylnonylphenol (*ca.* 4%) and possibly a isopropyldi-nonylphenol (*ca.* 3%). Product identifications were based on GC/MS results and comparison of retention times and mass spectra with previously characterised products (*i.e.* **4** and **9**). The addition of a second mole equivalent of 2-chloropropane failed to improve the yield of 2-isopropyl-4-nonylphenol (**4**).

2.3 Synthesis of 1-Isopropyl-2-naphthol (**6**)

The strategy chosen for the synthesis of 1-isopropyl-2-naphthol (**6**) involved direct isopropylation of 2-naphthol (**31**) at the 1-position *via* the reaction of sodium-2-naphthoxide with 2-chloropropane under heterogeneous conditions (Scheme 2.5). Carnduff and Leppard (264) have previously been prepared 1-isopropyl-2-naphthol (**6**) by this method with the exception that 2-bromopropane was used instead of 2-chloropropane. It was appreciated that the target compound, 1-isopropyl-2-naphthol (**6**) would be separable from its by-product, 2-(1-methylethoxy)naphthalene (**32**) by column chromatography.



31 **6** **32**

Scheme 2.5: Pathway to 1-isopropyl-2-naphthol (**6**) from 2-naphthol (**31**).

The 2-napthoxide ion is an ambident nucleophile, capable of forming covalent bonds at the oxygen or at the α-carbon. By choosing the right conditions it is possible to alkylate 2-napthoxide in a regioselective manner. The work of Kornblum and colleagues (265, 266, 267, 268) highlighted a number of factors that influence which atom of a phenoxide or naphthoxide ambident nucleophile attacks the alkyl halide. It is possible to carry out C-alkylation of 2-naphthoxide with allyl and benzyl halides under homogeneous conditions in high yield (*ca.* 85%) using protic solvents, such as water or 2,2,2-trifluoroethanol (268). However the same reaction with *n*-propylbromide under identical conditions gave mainly O-alkylated product (80-90%) (268). Kornblum and Lurie discovered that under heterogeneous reaction conditions (*i.e.* using a solvent in which the phenoxide is insoluble, *e.g.* toluene) C-alkylation of phenoxide ions is favoured (266). The reasons for this have been discussed by Kornblum and Lurie (266).

Using a modification of the method of Carnduff and Leppard (264) the isopropylation of 2-naphthoxide at C1 with 2-chloropropane was attempted. The reaction gave largely unreacted 2-naphthol (80%) after refluxing for 11 days. The starting material was separated from the two products of the reaction by partitioning with aqueous NaOH. 1-Isopropyl-2-naphthol (**6**) is a cryptophenol and is insoluble in aqueous NaOH. 2-(1-Methylethoxy)naphthalene (**32**) (*ca.* 3% yield) and 1-isopropyl-2-naphthol (**6**) (*ca.* 9% yield) were recovered from the ether partition and separated by column chromatography. Spectroscopic analysis confirmed the structure of the major product as 1-isopropyl-2-naphthol (**6**) (Section 7.2.5). The mass spectrum exhibits a strong molecular ion of *m/z* 186 ($C_{13}H_{14}O^{\cdot+}$, 48%) and base peak of *m/z* 171, formed by benzylic cleavage of CH_3. Structurally significant ^1H-^1H and ^{13}C-^1H NMR correlations observed in the 2D gCOSY, HMQC and HMBC spectra are tabulated in Table 2.5.

Table 2.5: Selected gCOSY, HMQC and HMBC correlations observed for 1-isopropyl-2-naphthol (**6**).

Signal	**δ (ppm)**	**Correlated ^1H signals, δ (ppm)**	**^{13}C**	**δ (ppm)**	**Correlated ^1H signals**
H10 & H11	1.51	H9	C10 & C11	21.1	H9, H11 & H10
H3	6.97	H4	C9	26.3	H10, H11
			C1	125.5	H3, H4, H8, H9, H10,
C10 & C11	21.1	H10 & H11, 1.51			H11
C9	26.3	H9, 3.90	C4	127.7	H5
C3	118.8	H3, 6.97	C5	128.8	H4
C8	123.2	H8, 8.11	C4a	129.7	H3, H6, H8
C4	127.7	H4, 7.58	C8a	132.9	H4, H5, H7, H9
C5	128.8	H5, 7.75	C2	151.0	H3, H4, H9

As mentioned above heterogeneous reaction conditions favour C-alkylation of 2-naphthoxide. 2-(1-Methylethoxy)naphthalene (**32**) is formed as a by-product as truly heterogeneous reaction conditions can not be maintained (266). As the reaction proceeds and 1-isopropyl-2-naphthol (**6**) is formed it is able to protonate 2-naphthoxide ions thereby solubilising them. The resulting homogeneous reaction yields the O-alkylated 2-(1-methylethoxy)naphthalene (**32**). Although the yield of 1-isopropyl-2-naphthol (**6**) was poor (9%), the reaction had only proceeded to 20% competition. Forcing the reaction further would undoubtedly result in the formation of more 2-(1-methylethoxy)naphthalene (**32**) as the reaction became more homogeneous. It is noteworthy that the competition between O-alkylation and C-alkylation is more evenly balanced for 2-naphthoxide ions compared to phenoxide ions for reasons

expressed by Kornblum and Lurie (266). Hence, this type of alkylation reaction was not a viable option for the isopropylation of *p-n*-nonylanisole (**7**).

Solid 1-isopropyl-2-naphthol (**6**) underwent a slow autoxidation when left to stand in the presence of air at room temperature. A pure sample after exposure to air for 18 days was found to consist of *ca.* 63% 1-isopropyl-2-naphthol (**6**) and *ca.* 27% oxidised product (based on GC/MS analysis). Carnduff and Leppard (264, 269) have reported that 1-isopropyl-2-naphthol (**6**) as a solid or in solution in benzene is autoxidised to 1-hydroperoxy-1-isopropyl-2-(1*H*)-naphthalenone (**33**). The oxidation product was separated the 1-isopropyl-2-naphthol (**6**) by column chromatography on alumina and further purified by recrystallisation. The IR, GC/MS and ^{1}H and ^{13}C NMR spectroscopic evidence was consistent with 1-hydroperoxy-1-isopropyl-2-(1*H*)-naphthalenone (**33**) as the product. ^{1}H-^{1}H and ^{13}C-^{1}H NMR correlations from the gCOSY, HMQC and HMBC spectra, which assisted in structural characterisation, are shown in Table 2.6.

Table 2.6: Selected gCOSY, HMQC and HMBC correlations observed for 1-hydroperoxy-1-isopropyl-2-(1*H*)-naphthalenone (**33**).

Signal	**δ (ppm)**	**Correlated ^{1}H signal, δ (ppm)**	**^{13}C**	**δ (ppm)**	**Correlated ^{1}H signals**
H10 & H11	0.80, 0.85	H9	C10 & C11	16.0, 16.6	H9, H11 & H10
H3	6.12	H4	C9	39.6	H10, H11
			C1	91.3	H3, H8, H9, H10, H11
C10 & C11	16.0, 16.6	H10, H11; 0.85, 0.80	C5	129.4	H4, H7
C9	39.6	H9, 2.10	C4a	132.7	H3, H6, H8
C3	126.6	H3, 6.12	C8a	142.5	H4, H5, H7, H9
C8	127.8	H8, 7.68	C4	145.1	H5
C5	129.4	H5, 7.46	C2	199.6	H4, H9
C4	145.1	H4, 7.55			

The MS spectrum did not display a molecular ion but showed an ion of *m/z* 202 ion ($[M-16]^{\bullet+}$) in low intensity and a base peak of *m/z* 160. A characteristic feature of the mass spectra of organic hydroperoxides are ions of $[M-16]^{\bullet+}$ formed by unimolecular deoxygenation (270, 271). This process maybe thermally induced (270). The base peak is possibly formed by McLafferty rearrangement with the loss of a propene radical (MW = 42 Da) from the *m/z* 202

species to give a $C_{10}H_8O_2^+$ ion (*m/z* 160). The presence of the hydroperoxy group was confirmed by the rapid reduction of (**33**) by iodide ion or permanganate ion.

1-Isopropyl-2-naphthol (**6**) can exist in a dienone form (**34**). According to March (272) keto tautomers of phenols become important in systems of fused aromatic rings. By adopting this form C1 becomes tetrahedral and the compound can relieve strain caused by *peri*-interactions (273). In this form the 1-position is benzylic and hence susceptible to autoxidation (256). Brady and Carnduff (273) have reported that the autoxidation of 1-isopropyl-2-naphthol (**6**) shows characteristics of radical chain processes. 1-Hydroperoxy-1-isopropyl-2-(1*H*)-naphthalenone (**33**) is possibly formed by a pathway analogous to that depicted in Figure 2.6.

A man gazing at the stars is proverbially at the mercy of the puddles in the road

Alexander Smith

Chapter 3: Antifungal Sesquiterpenoids as Synthetic Leads

3.1 Introduction

Natural sesquiterpenoids with antifungal properties can potentially provide structural leads to novel antifungal agents. Antifungal oxygenated sesquiterpenoids from wood and other plant tissues were reviewed in Section 1.6. The isolation and identification of sesquiterpenoids from higher plants has received considerable research interest (274), while comparably few studies have investigated the antifungal activities of oxygenated sesquiterpenes and their role in plant defense mechanisms. The potential of oxygenated sesquiterpenes to provide leads to new compounds for wood protection remains largely unexplored. With the objective of exploring this concept from natural product to potential wood protection agent, within a manageable framework, a bioactivity-led investigation of oxygenated sesquiterpenes in the foliage oils of three softwood species native to New Zealand was embarked upon. The three species investigated were totara (*Podocarpus totara*), kahikatea (*Dacrycarpus dacrydioides*) and rimu (*Dacrydium cupressinum*). The foliage essential oil from *Podocarpus totara* has recently been studied (177) and although the oil was found to be a rich source of a complex array of oxygenated sesquiterpenoids these compounds were not identified or tested for antifungal activity. Berry *et al.* (275) studied the foliage essential oil of *Dacrydium cupressinum* and found caryophyllene oxide (**35**) and longibornyl acetate to be the two major oxygenated sesquiterpenoids. To the author's knowledge no studies on sesquiterpenoids from *Dacrycarpus dacrydioides* foliage have been published.

3.2 Natural Sesquiterpenoids

3.2.1 Totara Foliage Oil

Franich and Cambie (177) recently identified 20 monoterpenoids, 6 sesquiterpenes and 9 diterpenoids as major constituents of *Podocarpus totara* foliage oil. The oil, fractionated by distillation under reduced pressure, contained 28% monoterpenoids (fractions 1-7), 29% sesquiterpenoids (fractions 8-11 (TF8-TF11)) and 43% diterpenoids (fractions 11-16). The sesquiterpenoid component was composed of 60% sesquiterpenes, of which β-elemene, β-caryophyllene, α-humulene and β-selinene were the most abundant (177). The remaining 40% consisted of a complex mixture of unidentified oxygenated sesquiterpenes with two major and numerous minor components. The proportion of oxygenated sesquiterpenes in each of the distillation fractions (approximations based on GC/MS peak areas) along with the results of antifungal assays (Section 7.3.1) are given in Table 3.1. The results are presented in terms of the extent of the fungus growth at various concentrations (% m/m) of the oils on the papers.

Fractions 8 and 9 (TF8 and TF9), which were rich in oxygenated sesquiterpenes, completely inhibited the growth of the brown rot fungus *Coniophora puteana* at a concentration loading of 0.6%, but were relatively ineffective against the white rot fungus *Trametes versicolor* at loadings as high as 2%. Fraction 11 (TF11), which was mainly composed of diterpenes and contained comparatively few oxygenated sesquiterpenoids, was inactive against both fungi.

Table 3.1: Percentage oxygenated sesquiterpenoid content and antifungal activity of distillation fractions of the essential oil of *Podocarpus totara* leaves.

Distillation fraction	**% Oxygenated Sesquiterpenoids**	**Av. radial growth of *Coniophora puteana* (cm)**			**Av. radial growth of *Trametes versicolor* (cm)**		
		0.2%	**0.6%**	**2.0%**	**0.2%**	**0.6%**	**2.0%**
TF8	52	8.5	0	0	8.0	7.2	6.6
TF9	52	8.1	0	0	7.7	7.9	4.7
TF10	37	8.5	5.7	0	8.0	7.4	5.2
TF11	10			7.9			8.0
Water			8.5			8.0	

Attempts to identify the oxygenated sesquiterpenoids of totara on the basis of their GC retention indices and mass spectra were in most cases unsuccessful. Fractions TF8 and TF10 were similar in composition and as TF8 showed greater activity only TF8 and TF9 were further fractionated by column chromatography in an attempt to separate the components and facilitate their identification (Table 3.2). Column chromatography on silica gel broadly separated the sesquiterpenoids into various classes based on functional group polarity. On the basis of GC/MS and FTIR results the column fractions were broadly classified as follows:

TF8 column fraction 1 (TF8f1) - sesquiterpenes

TF8f2 - sesquiterpene epoxides

TF8f3 - primarily sesquiterpene alcohols

A similar classification applied to the chromatography fractions of TF9 (Tables 3.2, 3.3).

Table 3.2: Column chromatography fractions of totara essential oil distillation fractions 8 and 9.

Fraction	Eluting solvent	Mass (mg)	Cumulative %
TF8f1	hexane (150 ml)	258	26
TF8f2	hexane:ether 7:1 (100 ml)	337	60
TF8f3	hexane:ether 3:1 (100 ml)	141	74
TF8f4	hexane:ether 5:3 (100 ml)	77	82
TF9f1	hexane (100 ml)	189	13
TF9f2	hexane:ether 7:1 (100 ml)	123	21
TF9f3	hexane:ether 3:1 (100 ml)	224	36
TF9f4	hexane:ether 1:1 (100 ml)	279	54
TF9f5	ether (100 ml)	246	71

The results of antifungal assays of these various fractions against *C. puteana* and *T. versicolor* are presented in Table 3.3. The fraction containing sesquiterpenes (TF8f1) was inactive against both fungi at the concentrations tested. Fractions containing sesquiterpenoid epoxides (TF8f2, TF9f2) exhibited minimum inhibitory concentrations (MICs) of *ca.* 0.6% against *C. puteana* and yet were ineffective in inhibiting the growth of *T. versicolor*. Fractions containing sesquiterpenoid alcohols showed potent to good activity against *C. puteana* and *T. versicolor* at loadings of 0.6% and 2% respectively (Table 3.3).

Table 3.3: Mean colony diameter (cm) of *Coniophora puteana* and *Trametes versicolor* grown on papers treated with the fractionated foliage oil of *Podocarpus totara*.

Fraction	Av. radial growth of *Coniophora puteana* (cm)				Av. radial growth of *Trametes versicolor* (cm)			
	0.1%	**0.2%**	**0.6%**	**2.0%**	**0.1%**	**0.2%**	**0.6%**	**2.0%**
TF8f1 (hydrocarbons)				5.6			7.9	8.0
TF8f2 (epoxides)	8.5	8.5	0	0	8.0	8.0	8.0	7.3
TF8f3 (alcohols)	7.3	4.5	0	0	8.0	7.3	5.7	1.7
TF9f2 (epoxides)	8.5	8.5	0	0	8.0	8.0	8.0	7.8
TF9f3 (alcohols)	7.1	5.9	0	0	8.0	7.8	7.4	1.8
TF9f4 (alcohols)	6.9	5.6	0	0	8.0	8.0	6.3	2.2
CH_2Cl_2	8.5				8.0			
Water	8.5				8.0			

Several sesquiterpene alcohols and quinones have being reported to have antifungal character (Section 1.6); the antifungal activities of sesquiterpene epoxides are not as well documented. Analyses by GC/MS and FTIR spectroscopy suggested that the two major oxygenated sesquiterpenes in the foliage oil were epoxides. Identification of these major oxygenated sesquiterpenes was considered practicable. The identification of the numerous minor sesquiterpene alcohols was considered impractical, within the scope of this study, considering attempts to identify on the basis of GC retention indices and mass spectra were unsuccessful.

Fraction TF8f2, which displayed antifungal activity against *C. puteana* (Table 3.3), contained the highest concentration of the two major sesquiterpene epoxides, *ca.* 58% and *ca.* 24% respectively. Interpretation of ^{1}H and ^{13}C NMR spectra of the mixture in conjunction with the retention indices and mass spectra data allowed identification of these two sesquiterpenes epoxides as caryophyllene oxide (**35**) (58%) and humulene epoxide II (**36**) (24%). Interpretation of the 2D ^{1}H-^{1}H and ^{13}C-^{1}H correlation NMR spectra confirmed the assigned structures (Appendix A). Caryophyllene oxide (**35**) and humulene epoxide II (**36**) were also the main components of TF9f2 and overall constituted *ca.* 2.5% and *ca.* 1.7% of the entire oil respectively. Only three of the remaining minor oxygenated sesquiterpenes could be identified with confidence by comparing retention indices (on methyl silicone (Ultra-2) and EC™-Wax columns) and mass spectra with literature data (Table 3.4). Where tentative identifications have been made the compound name is given in brackets.

Caryophyllene oxide (**35**) and humulene epoxide II (**36**) are relatively common sesquiterpenoid epoxides having been found in essential oils of a number of higher plants (276-280). However, little has been published on their occurrence in species of Podocarpaceae. Berry *et al.* (275) have reported the occurrence of caryophyllene oxide (**35**) in rimu (*Dacrydium cupressinum*). They found levels of the sesquiterpenoid varied significantly between different populations (0-20% of total sesquiterpenes). A trend of high inter and intra-population variability in terpene composition has been found in a number of New Zealand conifers and *Podocarpus totara* is among those species which show the greatest levels of variability (281, 282, 283). Isolation and characterisation of sesquiterpenoid components from this single source would consequently produce results of little phytochemical value.

Table 3.4: Oxygenated sesquiterpenes in *Podocarpus totara* foliage oil.

Sesquiterpenoid	**% of oil**	**Retention Indices**		**Mass Spectrum**
		Ultra 2[1]	**EC™-WAX**[2]	**($M^{\bullet+}$, Base peak)**
unknown 1	0.3	1582	1923	220, 79
spathulenol	0.1	1607 (1576)	2120 (2153)	220, 91
unknown 2 (Globulol)[3]	0.1	1616 (1583)	2066 (2104)	222, 43
caryophyllene oxide	2.5	1618 (1581)	1982 (2000)	220, 79
unknown 3	0.2	1630	2013	220, 93
humulene epoxide II	1.7	1646 (1606)	2037	222, 109
unknown 4	0.5	1665	2050	220, 81
unknown 5	0.5	1668	2308	220, 136
α-muurolol	0.08	1671 (1645)	2194	222, 161
unknown 6	0.4	1684	2104	220, 91
unknown 7	0.7	1688	2342	222, 109
(caryophyllenol II)	0.7	1700	2382	220, 93
unknown 8	0.3	1718	2350	222, 96
unknown 9	0.2	1761	2360	222, 111
α-cryperone	0.2	1785	2350	218, 93

[1] Values in parenthesis from Adams (255); [2] Values in parenthesis from Davies (284); [3] Compounds tentative identified are given in brackets.

The majority of studies on antifungal plant extracts report the antimicrobial properties of crude essential oils rather than individual components (155). Literature on the antifungal activity of sesquiterpene epoxides is limited, although some studies on caryophyllene oxide (**35**) have been reported. Dellar *et al.* (285) found caryophyllene oxide (**35**) to be only weakly antifungal against the fungal pathogen *Cladosporium cucumerinum*. Nabeta *et al.* (277)

reported caryophyllene oxide (**35**) and humulene epoxide II (**36**) showed no activity against the mould fungi *Aspergillus niger* and *Penicillium notatum* in a nutrient agar based zone inhibition assay using 6 mm antibiotic assay (AA) disks treated with 0.1 mg of the sesquiterpenoids. The foliage essential oil of *Buddleia asiatica*, which is rich in caryophyllene oxide (**35**) (22%), citronellol (17%) and caryophyllene (16%), has been found to possess good antifungal activity against *Asgergillus* spp., *Curvularia prasadii*, *Trichoderma viride* and *Trichophyton rubrum* (286). Other biological properties reported for caryophyllene oxide (**35**) include antibacterial (287), antimite (288), insect larvae antifeedant (289), insecticidal (290) and antimalarial activities (291). No research reporting on the antifungal properties of humulene epoxide II (**36**) could be found in the literature. The identification of caryophyllene oxide (**35**) and humulene epoxide II (**36**) in fractions TF8f2 and TF9f2, which displayed antifungal activity against *Coniophora puteana*, suggested that a simple synthetic compound with an epoxide functionality was worth exploring from the point of view of a novel wood protection agent (Section 3.3).

3.2.2 Kahikatea Foliage Oil

The kahikatea foliage oil fraction was composed of 42% sesquiterpenes, 56% oxygenated sesquiterpenoids and 2% diterpenes. Column chromatography of the oil fraction produced six fractions (Kf1-Kf6, Table 3.5) of which five were tested for antifungal activity. The major sesquiterpenes β-selinene, *cis*-β-guaiene, α-selinene, γ-cadinene, δ-cadinene, *trans*-calamenene, α-cadinene, α-calacorene and cadalene were identified from their mass spectra and retention indices (Appendix B).

Table 3.5: Column chromatography fractions of kahikatea foliage oil.

Fraction	Eluting solvent	Mass (mg)	Cumulative %
Kf1	hexane (80 ml)	200	20
Kf2	hexane:CH_2Cl_2 7:1 (80 ml)	25	23
Kf3	hexane:CH_2Cl_2 3:1 (80 ml)	83	31
Kf4	hexane:CH_2Cl_2 1:1 (80 ml)	150	46
Kf5	hexane:CH_2Cl_2 1:1 (80 ml)	209	67
Kf6	hexane:CH_2Cl_2 1:3 (80 ml)	106	77

The column fraction containing the above sesquiterpenes (Kf1) was essentially inactive against *C. puteana* and *T. versicolor* (Table 3.6). Column fraction 6 (Kf6) was the most active. This fraction completely inhibited growth of the brown rot fungus at a loading of 0.1% and severely restricted growth of the white rot fungus at the 2% level (Table 3.6). Fractions Kf4 and Kf5 were active against *C. puteana* at the 0.6% level and *T. versicolor* at a 2% loading. Kf3 was inactive against both fungi at the 0.6% loading.

Table 3.6: Mean colony diameter (cm) of *Coniophora puteana* and *Trametes versicolor* grown on papers treated with the fractionated foliage oil of *Dacrycarpus dacrydioides*.

Fraction	**Av. radial growth of *Coniophora puteana* (cm)**					**Av. radial growth of *Trametes versicolor* (cm)**			
	0.05%	**0.1%**	**0.2%**	**0.6%**	**2.0%**	**0.1%**	**0.2%**	**0.6%**	**2.0%**
K (original fraction)			7.5	5.7	0		8.0	8.0	6.9
Kf1					5.1				8.0
Kf3		8.5	8.5	8.2		8.0	8.0	8.0	
Kf4		7.2	6.7	0		8.0	8.0	8.0	2.1
Kf5		6.8	5.1	0		8.0	7.6	6.8	1.4
Kf6	8.0	0	0	0		7.8	7.6	5.2	1.4
α-Cadinol		4.4	0	0		8.0	7.3	3.8	1.6
CH_2Cl_2			8.5				8.0		
Water			8.5				8.0		

The major oxygenated sesquiterpenoids in the oil were identified, where possible, from their mass spectra and retention indices (RIs) (Table 3.7). The percentage compositions quoted for each of the components are approximations based on GC/MS peak areas. Kf6 was composed of two major components, globulol (**37**) (43%) and α-cadinol (**38**) (35%) and three minor components (γ-eudesmol (3%), α-muurolol (8%) and unknown 16 (8%), possibly selin-11-en-4-α-ol). Globulol (**37**) (6.3%) and α-cadinol (**38**) (6.9%) were the two major oxygenated sesquiterpenes in the original kahikatea oil fraction. They were identified by comparison of the ^{1}H and ^{13}C NMR spectra of the mixture with that of authentic α-cadinol (**38**) (from *Araucaria imbricata*) and published NMR data (292) in addition to their mass spectra and RIs (Appendix C). 2D ^{1}H-^{1}H and ^{13}C-^{1}H correlation NMR spectroscopy confirmed these identifications. Synergism between the sesquiterpene alcohols of Kf6 is possibly responsible for the lower MIC of Kf6 compared to pure α-cadinol (**38**) in the case of *C. puteana.* The efficacy of Kf6 against *T. versicolor* was comparable with that of α-cadinol (**38**). The antifungal activity of Kf6 against *C. puteana* was of a fungistatic rather than fungicidal nature.

Table 3.7: Oxygenated sesquiterpenes in kahikatea (*Dacrycarpus dacrydioides*) foliage oil.

Sesquiterpenoid	% of oil fraction	Retention Indices		Mass Spectrum
		Ultra 2[1]	EC™-WAX[2]	($M^{\bullet+}$, Base peak)
epi-globulol	1.2	1590	2007	222, 82
palustrol	2.2	1600	1917	222, 111
spathulenol	1.4	1607 (1576)	2120 (2153)	220, 119
globulol	6.3	1616 (1583)	2066 (2104)	222, 43
viridiflorol	2.4	1624 (1590)	2076 (2112)	222, 43
unknown 10 (guaiol)[3]	3.0	1624 (1595)	2048	222, 163
unknown 11 (β-eudesmol)	2.8	1632	2098	222, 149
unknown 12	2.5	1644	2131	222, 81
unknown 13 (α- or β-eudesmol)	1.9	1651	2107	222, 149
unknown 14 (epi-cubenol)	1.8	1655 (1627)	2057 (2037)	222, 119
unknown 15 (junenol)	1.0	1655	2052	222, 109
γ-eudesmol	2.6	1659 (1630)	2165 (2182)	222, 189
t-cadinol	2.7	1665 (1640)	2167 (2136)	222, 161
t-muurolol	3.7	1668 (1641)	2182	222, 95
cubenol	1.0	1669 (1642)	2052	222, 161
α-muurolol	2.7	1671 (1645)	2194	222, 161
α-cadinol	6.9	1682 (1653)	2229 (2224)	222, 95
unknown 16 (selin-11-en-4-α-ol)	5.2	1687 (1652)	2245	222, 81

[1] Values in parenthesis from Adams (255), [2] Values in parenthesis from Davies (284); [3] Compounds tentative identified are given in brackets.

Kf4 and Kf5 were both complex mixtures of sesquiterpene alcohols. Kf4 was composed of unknown 10 (17%), *t*-cadinol (13%), *t*-muurolol (12%), viridiflorol (11%), unknown 12 (9%), epi-globulol (5%) and unknowns 11, 13 and 14 (4%, 4% and 7% respectively). The major sesquiterpene alcohols of Kf5 were α-cadinol (**38**) (13%), unknown 16 (12%), *t*-muurolol (10%), γ-eudesmol (10%) and globulol (**37**) (8%). Other components included spathulenol (5%), viridiflorol (4%), α-muurolol (4%) and unknowns 10 and 11 (5% and 6%). As with Kf6, Kf4 and Kf5 exhibited fungistatic activity against *C. puteana*. Kf3 exhibited no antifungal activity at the concentrations tested even though its major components were the sesquiterpenoid alcohols palustrol (17%), cubenol (18%) and possibly junenol (22%). Palustrol and cubenol are structural isomers of globulol (**37**) and α-cadinol (**38**) (Figure 3.1), yet this fraction lacked antifungal activity, suggesting some specificity in structure for the displayed bioactivity.

Although it is impossible to draw clear conclusions from such complex mixtures of sesquiterpenoids the large difference in activity between Kf6 and Kf3 suggests a complex relationship between structure and activity. It is interesting to note that palustrol differs from globulol (**37**) and cubenol differs from α-cadinol (**38**) in that the hydroxy group is located at the bridge-head of the two-fused rings rather than on one of the rings (Figure 3.1). Few studies have been conducted into the structural/antifungal activity relationships of oxygenated sesquiterpenoids. Chang *et al.* (293) has explored the relationship between the structural isomers α-cadinol (**38**), *t*-cadinol and *t*-muurolol (see Figure 1.24, page 45) against the basidiomycetes *Trametes versicolor* and *Laetiporus sulpureus* on nutrient agar. They found the order of efficacy to be α-cadinol > *t*-cadinol > *t*-muurolol and concluded that the *trans* configuration at the ring junction was an important structural element for activity while the equatorial/axial configuration of the hydroxyl group was of secondary importance. In addition to antifungal properties, the α-cadinol (**38**) and *t*-cadinol have been shown to have bactericidal and termiticidal properties (294, 295).

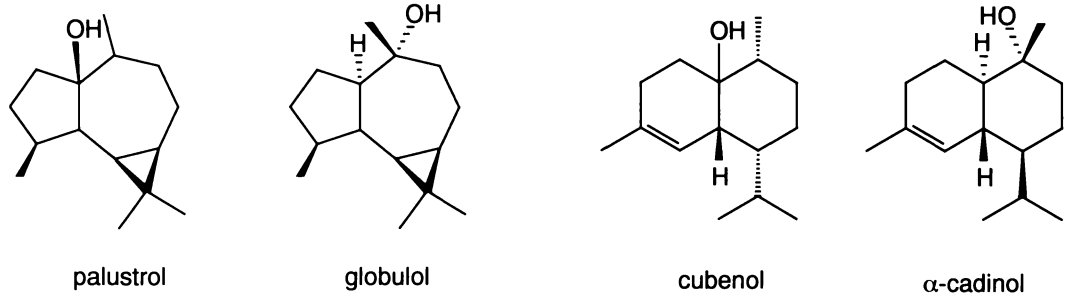


Figure 3.1: Palustrol, globulol, cubenol and α-cadinol.

Few studies have addressed the antifungal properties of globulol (**37**). Vallejo *et al.* (296) reported globulol (**37**) exhibits a strong fungistatic effect against the phytopathogenic fungus *Botrytis cinerea* which infects grapevine and tobacco plants. Wassmuth-Wagner *et al.* (297) identified globulol (**37**) and selin-11-en-4-α-ol as major components in the essential oil of the Indian medicinal plant *Murraya koenigii* which is thought to have antifungal and antibacterial properties. A few studies have been undertaken on compounds related to globulol (**37**). The fungicidal activity of an ether extract from the Vietnamese wood, *Tabauma gioi*, has been reported to be largely due to the sesquiterpenoid alcohol ledol, a diastereomer of globulol (**37**) (298). The related sesquiterpenoid spathulenol (Figure 1.24) has been reported to have antifungal and termiticidal properties (140).

3.2.3 Rimu Foliage Oil

The rimu foliage oil was composed of 46% sesquiterpenes (Appendix B), 21% oxygenated sesquiterpenoids and 32% diterpenes. The sesquiterpenes and diterpenes eluted in column fractions 1 and 2 (Rf1, Rf2, Table 3.8) and were not bioassayed as only fractions containing oxygenated sesquiterpenes (*i.e.* Rf3-Rf6) were of interest. As with the kahikatea oil the most polar fraction (Rf6) was the most active, completely inhibiting the growth of *C. puteana* at 0.1%, while retarding the growth of *T. versicolor* partially and significantly at loadings of 0.6% and 2% respectively (Table 3.9).

Table 3.8: Column chromatography fractions of rimu foliage oil.

Fraction	Eluting solvent	Mass (mg)	Cumulative %
Rf1	hexane (140 ml)	1900	63
Rf2	hexane:CH_2Cl_2 7:1 (140 ml)	42	65
Rf3	hexane:CH_2Cl_2 3:1 (140 ml)	187	72
Rf4	hexane:CH_2Cl_2 1:1 (140 ml)	216	80
Rf5	hexane:CH_2Cl_2 1:1 (100 ml)	145	84
Rf6	hexane:CH_2Cl_2 1:3 (100 ml)	169	90

Table 3.9: Mean colony diameter (cm) of *Coniophora puteana* and *Trametes versicolor* grown on papers treated with the fractionated foliage oil of *Dacrydium cupressinum.*

Fraction	Radial growth of *C. puteana* (cm)				Radial growth of *T. versicolor* (cm)			
	0.1%	0.2%	0.6%	2.0%	0.1%	0.2%	0.6%	2.0%
R		8.5	8.0	3.3		8.0	8.0	6.9
Rf3	8.5	8.1	7.3	2.3	8.0	8.0	8.0	8.0
Rf4	8.4	8.4	6.6	0	8.0	8.0	7.9	3.3
Rf5	5.8	5.7	0		7.9	7.8	7.8	3.5
Rf6	0	0	0		8.0	7.4	4.5	1.8
CH_2Cl_2	8.5				8.0			
Water	8.5				8.0			

Identification of the major oxygenated sesquiterpenoids in the oil was made from mass spectra and retention indices (Table 3.10). The percentage compositions quoted for each of the components are approximations based on GC/MS peak areas. Rf6 was a complex mixture of sesquiterpene alcohols including spathulenol (18%), clovanol (13%), unknown 10 (10%), longiborneol (10%), viridiflorol (6%) *t*-muurolol (4%) and unknowns 11 and 13 (*ca.* 4%

each). It is likely these 2° and 3° sesquiterpenoid alcohols are acting synergistically to achieve the observed antifungal activity rather one or two of these components being highly active. Activity was of a fungistatic nature.

Table 3.10: Oxygenated sesquiterpenes in rimu (*Dacrydium cupressinum*) foliage oil.

Sesquiterpenoid	% of oil fraction	Retention Indices		Mass Spectrum
		Ultra 2[1]	EC™-WAX[2]	($M^{\bullet+}$, Base peak)
unknown 1	0.5	1582	1925	220, 79
unknown 17	0.5	1589	1949	220, 96
spathulenol	1.3	1607 (1576)	2120 (2153)	220, 119
caryophyllene oxide	4.1	1616 (1581)	1979 (2000)	220, 79
viridiflorol	0.9	1624 (1590)	2076 (2112)	222, 43
unknown 10 (guaiol)[3]	0.6	1624	2048	222, 107
longiborneol	2.6	1635 (1592)	2150	222, 85
humulene epoxide II	1.1	1643 (1606)	2033 (2011)	220, 109
unknown 18	1.5	1651	2123	220, 123
unknown 19	1.0	1655	2250	220, 119
clovanol	0.7	1673	2235	222, 166
unknown 16 (selin-11-en-4-α-ol)	1.3	1686 (1652)	2245	222, 81
longibornyl acetate	3.2	1714 (1679)	2024	264, 204

[1] Values in parenthesis from Adams (255), [2] Values in parenthesis from Davies (284); [3] Compounds tentative identified are given in brackets.

Rf5 was strongly inhibitory toward the growth of *C. puteana* at the 0.6% loading, but only weakly active against *T. versicolor* at a 2% loading. Fraction Rf4 showed good activity against *C. puteana* at the 2% loading but was only weakly active against *T. versicolor* at the same concentration. Rf3 displayed moderate activity against *C. puteana* at the 2% concentration but was completely ineffectual against the white rot fungus. Rf5 was composed mainly of longiborneol (60%), caryophyllene oxide (**35**) (7%), viridiflorol (5%) and unknown 12 (4%). Rf4 was a mixture of caryophyllene oxide (**35**) (51%), humulene epoxide II (**36**) (13%) and an unknown epoxide (11%). The essentially inactive fraction Rf3 was mainly longibornyl acetate (71%) and unknowns 1 and 17 (6% each). It is apparent that esterification of the alcohol functionality leads to inactivity in the case of longiborneol (Rf5 compared to Rf3). The caryophyllene oxide (**35**) rich fraction, Rf4, showed inferior activity against *C. puteana* compared with its counterpart from totara, TF8f2 (MICs of >0.6%≤2% and *ca.* 0.6% respectively) and yet exhibited enhanced efficacy against *T. versicolor*. Synergistic effects with minor sesquiterpenoid components could possibly account for these differences.

Anti-basidomycetes activity of the foliage oil fractions was, in general, related to the levels of sesquiterpenoid epoxides or alcohols. These oxygenated sesquiterpenes were considerably more active against the brown rot fungus *Coniophora puteana* than the white rot fungus *Trametes versicolor*. Caryophyllene oxide (**35**), humulene epoxide II (**36**), globulol (**37**) and α-cadinol (**38**) were identified in fractions displaying good activity against *C. puteana*. Simple synthetic compounds that possess similar epoxide and tertiary alcohol functionalities as these sesquiterpenoids could potentially display antifungal properties. To investigate this hypothesis syntheses towards compounds of this type were undertaken.

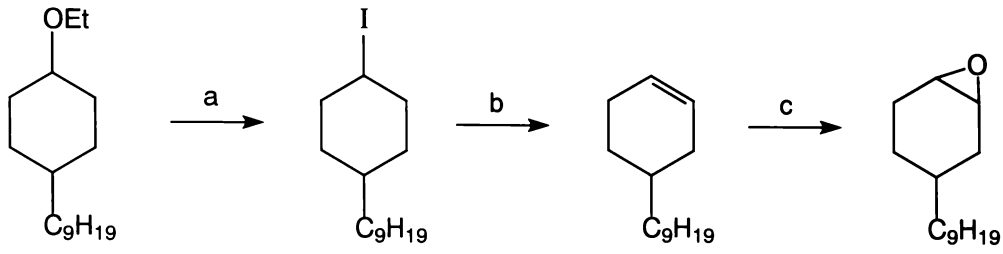
3.3 Synthetic Compounds from Sesquiterpenoid Leads

Two synthetic compounds 4-nonylcyclohexan-1,2-epoxide (**39**) and 4-ethyl-1-methyl-1,2,3,4-tetrahydronaphth-1-ol (**40**) were selected as synthetic targets. In the case of 4-nonylcyclohexan-1,2-epoxide (**39**) the structure bears no resemblance to the natural sesquiterpenoid epoxides (**35**, **36**) but rather is a simple C15 epoxide with a nonylcyclohexane backbone analogous to the compounds investigated in Chapter 2. The structural elements considered to be important were the epoxide ring and the fifteen-carbon skeleton. The second target compound 4-ethyl-1-methyl-1,2,3,4-tetrahydronaphth-1-ol (**40**) can be viewed as simplified 'analogue' of α-cadinol (**38**). In this case the goal was a tertiary alcohol functional group within the fused-ring system of approximately fifteen carbons. Understandably in order to keep the syntheses simple (and hence ultimately commercial viable) the synthesis of close analogues of the sesquiterpenoids was not practicable and possibly not necessary from the point of view of antifungal properties. The results of syntheses towards the aforementioned target compounds are discussed in this Section while antifungal assay results are discussed in Chapter 5.

3.3.1 4-Nonylcyclohexan-1,2-epoxide (39)

The strategy devised for the synthesis of 4-nonylcyclohexan-1,2-epoxide (**39**) utilised 1-ethoxy-4-nonylcyclohexane (**27**), the preparation of which was discussed in Chapter 2 (Section 2.2.3.3, page 79). The proposed pathway involved cleavage of the ethoxy group of 1-ethoxy-4-nonylcyclohexane (**27**) with HI to give 1-iodo-4-nonylcyclohexane (**41**) (Scheme

3.1). The iodoalkane (**41**) could then be dehydrohalogenated with alcoholic KOH to give 4-nonylcyclohex-1-ene (**42**). The alkene (**42**) could then be epoxidised with *m*-chloroperbenzoic acid to give 4-nonylcyclohexan-1,2-epoxide (**39**) (Scheme 3.1).



27 **41** **42** **39**

Scheme 3.1: Synthetic strategy to 4-nonylcyclohexan-1,2-epoxide (**39**) from 1-ethoxy-4-nonylcyclohexane (**27**) (a = 57% HI (aq), reflux; b = KOH/ethanol, reflux; c = *m*-chloroperbenzoic acid, CH_2Cl_2, 35°C).

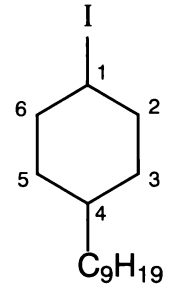
The starting material 1-ethoxy-4-nonylcyclohexane (**27**) was an unexpected by-product produced during the attempted catalytic hydrogenation of 4-nonylcyclohex-2-enone (**12**) and 4-nonylcyclohex-3-enone (**13**) (Section 2.2.3.3). Purification of the product mixture of this reaction by chromatography gave a fraction containing 1-ethoxy-4-nonylcyclohexane (**27**) in *ca.* 88% purity. The other component was identified as 4-nonylcyclohexane (**26**). Although it was undesirable to use a mixture of compounds as a starting material it was envisaged that 4-nonylcyclohexane (**26**) would not interfere with any of the proposed synthetic transformations and would be more easily separated from the final product 4-nonylcyclohexan-1,2-epoxide (**39**) rather than at any of the intermediate stages. As 1-ethoxy-4-nonylcyclohexane (**27**) was available as a starting material and 4-nonylcyclohexanone (**20**) was consumed in the synthesis of 1-methyl-4-nonylcyclohexan-1-ol (**5**) (Section 2.2.2) the synthetic strategy outlined in Scheme 3.1 was chosen over the synthesis of 4-nonylcyclohex-1-ene (**42**) by $NaBH_4$ reduction and dehydration of 4-nonylcyclohexanone (**20**).

Substitution of the ethoxy group of 1-ethoxy-4-nonylcyclohexane (**27**) with an iodo group was achieved by heating the starting material to reflux in 57% aqueous hydriodic acid. This was found to be superior to halo-de-alkoxylation by heating to reflux in 46% aqueous HBr, catalysed with 10% conc. H_2SO_4. GC/MS analysis of the reaction product suggested four products, three iodo-4-nonylcyclohexane isomers (*ca.* 78%) and a 4-nonylcyclohexene (*ca.*

7%) were formed in the reaction. The crude product also contained unreacted 4-nonylcyclohexane (**26**, *ca.* 12%). Molecular ions (*m/z* 336) were absent from the mass spectra of the iodo-4-nonylcyclohexanes although ions of *m/z* 209 ($M^{\bullet+}$- I) were prominent. The major iodo-4-nonylcyclohexane isomer (*ca.* 52%) was established as 1-iodo-4-nonylcyclohexane (**41**) by 2D ^{1}H-^{1}H and ^{13}C-^{1}H correlation NMR spectroscopy on the product mixture (Table 3.11).

Table 3.11: Selected gCOSY and HMBC correlations observed for 1-iodo-4-nonylcyclohexane (**41**).

Signal	δ (ppm)	Correlated ^{1}H signals
H3/H5	0.96	H2/H6, H2'/H6', H3'/H5', H4
H3'/H5'	1.61	H2/H6, H2'/H6'
H1	4.09	H2/H6, H2'/H6'
C1	30.9	H2/H6
C3/C5	35.5	H2/H6
C2/C6	40.5	H1, H3/H5, H3'/H5'



The mechanism for the halo-de-alkoxylation of 1-ethoxy-4-nonylcyclohexane (**27**) is likely to involve several pathways to 1-iodo-4-nonylcyclohexane (**41**) (Figure 3.2). According to the discussions outlined in March (299) and Staude and Patat (231) ether cleavage of a 1° and 2° dialkyl ether under strongly acidic conditions is most likely to occur by a S_N1cA mechanism, where cA stands for conjugated acid. Protonation of the ethoxy group would be followed by nucleophilic substitution *via* an S_N1 mechanism, with both EtOH (pathway **i**) and 1-hydroxy-4-nonylcyclohexane (pathway **ii**) as viable leaving groups in the first step (Figure 3.2). Addition of iodide to the resultant carbocations in the second step would yield ethyl iodide and 1-iodo-4-nonylcyclohexane (**41**). As the reaction was carried out in excess HI the hydroxyl group of 1-hydroxy-4-nonylcyclohexane would become protonated and could undergo S_N1 substitution with iodide to yield 1-iodo-4-nonylcyclohexane (**41**) (Figure 3.2).

The first step of the S_N1 mechanism, in both scenarios above, generates a 4-nonylcyclohexane carbocation with the positive charge residing at C1 (Figure 3.2). It is possible for this carbocation, in addition to undergoing nucleophilic attack by iodide (pathway **iii**), to undergo abstraction of an α-proton to yield 4-nonylcyclohex-1-ene (**42**) (pathway **iv**). Hence an E1 mechanism could be in competition with the S_N1 mechanism. As the reaction was carried out in excess HI any 4-nonylcyclohex-1-ene (**42**) that was formed will undergo electrophilic

addition of HI to give 1-iodo-4-nonylcyclohexane (**41**) and 1-iodo-3-nonylcyclohexane (**43**) as possible products (Figure 3.2).

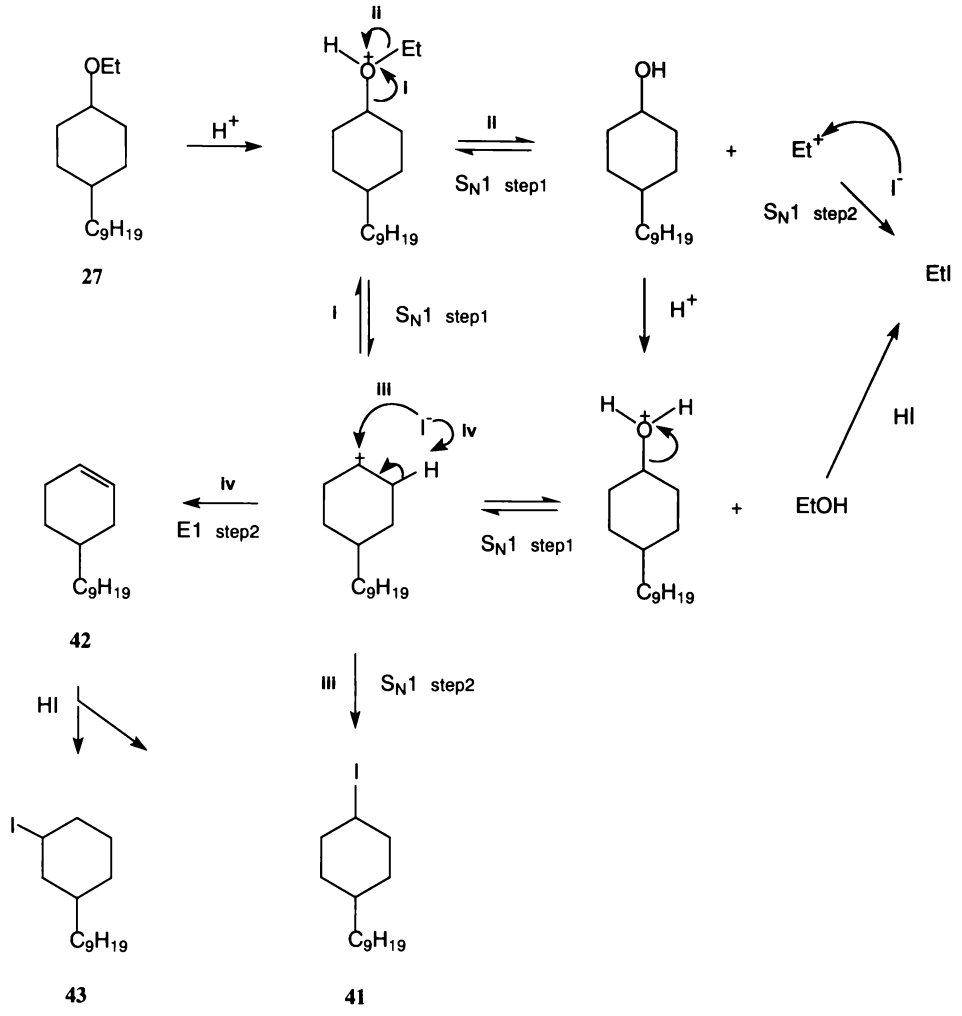


Figure 3.2: Proposed mechanism for the halo-de-alkoxylation of 1-ethoxy-4-nonylcyclohexane (**27**) with HI.

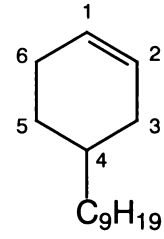
As mentioned above GC/MS analysis of the crude product indicated three peaks (GC retention times: 38.9 min (*ca.* 16%), 39.1 min (*ca.* 10%) and 39.5 min (*ca.* 52%)) which were assignable to iodo-4-nonylcyclohexane products. As the *cis* and *trans* diastereomers of 1-iodo-4-nonylcyclohexane (**41**) can only account for two of these peaks the GC/MS results point towards some 1-iodo-3-nonylcyclohexane (**43**) being formed in the reaction. The NMR evidence indicates that the major product (*ca.* 52%) was a diastereomer of 1-iodo-4-nonylcyclohexane. It follows that one of the minor iodo-4-nonylcyclohexane products (*ca.* 16% or 10%) is the other diastereomer of 1-iodo-4-nonylcyclohexane (**41**) and the other is possibly a diastereomer of 1-iodo-3-nonylcyclohexane (**43**). The GC/MS results suggested that a 4-nonylcyclohexene was a minor reaction product. As the reaction was carried out in

excess HI any 4-nonylcyclohex-1-ene (**42**) generated should have undergone further reaction. The ^{1}H NMR spectra of the reaction product showed no olefinic proton signals suggesting that the 4-nonylcyclohexene observed was formed only under the conditions of GC/MS analysis.

4-Nonylcyclohex-1-ene (**42**) was expected as the major product of dehydrohalogenation of the aforementioned mixture of iodo-4-nonylcyclohexanes. Refluxing in ethanolic KOH successfully brought about the dehydrohalogenation in good yield (86%) (Scheme 3.1). The GC/MS chromatogram of the product showed a single product peak which could be assigned to 4-nonylcyclohex-1-ene (**42**) (*ca.* 85%) (and unreacted 4-nonylcyclohexane (**26**) (*ca.* 15%) carried over from the starting material). The mass spectrum of this product exhibited a molecular ion of *m/z* 208 and a base peak of *m/z* 81. This fragment is possibly formed by loss of a C_9H_{17} radical (MW = 127 Da) due to isomerisation of the double bond and allylic cleavage. The ^{1}H and ^{13}C NMR spectra of the product indicated 4-nonylcyclohex-1-ene (**42**) was the major product. Couplings that were observed in the 2D ^{1}H-^{1}H and ^{13}C-^{1}H correlation NMR spectra supported this conclusion (Table 3.12).

Table 3.12: Selected gCOSY and HMBC correlations observed for 4-nonylcyclohex-1-ene (**42**).

Signal	δ (ppm)	Correlated ^{1}H signals
H5, H5'	1.50, 1.67	H6'
H6, H6'	1.61, 2.07	H1
H4	1.71	H3, H5'
H3	2.01	H2
C3	25.3	H2, H4
C4	29.0	H2, H6'
C6	32.0	H1, H4
C5	33.5	H1, H4, H6, H6'
C1	126.7	H6
C2	127.0	H3



As described above 1-iodo-3-nonylcyclohexane (**43**) was proposed as a minor component of the starting material used in this reaction. Dehydrohalogenation of 1-iodo-3-nonylcyclohexane (**43**) could yield both 4-nonylcyclohex-1-ene (**42**) and 3-nonylcyclohex-1-ene (**16**). Minor vinyl carbon signals at δ 126.9 and 132.8 ppm were observed in the ^{13}C NMR spectra of the product and can possibly be assigned to C1 and C2 in 3-nonylcyclohex-1-ene (**16**). This

assertion requires that the 4-nonyl and 3-nonyl isomers co-elute on GC/MS as only one product peak was observed in the chromatogram.

Epoxidation of 4-nonylcyclohex-1-ene (**42**) was achieved by oxidation of the double bond with *m*-chloroperbenzoic acid (Scheme 3.1). The separation of the main epoxidation product from the alkane 4-nonylcyclohexane (**26**) carried over from the starting material was achieved by column chromatography. Elution with 1:1 hexane:CH_2Cl_2 gave 4-nonylcyclohexan-1,2-epoxide (**39**) in 48% yield and 97% purity as indicated by GC/MS analysis. A molecular ion of *m/z* 224 ($C_{15}H_{28}O$) and base peak ion of *m/z* 97 ($M^{\bullet+}$-127 (C_9H_{19})) were observed in the mass spectrum. The GC/MS chromatogram showed a single peak, however the presence of diastereomers was confirmed by interpretation of the ^1H and ^{13}C NMR spectra of the product. It was possible to assign H1-H6 and C1-C7 for the two diastereomers using 2D ^1H-^1H and ^{13}C-^1H correlation NMR spectroscopy to resolve the signals (Table 3.13). Two diastereomeric forms of 4-nonylcyclohexan-1,2-epoxide (**39**) are possible from the epoxidation of 4-nonyl-cyclohex-1-ene (**42**) as oxygen can be added from above or below the plane of the cyclohexene ring.

Table 3.13: gCOSY and HMBC correlations observed for two diastereomers of 4-nonylcyclohexan-1,2-epoxide (**39**) for H1-H6 and C1-C7.

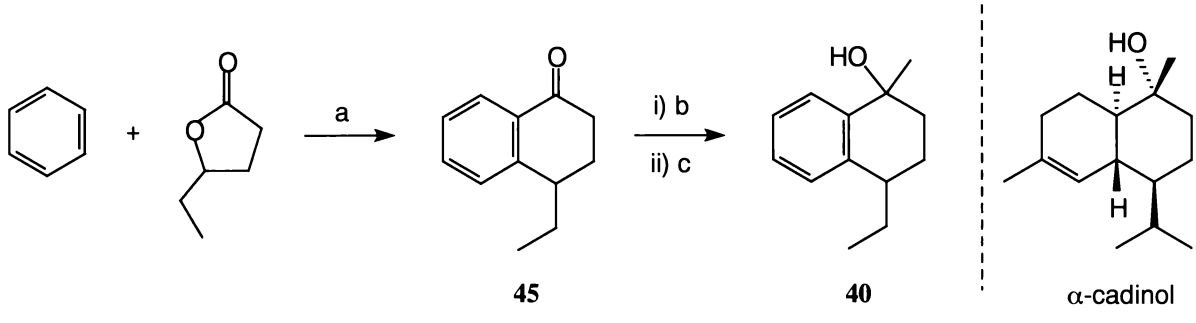
Diastereomer 1			**Diastereomer 2**		
Signal	**δ (ppm)**	**Correlated ^1H signals**	**Signal**	**δ (ppm)**	**Correlated ^1H signals**
H6	1.67	H1, H5'	H6	1.80	H5, H5'
H3'	2.01	H2	H6'	1.95	H1, H5
H6'	2.11	H1, H5	H2	3.14	H3, H3'
C5	24.5	H1, H3', H6, H6'	C6	23.6	H1, H5
C6	25.4	H1, H5	C5	27.2	H1, H3, H6, H6'
C3	30.8	H2	C4	29.4	H2
C4	32.7	H2, H3, H3', H5, H6'	C3	31.9	H2, H3', H5, H6'
C7	36.9	H3	C7	36.3	H3, H5
C2	51.9	H3	C1	51.9	H3, H6'
C1	52.6	H3	C2	53.1	H3', H6

High resolution probe MS confirmed the product was 4-nonylcyclohexan-1,2-epoxide (**39**) although elemental analysis did not give satisfactory data (Section 7.2.6). This is the first reported synthesis of 4-nonylcyclohexan-1,2-epoxide (**39**). Column chromatography of the

crude product also gave some other minor epoxide products in *ca.* 7% yield in a fraction eluted from the column with 1:3 hexane:CH_2Cl_2. GC/MS analysis of this fraction indicated a mixture of three epoxides, one of which appeared to be 4-nonylcyclohexan-1,2-epoxide (**39**) (based on identical GC retention times). The two additional epoxides (MW = 224 Da) were possibly products of the epoxidation of 3-nonylcyclohex-1-ene (**16**) which was proposed as a minor component of the starting material.

3.3.2 4-Ethyl-1-methyl-1,2,3,4-tetrahydronaphth-1-ol (40) and 5,9-Dimethyl-6,7,8,9-tetrahydrobenzocyclohepten-5-ol (44)

4-Ethyl-1-methyl-1,2,3,4-tetrahydronaphth-1-ol (**40**) was selected as a synthetically achievable simplified 'analogue' of α-cadinol (**38**). α-Cadinol (**38**) was found to displayed good and moderate antifungal properties against the decay fungi *Coniophora puteana* and *Trametes versicolor* respectively (Section 3.2.2). It was proposed 4-ethyl-1-methyl-1,2,3,4-tetrahydronaphth-1-ol (**40**), could be prepared through the pathway depicted in Scheme 3.2. The first step in the proposed strategy involves the reaction of γ-caprolactone with benzene under Freidel-Crafts conditions to give 4-ethyl-α-tetralone (**45**) in an analogous fashion to the reaction of γ-butyrolactone with benzene, which yields α-tetralone (300). The tertiary alcohol (**40**) could then be produced by reaction with a methyl Grignard reagent (Scheme 3.2). As 1-isopropylbutyrolactone is not commercially available the 4-isopropyl variant of (**40**) was not considered a variable synthetic target.



Scheme 3.2: Proposed synthetic pathway to 4-ethyl-1-methyl-1,2,3,4-tetrahydronaphth-1-ol (**40**), an analogue of α-cadinol (**38**) (a = $AlCl_3$, Δ; b = Mg, MeI, ether; c = NH_4Cl/H_2O).

γ-Caprolactone reacted with benzene in the presence of a 4 mol excess of $AlCl_3$ to give two products which co-distilled, in a yield of 82%. These products were identified as 4-ethyl-α-tetralone (**45**) (yield *ca.* 37%) and 9-methyl-6,7,8,9-tetrahydrobenzocyclohepten-5-one (**46**) (yield *ca.* 45%) from the infrared, GC/MS and ^{1}H and ^{13}C NMR spectra of the mixture. The infrared spectrum of the mixture exhibited a strong carbonyl stretching vibration at 1685 cm^{-1}. The GC/MS chromatogram showed two peaks with molecular ions of *m/z* 174 and base peaks of *m/z* 131 and 145 respectively. These ions were attributed to losses of a CH_3CH_2 radical and a CH_3 radical + CO from the α-tetralone (**45**) and benzocycloheptenone (**46**) molecular ions respectively (Figure 3.3).

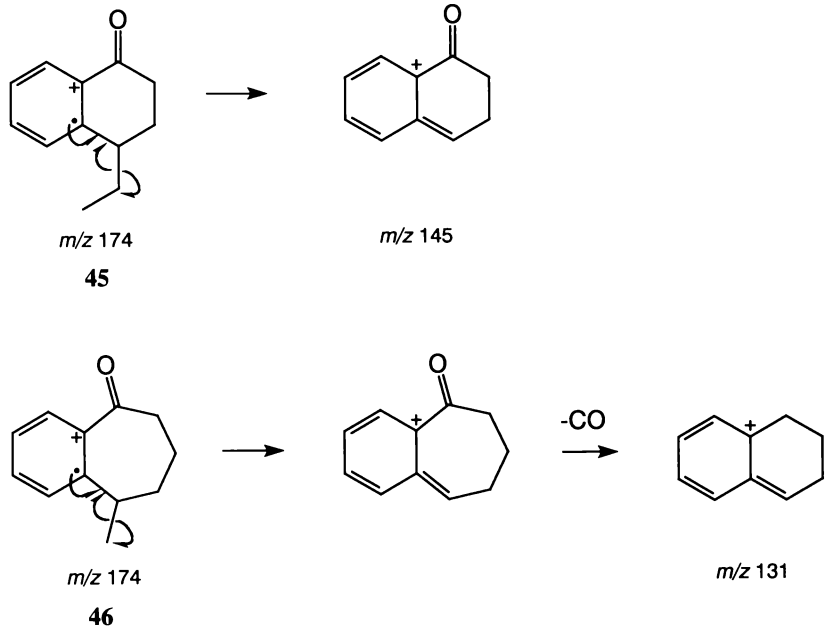


Figure 3.3: Proposed base peak MS fragmentations of 4-ethyl-α-tetralone (**45**) and 9-methyl-6,7,8,9-tetrahydrobenzocyclohepten-5-one (**46**).

The ^{1}H NMR spectrum of the mixture exhibited signals attributed to both methyl and ethyl groups and two carbonyl signals were observed at δ 198.2 and 208.3 ppm in the ^{13}C NMR spectrum. Interpretation of the gCOSY, HMQC and HMBC NMR spectra of the mixture enabled assignment of all ^{1}H and ^{13}C NMR signals and provided additional evidence for 4-ethyl-α-tetralone (**45**) and 9-methyl-6,7,8,9-tetrahydrobenzocyclohepten-5-one (**46**) as the reaction products (Table 3.14). Good high resolution GC/MS results validated these structural identifications (Section 7.2.7).

Table 3.14: gCOSY and HMBC correlations observed for 4-ethyl-α-tetralone (**45**) and 9-methyl-6,7,8,9-tetrahydrobenzocyclohepten-5-one (**46**).

4-ethyl-α-tetralone			9-methyl-6,7,8,9-tetrahydrobenzocycloheptan-5-one		
Signal	**δ (ppm)**	**Correlated ^{1}H signals**	**Signal**	**δ (ppm)**	**Correlated ^{1}H signals**
H10	1.00	H9	H10	1.36	H9
H3	2.05	H2', H3'	H8	1.51	H7', H8', H9
H2'	2.74	H2	H7	1.58	H6, H7', H8',
C10	12.0	H9	H6'	2.71	H6, H7'
C3	26.2	H2, H2', H9	C10	19.2	H8, H9
C9	27.3	H3, H3', H4, H10	C7	20.3	H6', H8, H8'
C2	34.8	H3, H3', H4	C8	34.1	H6, H7, H7', H9
C4	39.4	H2, H2', H3, H3', H5,	C9	34.2	H1, H8, H8', H10
		H9, H10	C6	41.1	H7
C7	126.4	H5	C1	125.2	H2, H3, H9
C8	127.2	H6	C3	126.4	H1
C5	128.2	H4, H7	C4	127.7	H2
C8a	131.8	H2, H4, H5	C2	131.9	H3, H4
C6	133.2	H5, H7, H8	C4a	139.4	H1, H3, H6', H9
C4a	148.1	H3, H3', H4, H9, H6,	C9a	143.1	H2, H4, H8, H9, H10
		H8			
C1	198.3	H2, H2', H3, H3', H8	C5	208.3	H4, H6, H7, H7'

It is proposed that the reaction of benzene with γ-caprolactone in an excess of $AlCl_3$ proceeds *via* an inter-molecular Friedel-Crafts acylation followed by an intra-molecular alkylation (Figure 3.4). The possible pathway involves formation of a complex between γ-caprolactone and $AlCl_3$, which results in opening of the lactone and formation of a carbocation. This carbocation can attack benzene and by substituting for hydrogen yield the alcohol (**i**) (Figure 3.4). Dehydration of the alcohol to form the alkene (**ii**) followed by intramolecular Friedel-Crafts alkylation to effect ring closure could yield both 4-ethyl-α-tetralone (**45**) and 9-methyl-6,7,8,9-tetrahydrobenzocyclohepten-5-one (**46**). Danheiser and Helgason (301) proposed this pathway to account for the formation of 4-ethyl-α-tetralone (**45**) in the analogous reaction of benzene with δ-caprolactone. They found that Friedel-Crafts annulation of benzene and δ-caprolactone gives a 7:3 mixture of 9-methyl-6,7,8,9-tetrahydrobenzocyclohepten-5-one (**46**) and 4-ethyl-α-tetralone (**45**) in 54% yield (301). Danheiser and Helgason (301) produced pure 9-methyl-6,7,8,9-tetrahydrobenzocyclohepten-5-one (**46**) by treating 2-methylcyclo-pentanone with $NaNH_2$ and reacting with bromobenzene.

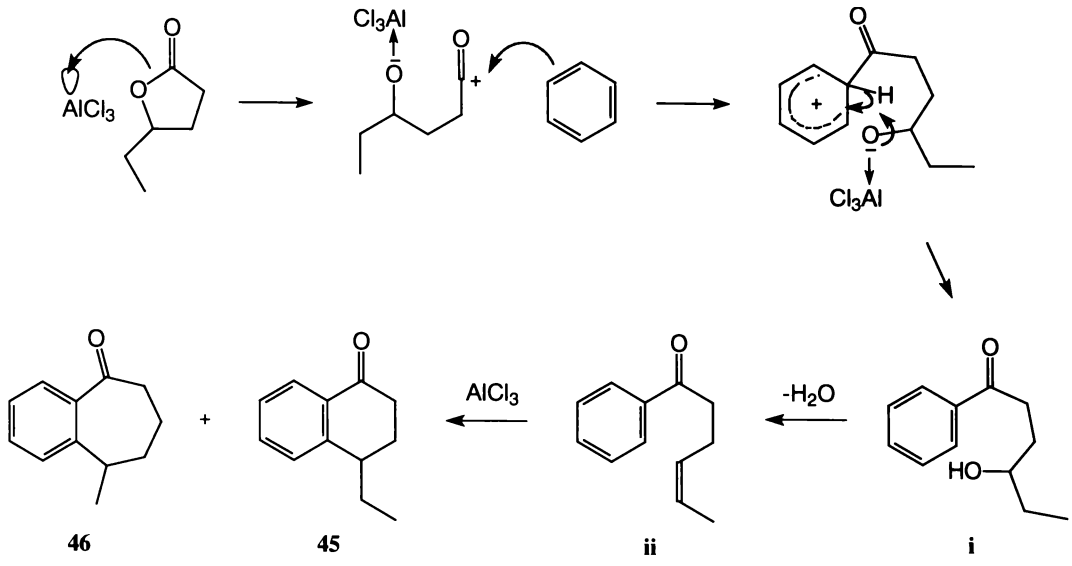


Figure 3.4: Proposed mechanistic pathway for the reaction of γ-caprolactone with benzene.

The 9-methyl-6,7,8,9-tetrahydrobenzocyclohepten-5-one (**46**) product is a suitable precursor to 5,9-dimethyl-6,7,8,9-tetrahydrobenzocyclohepten-5-ol (**44**), which can be viewed as a simplified 'analogue' of himachalol (Figure 3.5). Himachalol has been reported as an antifungal sesquiterpenoid (141) (see Section 1.6.2.2).

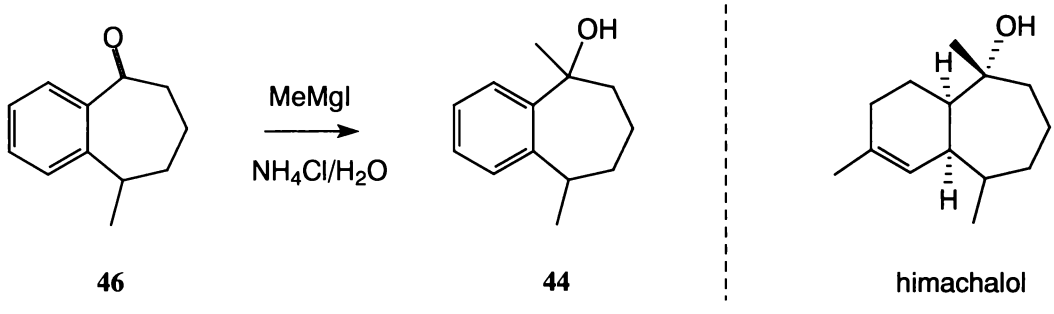


Figure 3.5: 9-Methyl-6,7,8,9-tetrahydrobenzocyclohepten-5-one (**46**) is a precursor to 5,9-dimethyl-6,7,8,9-tetrahydrobenzocyclohepten-5-ol (**44**), a simplified 'analogue' of himachalol.

The reaction of MeMgI with a 1:1.22 mixture of 4-ethyl-α-tetralone (**45**) and 9-methyl-6,7,8,9-tetrahydrobenzocyclohepten-5-one (**46**) gave the tertiary alcohols 4-ethyl-1-methyl-1,2,3,4-tetrahydronaphth-1-ol (**40**) and 5,9-dimethyl-6,7,8,9-tetrahydrobenzocyclohepten-5-ol (**44**) which were broadly separated by column chromatography on silica gel. Chromatography of the crude Grignard reaction product, eluting with 1:1 hexane:CH_2Cl_2 gave unreacted 9-methyl-6,7,8,9-tetrahydrobenzocyclohepten-5-one (**46**) (*ca.* 35% of the original amount in the

starting material). Hence the benzocycloheptenone (**46**) was less reactive than the tetralone (**45**) which reacted completely.

A column fraction obtained by eluting with 1:3 hexane:CH_2Cl_2 contained mainly 5,9-dimethyl-6,7,8,9-tetrahydrobenzocyclohepten-5-ol (**44**) (*ca.* 79% (**44**) and *ca.* 21% (**40**)). A column fraction obtained by eluting with 100% CH_2Cl_2 contained mainly 4-ethyl-1-methyl-1,2,3,4-tetrahydronaphth-1-ol (**40**) (*ca.* 85% (**40**) and *ca.* 15% (**44**)). Based on the mass and approximate composition of each fraction (estimated from GC/MS peak areas) yields of the two tertiary alcohols were calculated. 5,9-Dimethyl-6,7,8,9-tetrahydrobenzocyclohepten-5-ol (**44**) and 4-ethyl-1-methyl-1,2,3,4-tetrahydronaphth-1-ol (**40**) were produced in 34% and 40% yields respectively. In a subsequent chromatographic purification the tertiary alcohols were successful separated from each other to give small amounts of 5,9-dimethyl-6,7,8,9-tetrahydrobenzocyclohepten-5-ol (**44**) and 4-ethyl-1-methyl-1,2,3,4-tetrahydronaphth-1-ol (**40**) in *ca.* 93% and *ca.* 94% purity respectively (Section 7.2.7).

Spectroscopic evidence provided the basis for the structural identifications of 5,9-dimethyl-6,7,8,9-tetrahydrobenzocyclohepten-5-ol (**44**) and 4-ethyl-1-methyl-1,2,3,4-tetrahydronaphth-1-ol (**40**). The GC/MS and ^1H and ^{13}C NMR spectra of the above column fractions indicated that 5,9-dimethyl-6,7,8,9-tetrahydrobenzocyclohepten-5-ol (**44**) consisted of major and minor diastereomers in an approximate ratio of 7.6:1. The mass spectrum of the major diastereomer of (**44**) displayed a molecular ion of *m/z* 190 and base peak of *m/z* 175 formed by loss of a CH_3 radical through benzylic cleavage. Loss of water to give a *m/z* 172 ion was also observed. Interpretation of the 2D ^1H-^1H and ^{13}C-^1H correlation NMR spectra enabled complete assignment of the ^1H and ^{13}C NMR spectra of the major diastereomer of (**44**). The coupling correlations provided strong evidence for the 5,9-dimethyl-6,7,8,9-tetrahydrobenzocyclohepten-5-ol (**44**) structure (Table 3.15). The minor diastereomer of (**44**) was tentatively identified from the GC/MS results and minor signals in the ^1H NMR spectrum.

The GC/MS and ^1H and ^{13}C NMR spectroscopic results of the above column fractions indicated 4-ethyl-1-methyl-1,2,3,4-tetrahydronaphth-1-ol (**40**) consisted of major and minor diastereomers in an approximate ratio of 3:1. Both diastereomers co-eluted on GC/MS analysis, but separate signals in the ^1H and ^{13}C NMR spectra indicated the presence of diastereomers. Relative integrals of the H4 methine signal in the ^1H NMR spectrum were used

to estimate the ratio of two diastereomers. Structural identification of 4-ethyl-1-methyl-1,2,3,4-tetrahydronaphth-1-ol (**40**) was assisted by the interpretation of the 2D gCOSY and HMBC NMR spectra. The 2D spectra enabled the assignment of all ^{1}H and ^{13}C NMR signals associated with the major diastereomer (Table 3.15).

Table 3.15: gCOSY and HMBC correlations observed for major diastereomers of 4-ethyl-1-methyl-1,2,3,4-tetrahydronaphth-1-ol (**40**) and 5,9-dimethyl-6,7,8,9-tetrahydrobenzocyclohepten-5-ol (**44**) formed in the Grignard reaction.

4-ethyl-1-methyl-1,2,3,4-tetrahydronaphth-1-ol (40)			**5,9-dimethyl-6,7,8,9-tetrahydrobenzocyclohepten-5-ol (44)**		
Signal	**δ (ppm)**	**Correlated ^{1}H signals**	**Signal**	**δ (ppm)**	**Correlated ^{1}H signals**
H11	0.98	H10	H8	1.36	H8'
H2	1.86	H2'	H11	1.37	H9, H8'
H4	2.64	H3, H10	H7	1.64	H6', H7', H8'
C11	12.2	H10	H6	1.90	H6'
C3	23.9	H2, H2', H4, H10	C11	20.6	H9
C10	29.3	H3, H3', H4, H11	C7	23.1	H6, H6', H8, H8'
C9	31.1	H2, H2'	C10	29.6	H6'
C2	36.0	H3, H3', H4, H9	C9	34.9	H1, H11
C4	39.4	H2, H2', H3, H3', H5, H10, H11	C8	35.9	H6, H6', H7, H9, H11
			C6	42.3	H7, H7', H8, H10
C1	71.0	H2, H2', H3, H3', H8, H9	C5	76.4	H4, H6, H6', H7, H7', H10
C7	126.1	H5	C1	125.2	H3, H9
C8	126.2	H6	C4	125.8	H2
C6	127.0	H8	C3	126.1	H1
C5	128.4	H7	C2	127.0	H4
C4a	140.4	H3, H3', H4, H6, H8	C9a	141.1	H2, H4, H9, H11
C8a	142.8	H2, H2', H5, H7,H9	C4a	146.9	H1, H3, H6, H6', H10, H9

The mass spectrum of 4-ethyl-1-methyl-1,2,3,4-tetrahydronaphth-1-ol (**40**) exhibited a molecular ion of *m/z* 190 and a base peak of *m/z* 175. A prominent ion of *m/z* 143 was possibly formed by benzylic cleavage of the ethyl group following loss of water. The spectroscopic characterisation of 4-ethyl-1-methyl-1,2,3,4-tetrahydronaphth-1-ol (**40**) and 5,9-dimethyl-6,7,8,9-tetrahydrobenzocyclohepten-5-ol (**44**) was verified by high resolution GC/MS analysis. Masses of 190.1354 Da were recorded for both diastereomers of 5,9-

dimethyl-6,7,8,9-tetrahydrobenzocyclohepten-5-ol (**44**) ($C_{13}H_{18}O$ requires 190.1358 Da). The two diastereomers of 4-ethyl-1-methyl-1,2,3,4-tetrahydronaphth-1-ol (**40**) co-eluted on the GC/MS with a measured mass of 190.1361 Da ($C_{13}H_{18}O$ requires 190.1358 Da). The Grignard reaction results in diastereomeric forms of 4-ethyl-1-methyl-1,2,3,4-tetrahydronaphth-1-ol (**40**) and 5,9-dimethyl-6,7,8,9-tetrahydrobenzocyclohepten-5-ol (**44**) as the approaching nucleophile can attack either diastereotopic face of the substrates 4-ethyl-α-tetralone (**45**) and 9-methyl-6,7,8,9-tetrahydrobenzocyclohepten-5-one (**46**). This is the first reported synthesis of 4-ethyl-1-methyl-1,2,3,4-tetrahydronaphth-1-ol (**40**) and 5,9-dimethyl-6,7,8,9-tetrahydrobenzocyclohepten-5-ol (**44**).

If we knew, what we were doing it wouldn't be called research, would it?

Albert Einstein

Chapter 4: Synthesis of Tropolone Analogues

4.1 Introduction

The discussion in Section 1.7 identified some antifungal tropolones found in the heartwood of durable timbers. The thujaplicins (Figures 1.32 and 1.33, pages 53 and 54) were among the simplest of these tropolones. They possessed antifungal activities similar to that of the hydroxytropolones, the thujaplicinols (Figure 1.33) and superior to that of the methoxytropolone, pygmaein (Figure 1.33) (193, 205). It is proposed that synthetic analogues of α-thujaplicin and β-thujaplicin (**47**) could potentially be useful wood protection agents. Tropolone (**48**) is too water soluble to be suitable for wood preservation. The thujaplicins are sufficiently water soluble to be leached from western red cedar heartwood shakes (roofing shingles) during natural weathering (302). The depletion of the thujaplicins from the shakes as a result of weathering resulted in their eventual biodeterioration (302). For this reason increased lipidophilic character would be desirable in a thujaplicin analogue. 3-Alkyl and 4-alkyltropolones with various hydrocarbon chain lengths were identified as potential synthetic targets.

An additional benefit of adding extra hydrocarbon character to the tropolone molecule is a possible decline in mammalian toxicity. Tropolone (**48**) has reasonably mammalian toxicity with a LD_{50} of 233 mg kg^{-1} for subcutaneous exposure to mice (303). This places it at a similar level of toxicity to phenol (oral LD_{50}, mouse = 270 mg kg^{-1} (226)), but less toxic than some current compounds used in wood preservation (see Section 2.1). β-Thujaplicin (**47**) is considerably less toxic than tropolone with a subcutaneous LD_{50} (mouse) of 541 mg kg^{-1} (303). As discussed in Section 2.1, when phenol is alkylated with hydrocarbon chains of increasing chain length a decline in mammalian toxicity is observed. A similar trend could be expected for alkylated tropolones.

Three different approaches were investigated for the synthesis of alkyltropolones and related compounds. These included syntheses using the diacid tropolone 3-carboxy-4-

carboxymethyltropolone (**49**) as a starting material (Section 4.2), the attempted oxidation and decarboxylation of an alkyltrihydroxybenzocyclohepten-5-one (Section 4.4) and direct alkylation of tropolone (**48**) with diethyl acetals (Section 4.5). In addition to the synthesis of thujaplicin analogues the syntheses of various 4-styryltropolones were investigated. 4-Styryltropolones can be viewed as tropolone analogues of stilbenes. Stilbenes with hydroxyl and methoxyl substituents are known antifungal heartwood extractives (Section 1.5.2.3). Compounds containing a tropolone ring and partial stilbene structure were considered interesting target compounds for synthesis and antifungal activity testing. 4-Styryltropolones can be prepared from 3-carboxy-4-carboxymethyltropolone (**49**) and hence were discussed in Section 4.3 following the synthesis of alkyltropolones using this starting material. The results of syntheses towards the tropolone analogues described above are discussed in this Chapter while the results of antifungal activity testing against decay and sapstain fungi are discussed in Chapter 5.

4.2 Preparation of Alkyltropolones from 3-Carboxy-4-carboxymethyltropolone (49)

4.2.1 Preparation of 3-Carboxy-4-carboxymethyltropolone (49) and Derivatives

3-Carboxy-4-carboxymethyltropolone (**49**) was first prepared by Haworth *et al.* (304) by oxidation of the benzotropolone, purpurogallin (**50**) with oxygen under alkali conditions. Haworth and Hobson later improved the yield by using hydrogen peroxide as the oxidising agent (305). Purpurogallin (**50**) has been obtained in good yield by $NaIO_3$ oxidation of pyrogallol (306). Following the method of Evans and Dehn (306) crude purpurogallin (**50**) was obtained in a yield of 73%. Recrystallisation from anisole (305) gave pure purpurogallin (**50**) in 49% yield. The structure of purpurogallin (**50**) was verified from the MS and ^1H and ^{13}C NMR spectroscopic evidence. The mass spectrum showed a *m/z* 220 molecular ion base peak ($C_{11}H_8O_5^{\cdot+}$) and a prominent ion of *m/z* 192 ($M^{\cdot+}$-CO). Loss of carbon monoxide is characteristic of tropolones and benzotropolones (307). The most interesting feature of the ^1H NMR spectrum was a singlet observed at δ 15.2 ppm, which was attributed to the hydroxyl group *alpha* to C4 on account of ^{13}C-^1H correlations observed in the HMBC spectrum.

Efficient intra-molecular H-bonding with the C5 carbonyl results in deshielding of the phenolic H and causes its shift to higher frequency. The chemical shifts for C5 (154.6 ppm) and C6 (182.2 ppm) in the ^{13}C NMR spectrum reflect the non-tautomeric nature of benzotropolones.

3-carboxy-4-carboxymethyltropolone (**49**) was obtained by oxidation of purpurogallin (**50**) with alkaline hydrogen peroxide according to the method of Haworth and Hobson (305) with the modifications described in Section 7.2.8. When crude purpurogallin (**50**) was used 3-carboxy-4-carboxymethyltropolone (**49**) was obtained in yields of 20-30% at best. When recrystallised purpurogallin (**50**) was used 3-carboxy-4-carboxymethyltropolone (**49**) was obtained in yields of 50-55%. The structure was established from the MS and ^{1}H and ^{13}C NMR spectra. The mass spectrum was characterised by a *m/z* 224 molecular ion, prominent ions of *m/z* 180 (85%) and *m/z* 162 (95%) and a base peak of *m/z* 134. These fragments represent losses of CO_2, CO_2 + H_2O and CO_2, CO + H_2O respectively. ^{1}H-^{1}H and ^{13}C-^{1}H NMR correlations from the 2D NMR spectra are tabulated in Table 4.1.

Table 4.1 gCOSY, HMQC and HMBC correlations observed for 3-carboxy-4-carboxymethyltropolone (**49**).

Signal	**δ (ppm)**	**Correlated ^{1}H signals, δ (ppm)**	**^{13}C**	**δ (ppm)**	**Correlated ^{1}H signals**
H9	3.79	H5	C9	43.2	H5
H6	7.42	H5, H7	C7	118.9	H5
			C5	133.1	H7, H9
C9	43.2	H9, 3.79	C3	137.7	H5, H9
C7	118.9	H7, 7.29	C4	143.1	H6, H9
C5	133.1	H5, 7.17	C1	168.4	H6, H7
C6	136.7	H6, 7.42	C10	170.7	H9
			C2	173.5	H7

Methyltropolone (**51**) was prepared by decarboxylation of 3-carboxy-4-carboxymethyl-tropolone (**49**) (305) and was characterised from GC/MS and ^{1}H and ^{13}C NMR spectra. The mass spectrum exhibits an intense molecular ion of *m/z* 136 and fragments representing losses of CO and a CHO radical (*m/z* 108 and *m/z* 107). Intense molecular ions are characteristic of simple tropolones due to their ability to accommodate the positive charge by hydrogen transfer between the two oxygens (307). The almost identical chemical shifts of C1 and C2

(170.7 and 170.8 ppm) in the ^{13}C NMR spectrum are illustrative of the tautomeric nature of the tropolone.

The decarboxylation of 3-carboxy-4-carboxymethyltropolone (**49**) occurs readily on heating as the loss of CO_2 at C8 and C10 is facilitated by the α,β-double bond and β-carbonyl group in the case of C8 and the β,γ-double bond in the case of C10. Direct decarboxylations of β-keto acids and β,γ-unsaturated acids are believed to occur, in most cases, through a cyclic, six-centred mechanism (308). It is possible to postulate such a mechanism for the thermal decarboxylation of 3-carboxy-4-carboxymethyltropolone (Figure 4.1).

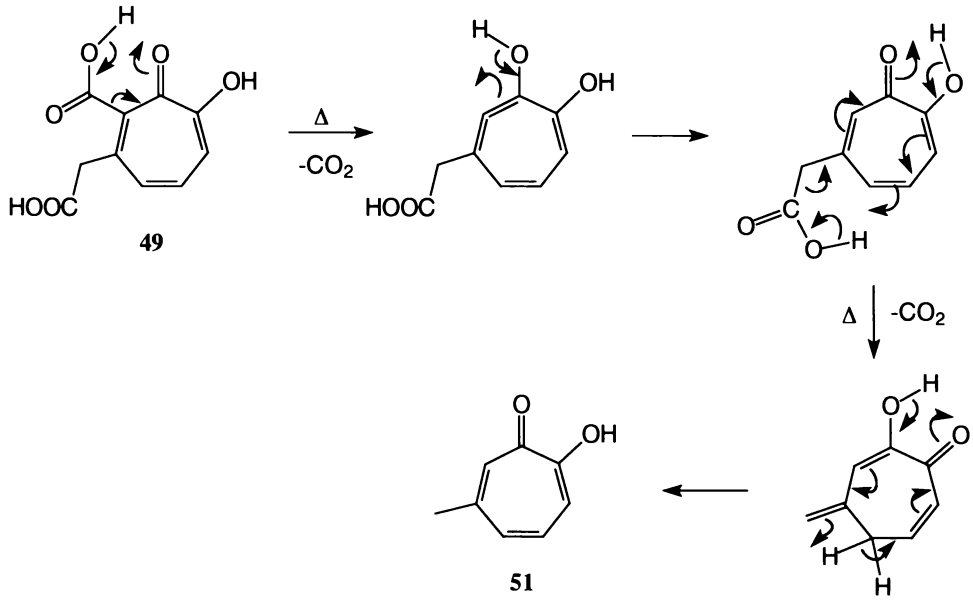


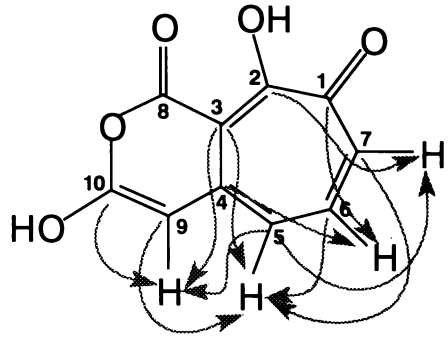
Figure 4.1: Proposed mechanism for decarboxylation of 3-carboxy-4-carboxymethyltropolone (**49**) to methyltropolone (**51**) on heating.

3-Carboxy-4-carboxymethyltropolone (**49**) was dehydrated with dicyclohexylcarbodiimide (DCC) in dioxane to give the tropolone anhydride (**52**) in 88% yield. This method was found to be superior to that of Haworth and Hobson (305), which consisted of warming the diacid tropolone (**49**) with concentrated sulphuric acid. The tropolone anhydride (**52**) was postulated by Crow *et al.* (309) to exist largely in its enol form (**52**). The spectroscopic evidence was consistent with an enol structure. The IR spectrum show ν O-H vibrations at 3400-3600 cm^{-1} in addition to the characteristic tropolone ν O-H vibration at *ca.* 3200 cm^{-1}. The ^{1}H NMR spectrum showed a singlet at δ 5.45 ppm (C9, 1H) and a broad singlet at δ 9.41 ppm (enol O-**H**). The ^{13}C NMR spectrum exhibits carbon signals at δ 97.4 ppm and δ 155.0 ppm, which are

assigned to C9 and C10 respectively. The ^{13}C-^{1}H NMR correlations of the HMBC spectrum confirmed an enol structure of type shown below (Table 4.2)[1]. The mass spectrum of the tropolone anhydride (**52**) showed a strong molecular ion of *m/z* 206 (56%) and ions associated with sequential losses of CO (*m/z* 178 (100%) and *m/z* 150 (62%)). Based on a mechanism proposed by Smith *et al.* (310) for the esterification of acids with DCC, it is proposed that the dehydration follows the pathway illustrated in Figure 4.2.

Table 4.2: HMBC correlations observed for the tropolone anhydride[1] (**52**).

^{13}C	ppm	Correlated ^{1}H signal
C9	97.4	H5
C3	100.6	H5, H9
C7	116.0	H5
C6	130.2	H5
C5	130.9	H7, H9
C4	149.9	H6
C2	152.9	H7
C10	155.0	H9
C1	171.0	H6



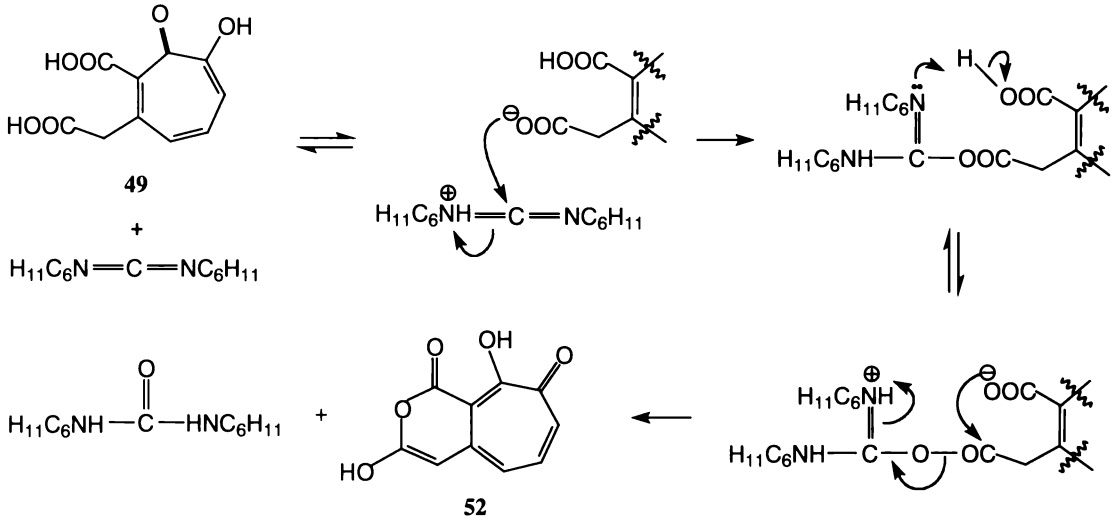


Figure 4.2: Proposed mechanistic pathway for the dehydration of 3-carboxy-4-carboxymethyl-tropolone (**49**) with DCC.

[1] The tropolone anhydride (**52**) was numbered for NMR assignment based on the system adopted for 3-carboxy-4-carboxymethyltropolone (**49**).

4.2.2 Preparation of Alkylcarboxytropolones

Crow *et al.* (309) reported that the tropolone anhydride (**52**) of 3-carboxy-4-carboxymethyltropolone when warmed with methanol afforded largely 3-methylcarboxy-4-carboxymethyltropolone. Heating this tropolone anhydride (**52**) in excess neat octanol yielded a mixture of two ester tropolones, 3-carboxy-4-octylcarboxymethyltropolone (**53**) and possibly 3-octylcarboxy-4-carboxymethyltropolone (**54**) which were partially decarboxylated depending on the severity of the reaction conditions (Section 7.2.8). The 4-carboxymethyl compound (**54**) appeared to be more susceptible to decarboxylation than the 3-carboxy compound (**53**) under the conditions employed. It was possible to isolate 3-octylcarboxy-4-methyltropolone (**55**) (formed by decarboxylation of (**54**)) and 3-carboxy-4-octylcarboxy-methyltropolone (**53**) from the product mixtures of certain reactions. As the possible product 3-octylcarboxy-4-carboxymethyltropolone (**54**) could not be isolated it was only tentatively identified based on ^{1}H and ^{13}C NMR signals in the spectra of a mixture of (**53**) and (**54**) (Section 7.2.8). 4-Octylcarboxymethyltropolone (**56**) was produced by pyridine catalysed decarboxylation of 3-carboxy-4-octylcarboxymethyltropolone (**53**). Hence reaction of the tropolone anhydride (**52**) with octanol and subsequent decarboxylations yielded three ester tropolones (**53**, **55** and **56**).

The ester tropolones (**53**, **55**, **56**) were characterised from their IR, MS and ^{1}H and ^{13}C NMR spectra. The IR spectrum of 3-carboxy-4-octylcarboxymethyltropolone (**53**) exhibited ν O-H vibrations at 3400-3600 cm^{-1} (acid) and 3200 cm^{-1} (tropolone). A strong band at 1729 cm^{-1} was the result of both the acid and ester carbonyl stretching vibrations. Four carbon signals, observed in the ^{13}C NMR spectrum in the δ 167.9-172.8 region were assignable to acid and ester carbonyls and the tautomeric tropolone carbonyls. The mass spectrum displays a *m/z* 336 $M^{\cdot+}$ ion ($C_{18}H_{24}O_6$) and a *m/z* 178 base peak. The base peak was possibly generated by allylic cleavage through the C9-C10 bond with H abstraction to give loss of $CH_3(CH_2)_7OCOH$.

The IR spectra of the decarboxylated tropolones (**55** and **56**) lacked ν O-H vibrations in the acid region and exhibited ν C=O vibrations for the esters at *ca.* 1732 cm^{-1}. The mass spectrum of 3-octylcarboxy-4-methyltropolone (**55**) displayed a molecular ion of *m/z* 292 $M^{\cdot+}$ ion, prominent ions of *m/z* 179 (loss of a C_8H_{17} radical) and 163 (loss of a OC_8H_{17} radical) and a base peak of *m/z* 134. The mass spectrum of 4-octylcarboxymethyltropolone (**56**) also

exhibited a molecular ion of *m/z* 292 and fragment ions of *m/z* 179 and 163. The base peak of *m/z* 136 is possibly formed by a McLafferty rearrangement at the carbonyl group resulting in the loss of a C_8H_{16} radical and formation of a 4-carboxymethyltropolone ion which in turn could decarboxylate to give a methyltropolone ion (*m/z* 136). A characteristic feature of the ^1H NMR spectrum of 3-octylcarboxy-4-methyltropolone (**55**) was a singlet at δ 2.40 ppm (H17, 3H). In the case of 4-octylcarboxymethyltropolone (**56**) a singlet at δ 7.30 ppm not observed in the ^1H NMR spectrum of (**53**) and assignable to H3 was an indicative feature. Both ester tropolones (**55, 56**) showed three carbon signals in the carbonyl region. Further evidence for the structures (**55**) and (**56**) was obtained through interpretation of the 2D ^1H-^1H and ^{13}C-^1H NMR spectra (selected HMBC correlations are shown Table 4.3).

Table 4.3: Selected HMBC correlations observed for 3-octylcarboxy-4-methyltropolone (**55**) and 4-octylcarboxymethyltropolone (**56**).

3-octylcarboxy-4-methyltropolone (55)			**4-octylcarboxymethyltropolone (56)**		
^{13}C	**δ (ppm)**	**Correlated ^1H signals**	**^{13}C**	**δ (ppm)**	**Correlated ^1H signals**
C17	24.7	H5	C8	46.0	H3, H5
C7	118.6	H5	C7	123.8	H5
C5	131.4	H7, H17	C3	125.2	H5, H8
C3	134.6	H5, H17	C5	129.9	H3, H7, H8
C6	136.4	H7	C6	137.1	H7
C4	146.5	H5, H6, H17	C4	144.9	H6, H8
C8	167.7	H9	C9	170.0	H8
C1	168.2	H7	C2	170.2	H3, H7
C2	171.4	H6	C1	171.8	H3, H6, H7

The reaction was carried out in the absence of a catalyst as the uncatalysed alcoholysis gives only monocarboxylic esters. Under acid or base catalysed conditions with excess octanol the possibly of forming diesterified products exists. The mechanism of alcoholysis involves a nucleophilic attack of the oxygen atom of the alcohol on either of the two anhydride carbonyl/enol carbons. The uncatalysed hydrolysis of anhydrides with H_2O follows a second order tetrahedral mechanism (311). It is possible that a similar mechanism operates in this reaction (Figure 4.3). The carboxyl ions that are generated (Figure 4.3) may undergo direct decarboxylation or gain protons to form acid functionalities. The C3 carboxylate ion may be more resistant to decarboxylation than the β-γ unsaturated carboxylate ion due to the

opportunity for sharing of the tropolonic proton, thus leading to the preference for 3-carboxy-4-octylcarboxymethyltropolone (**53**) and 3-octylcarboxy-4-methyltropolone (**55**) products.

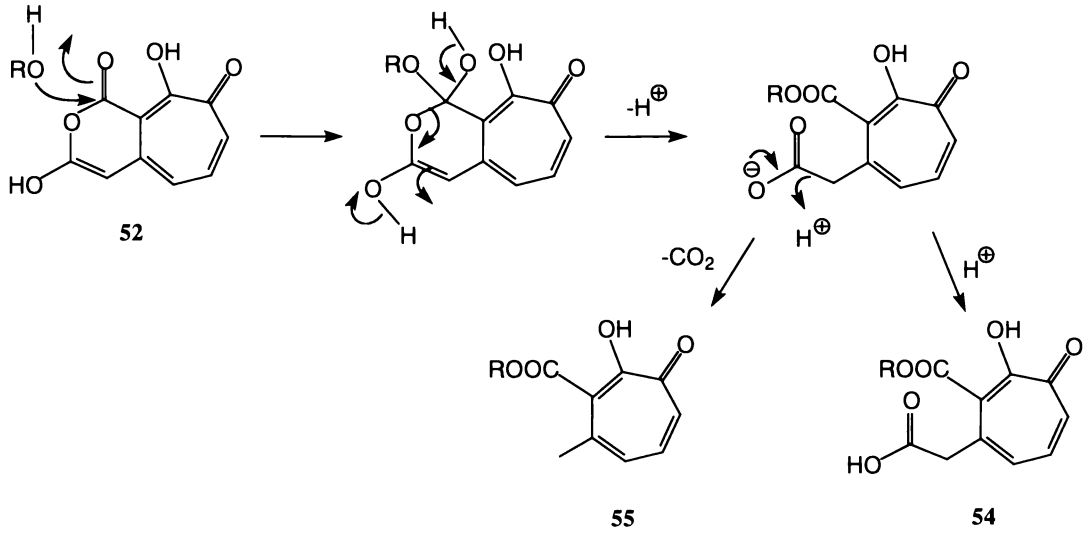


Figure 4.3: Proposed tetrahedral mechanism for alcoholysis of the anhydride (**52**) of 3-carboxy-4-carboxymethyltropolone (**49**) showing nucleophilic attack at the carbonyl group (R = C_8H_{17}).

4.2.3 Preparation of 4-Alkyltropolones

Nozoe *et al.* (312) and Crow *et al.* (309) have shown that the tropolone anhydride (**52**) undergoes a Perkin-like condensation reaction with aromatic aldehydes to give 3-carboxystyryltropolones (Figure 4.4). Besides vinylogs of aromatic aldehydes (*i.e.* ArCH=CHCHO) the Perkin condensation reaction is reported as unsuitable for aliphatic aldehydes (313). Nozoe *et al.* (314) discovered that an analogous condensation between the tropolone anhydride (**52**) and phenylacetaldehyde gave a lactone (Figure 4.4). Based on these findings the condensation of the tropolone anhydride (**52**) with aliphatic aldehydes appeared viable.

The condensation reaction was initially attempted by warming octanal and the tropolone anhydride (**52**) in a 3:1 mol ratio in pyridine. This reaction gave a good yield (83%) of 4-(2-hydroxynonyl)-3-carboxylic acid δ-lactone tropolone (**57**). Spectroscopic evidence for this structure included absorption maxima at 1723 cm^{-1} and 1617 cm^{-1} in the IR spectrum which were assignable to the lactone and tropolone carbonyl stretching vibrations. The MS spectrum exhibited a *m/z* 290 $M^{\bullet+}$ ($C_{16}H_{22}O_4$), a base peak of *m/z* 134 and significant ions of *m/z* 262,

191, 163, 148. Possible pathways to these ions are proposed in Figure 4.5. Loss of CO and cleavage of C_7H_{15} as depicted from the molecular ion could give the *m/z* 262 and *m/z* 191 ions respectively. Sequential losses of CO and a CHO radical from the *m/z* 191 fragment could lead to the *m/z* 163 and *m/z* 134 ions (Figure 4.5). The *m/z* 148 ion could have been generated by cleavage of CH_2CHO from the *m/z* 191 fragment (Figure 4.5).

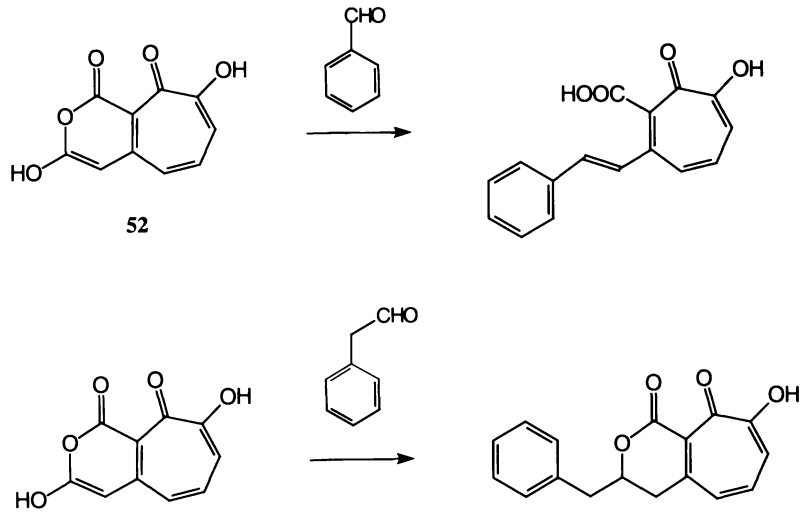


Figure 4.4: Perkin-like condensation reactions between the tropolone anhydride (**52**) and benzaldehyde and phenylacetaldehyde.

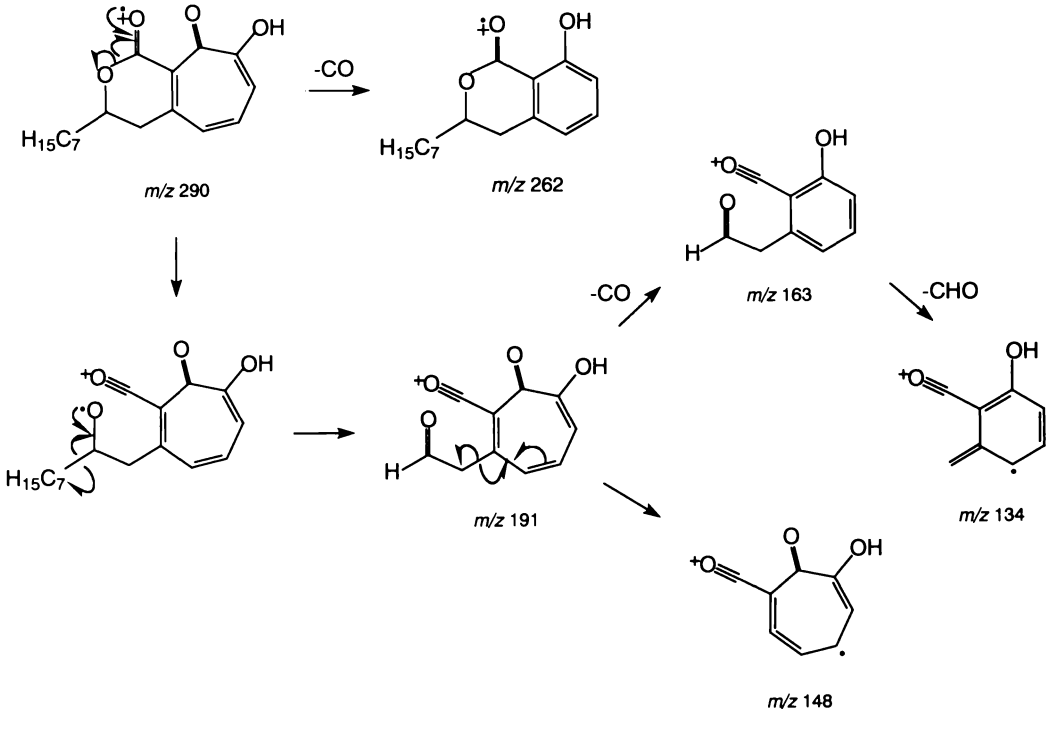


Figure 4.5: Proposed MS fragmentation of 4-(2-hydroxynonyl)-3-carboxylic acid δ-lactone tropolone (**57**).

Distinctive features of the ^{1}H NMR spectrum (in $CDCl_3$) were a one proton multiplet at δ 4.38 ppm (H10 methine proton) and two separate dd at δ 2.84 and 2.99 ppm for axial and equatorial protons at C9. The ^{13}C NMR spectrum showed a carbonyl signal at δ 167.2 ppm attributable to the lactone carbonyl and a signal at δ 78.6 ppm attributable to C10. The structure of the product was substantiated as 4-(2-hydroxynonyl)-3-carboxylic acid δ-lactone tropolone (**57**) by interpretation of ^{1}H-^{1}H and ^{13}C-^{1}H NMR correlations observed in the 2D gCOSY, HMQC and HMBC spectra (Table 4.4). The purity and structural identity of the product was confirmed by microanalytical analysis (Section 7.2.8). This is the first reported synthesis of 4-(2-hydroxynonyl)-3-carboxylic acid δ-lactone tropolone (**57**).

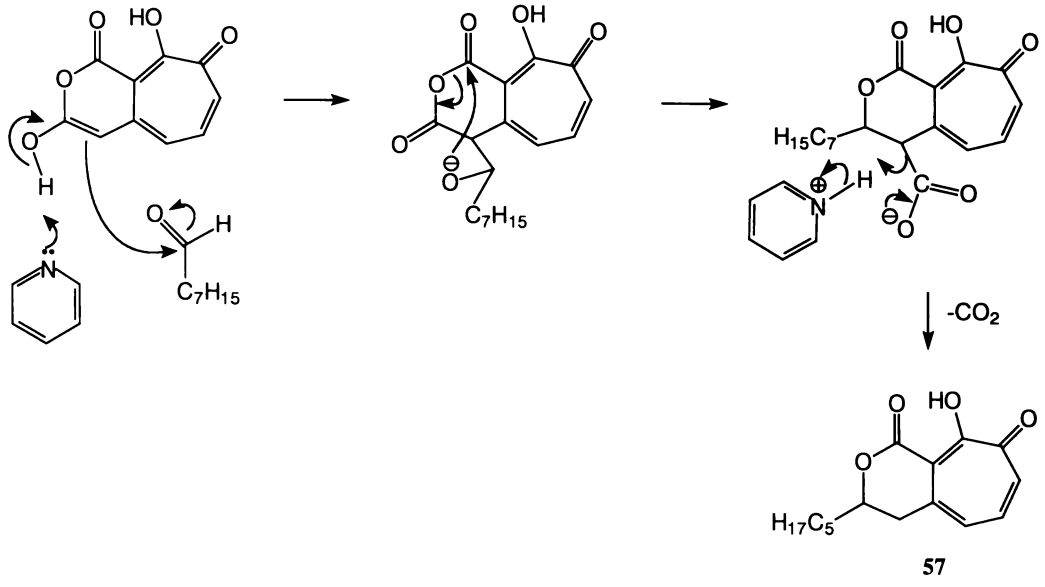
Table 4.4: Selected gCOSY, HMQC and HMBC correlations observed for 4-(2-hydroxynonyl)-3-carboxylic acid δ-lactone tropolone (**57**) in CD_3OD.

Signal	**δ (ppm)**	**Correlated ^{1}H signals, δ (ppm)**	**^{13}C**	**δ (ppm)**	**Correlated ^{1}H signals**
H9	3.02	H10	C11	35.2	H9
H10	4.48	H11	C9	40.4	H5
H6	7.41	H5, H7	C10	78.6	H9
			C7	118.8	H5
C9	40.4	H9, 3.02	C3	124.6	H5
C10	78.6	H10, 4.48	C5	128.8	H7
C7	118.8	H7, 7.15	C4	153.0	H6
C5	128.8	H5, 6.97	C1	171.1	H6, H7
C6	140.9	H6, 7.41	C2	176.2	H7

A hypothetical mechanism proposed for the formation of 4-(2-hydroxynonyl)-3-carboxylic acid δ-lactone tropolone (**57**) involves nucleophilic attack of the enolate (generated by enol proton abstraction by pyridine) on octanal (Figure 4.6). Nucleophilic attack of the resultant carbanion on the anhydride carbonyl results in lactone formation and subsequent ejection of CO_2 through the depicted pathway (Figure 4.6).

Although the lactone tropolone (**57**) was an intermediate worthy of antifungal activity testing, the objective was the development of a pathway to alkyltropolones analogous to β-thujaplicin (**47**). δ-Lactones upon saponification to γ-hydroxy acids are known to be unstable with respect to spontaneous relactonisation (315, 316). Nevertheless, the saponification of the lactone

tropolone (**57**) coupled with a decarboxylation was attempted with the aim of producing 4-(2-hydroxynonyl)tropolone.



57

Figure 4.6: Proposed mechanism for the condensation of the tropolone anhydride (**52**) with octanal in pyridine to give 4-(2-hydroxynonyl)-3-carboxylic acid δ-lactone tropolone (**57**).

A trial saponification of the lactone tropolone (**57**) gave a product in 15% yield which was tentatively identified as mainly 3-carboxy-4-(2-hydroxynonyl)tropolone (**58**) from the following spectoscopic evidence. The IR spectrum displayed acid ν O-H and ν C=O vibrations at 3400-3600 cm^{-1}, 2600 cm^{-1} and 1718 cm^{-1}. Two proton signals assigned to H9 in the ^{1}H NMR spectrum were observed at δ 2.74 ppm (dd) and δ 2.92 ppm (dd) and were coupled to each other (J = 13.4 Hz). On first inspection this seems unusual as the methylene protons are in a flexible chain and should be equivalent (rapid rotation leading to exchange of the two proton environments). The non-equivalence of the methylene protons can be explained in terms of the influence of the chiral group at C10 (Appendix F). The H10 methine signal found at δ 3.95 ppm was consistent with a α-proton in a secondary alcohol. The acid carbonyl signal in the ^{13}C NMR spectrum occurred at δ 168.4 ppm. 2D ^{1}H-^{1}H and ^{13}C-^{1}H NMR correlations (Appendix E, Table E1) provided further evidence for the proposed product 3-carboxy-4-(2-hydroxynonyl)tropolone (**58**).

The product obtained from the saponification reaction contained a small amount of the lactone tropolone (**57**), in addition to the proposed γ-hydroxy acid tropolone (**58**). The lactone

tropolone (**57**) was most likely formed by spontaneous lactonisation during workup rather than the result of incomplete saponification. The saponification was not optimised and complete characterisation of the 3-carboxy-4-(2-hydroxynonyl)tropolone (**58**) was not undertaken as the subsequent trial combined saponification/decarboxylation focused the research in a different direction.

In an attempt to product 4-(2-hydroxynonyl)tropolone, a combined saponification and decarboxylation was undertaken. The saponification gave a 1:1 mixture of the lactone tropolone (**57**) and γ-hydroxy acid tropolone (**58**) (yield = 48%) which upon Cu catalysed decarboxylation in quinoline gave crude 4-non-1-enyltropolone (**59**) in 19% yield. The lack of evidence of the lactone tropolone (**57**) in the ^{1}H and ^{13}C NMR spectra of the crude product suggested that (**57**) had been successfully decarboxylated under the reaction conditions. This result indicated that the saponification step was redundant. This was subsequently proved correct. Decarboxylation of the lactone tropolone (**57**) under the aforementioned conditions gave 4-non-1-enyltropolone (**59**), which on purification by column chromatography was obtained in an overall yield of 26%. The IR, GC/MS (TMSi derivative) and ^{1}H and ^{13}C spectroscopic evidence all pointed towards 4-non-1-enyltropolone (**59**) as the product. This was latter confirmed by comparison of the spectroscopic evidence with a completely characterised sample of 4-non-1-enyltropolone (**59**) (see below). Decarboxylation of 4-(2-hydroxynonyl)-3-carboxylic acid δ-lactone tropolone (**57**) gave only a poor yield of 4-non-1-enyltropolone (**59**) (26%). Optimisation of this reaction may have lead to a higher yield had this work been undertaken. However, at this time, an alternative method was discovered and the research was directed along this new approach.

During the attempted preparation of additional 4-(2-hydroxynonyl)-3-carboxylic acid δ-lactone tropolone (**57**) by the condensation of the tropolone anhydride (**52**) with octanal, the stoichiometry of the reaction was adjusted from that used originally (namely 1:3 anhydride:aldehyde) to 1:1 anhydride:aldehyde. Additionally, the temperature of the reaction was increased to 115°C rather than 60-65°C. The crude product of this condensation was a black oil rather than a brown solid, observed previously for this reaction. On purification by column chromatography, 4-non-1-enyltropolone (**59**) was obtained as an orange oil in 49% yield. Repeating the reaction using the same stoichiometry at 60-65°C also gave 4-non-1-enyltropolone (**59**). When the reaction was repeated using the original 1:3 stoichiometry at 60-

65°C crude 4-(2-hydroxynonyl)-3-carboxylic acid δ-lactone tropolone (**57**) was obtained in 83% yield and no 4-non-1-enyltropolone (**59**) was detected. The fact that the outcome of the condensation was dependent on the stoichiometry of the reactants was an unexpected result!

The spectroscopic evidence for 4-non-1-enyltropolone (**59**) as the product of the 1:1 condensation was as follows. The IR spectrum of the product exhibited ν O-H and ν C=O vibrations at maxima of 3200 cm^{-1} and 1601 cm^{-1}. The GC/MS spectrum displayed a molecular ion of *m/z* 246 and a base peak ion of *m/z* 120. The base peak ion could have been formed by two McLafferty rearrangement as depicted in Figure 4.8. The GC/MS spectrum of the TMSi derivative contained a molecular ion of *m/z* 318 (246 ($C_{16}H_{21}O_2$) + 73 ($SiMe_3$)) and a base peak of *m/z* 303 ($M^{\bullet+}$-CH_3). Two olefinic proton signals at δ 6.34 and 6.43 ppm assignable to H8 and H9 and a singlet at δ 7.44 ppm attributable to H3 in 4-non-1-enyltropolone (**59**) were observed in the ^1H NMR spectrum. Distinguishing features of the ^{13}C NMR spectrum were two olefinic carbon signals at δ 132.0 and 138.2 ppm (C8 and C9) and only two carbon signals in the carbonyl region (C1 and C2). The ^1H and ^{13}C NMR spectra were assigned with assistance from the 2D ^1H-^1H and ^{13}C-^1H correlation NMR spectra (Table 4.5) which provided strong evidence for 4-non-1-enyltropolone (**59**) as the product of the 1:1 condensation of tropolone anhydride (**52**) and octanal.

Table 4.5: Selected gCOSY, HMQC and HMBC correlations observed for 4-non-1-enyltropolone (**59**).

Signal	**δ (ppm)**	**Correlated ^1H signals, δ (ppm)**	**^{13}C**	**δ (ppm)**	**Correlated ^1H signals**
H8	6.34	H9	C11	29.0	H9, H10
H5	7.00	H3, H6	C10	33.0	H8, H9, H11
H7	7.17	H6	C3	119.7	H5, H8
			C7	123.8	H5, H6
C11	29.0	H11, 1.46	C5	126.8	H3, H7, H8
C10	33.0	H10, 2.24	C8	132.0	H3, H5, H9, H10
C3	119.7	H3, 7.44	C6	137.3	H7
C7	123.8	H7, 7.17	C9	138.2	H8, H10, H11
C5	126.8	H5, 7.00	C4	147.2	H3, H6, H8, H9
C8	132.0	H8, 6.34	C2	168.9	H3, H7
C6	137.3	H6, 7.31	C1	172.8	H3, H6, H7
C9	138.2	H9, 6.43			

High resolution MS data (found 246.1615 Da; $C_{16}H_{22}O_2$ requires 246.1620 Da) verified the product as 4-non-1-enyltropolone (**59**). This is the first reported synthesis of 4-non-1-enyl-tropolone (**59**).

The formation of 4-non-1-enyltropolone (**59**) in the 1:1 condensation of tropolone anhydride (**52**) and octanal compared to the lactone tropolone (**57**) observed in the 1:3 condensation is most probably the result of base catalysed opening of the lactone ring. Abstraction of a H9 proton by pyridine could result in formation of a tropolone carboxylate, which then undergoes decarboxylation (Figure 4.7). It is unclear why this occurs in the 1:1 condensation reaction and not the 1:3 condensation.

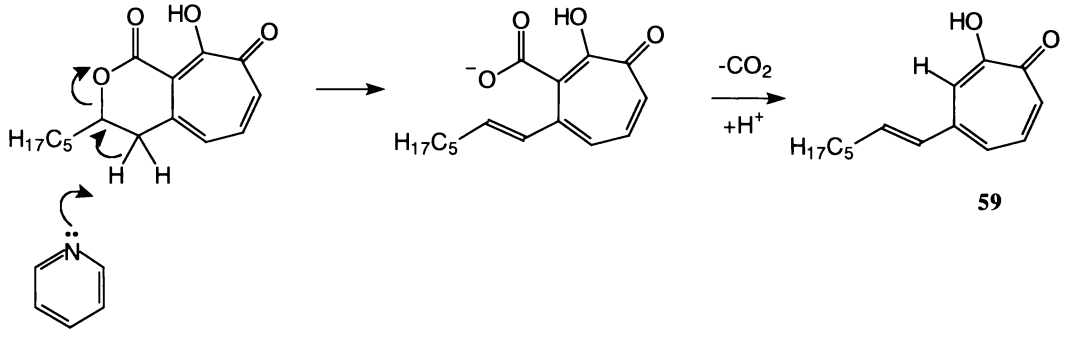


Figure 4.7: Proposed mechanism for the opening of the lactone of 4-(2-hydroxynonyl)-3-carboxylic acid δ-lactone tropolone (**57**) to give 4-non-1-enyltropolone (**59**) in the 1:1 condensation of the tropolone anhydride (**52**) with octanal in pyridine.

Having obtained 4-non-1-enyltropolone (**59**) in moderate yield (49%) the target compound, 4-nonyltropolone (**60**), could be obtained by catalytic hydrogenation of the double bond. According to Gardner (184) the tropolone nucleus is not reduced by hydrogen in the presence of palladium catalysts. The hydrogenation carried out in ethyl acetate over 10% Pd/C gave 4-nonyltropolone (**60**) in 82% yield. The mass spectrum of the product displayed a molecular ion of *m/z* 248 and a base peak ion of *m/z* 136. The base peak is explicable in terms of an allylic cleavage through the C9-C10 bond with proton abstraction from the C_8H_{17} leaving group as depicted in Figure 4.8. The ^1H and ^{13}C NMR spectra in conjunction with coupling correlations observed in 2D ^1H-^1H and ^{13}C-^1H correlation NMR spectra provided convincing evidence for 4-nonyltropolone (**60**) as the product (Table 4.6). High resolution MS data (found 248.17761 Da; $C_{16}H_{24}O_2$ requires 248.17763 Da) was consistent with identification of

the product as 4-nonyltropolone (**60**). This is the first reported synthesis of 4-nonyltropolone (**60**).

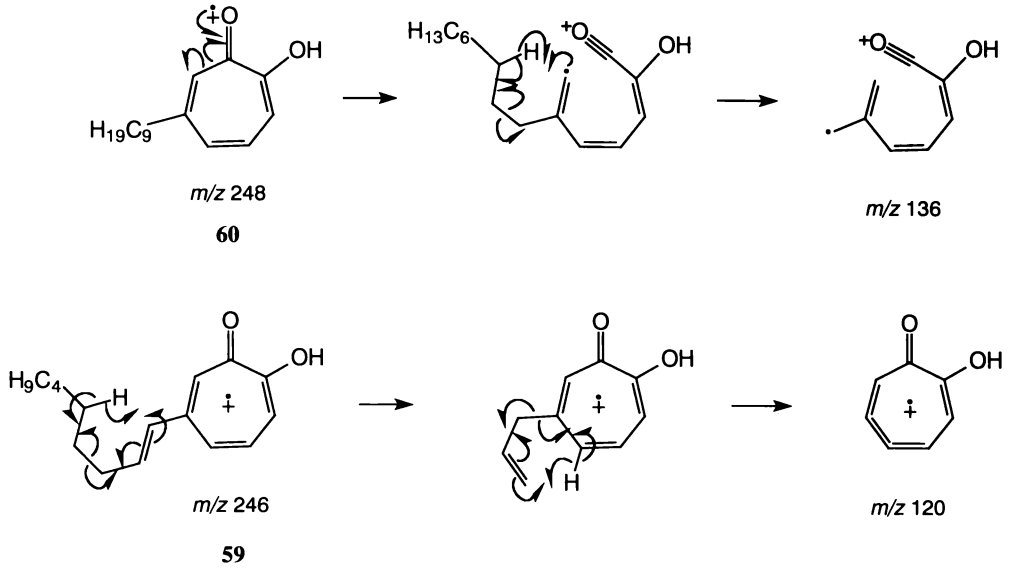


Figure 4.8: Proposed fragmentation pathways to base peak GC/MS ions for 4-nonyltropolone (**60**) and 4-non-1-enyltropolone (**59**).

Table 4.6: gCOSY, HMQC and HMBC correlations observed for 4-nonyltropolone (**60**).

Signal	**δ (ppm)**	**Correlated ^{1}H signal, δ (ppm)**	**^{13}C, δ (ppm)**	**Correlated ^{1}H signals**
H16	0.86	H14, H15	C16	H15
H9	1.62	H8, H10	C15	H14, H16
H6	7.24	H5, H7	C10, 29.1	H8
			C11, 29.2	H9
			C12, 29.4	H11
C16	14.0	H16, 0.86	C13, 29.4	H12
C15	22.6	H15, 1.25	C9	H8
C10-C13	29.1-29.4	H10-H13, 1.22-1.38	C14	H13, H15, H16
C9	31.3	H9, 1.64	C8	H3, H5, H9
C14	31.8	H14, 1.24	C7	H5
C8	41.4	H8, 2.59	C3	H5, H8
C7	122.1	H7, 7.18	C5	H3, H7, H8
C3	125.2	H3, 7.25	C6	H7
C5	129.4	H5, 6.88	C4, 154.6	H6, H8
C6	136.9	H6, 7.24	C2, 170.9	H7
			C1, 171.0	H3

Having established a viable pathway to 4-nonyltropolone (**60**) the versatility of the pathway was investigated by attempting the preparation of tropolones with shorter alkyl chains. The tropolone anhydride (**52**) was successfully condensed with hexanal and butanal in pyridine to give 4-hept-1-enyltropolone (**61**) and 4-pent-1-enyltropolone (**62**) in yields of 43% and 51% respectively after chromatographic purification. These structures were confirmed as the products from GC/MS, ^{1}H and ^{13}C NMR and high resolution probe MS evidence. The GC/MS spectrum of 4-hept-1-enyltropolone (**61**) showed at molecular ion of *m/z* 218 and base peak of *m/z* 120, which was formed by the same fragmentation pathway as that depicted for the base peak of 4-non-1-enyltropolone (**59**) (Figure 4.8). The GC/MS spectrum of 4-pent-1-enyltropolone (**62**) showed a molecular ion of *m/z* 190 and base peak of *m/z* 133. This base peak is possibly formed through losses of CO and a CH_3CH_2 radical. The GC/MS chromatograms of TMSi derivatives of (**61**) and (**62**) consisted of a single peak which exhibited molecular ions of *m/z* 290 ($C_{14}H_{17}O_2$ + $SiMe_3$) and 262 ($C_{12}H_{13}O_2$ + $SiMe_3$) respectively.

The signals in the ^{1}H and ^{13}C NMR spectra of 4-hept-1-enyltropolone (**61**) and 4-pent-1-enyltropolone (**62**) closely match corresponding signals in the spectra of 4-non-1-enyltropolone (**59**) (Section 7.2.8). ^{1}H-^{1}H and ^{13}C-^{1}H correlations in the 2D NMR spectra assisted with signal assignment and provided further evidence for the structures (**61**) and (**62**). High resolution MS analysis verified the products as 4-hept-1-enyltropolone (**61**) (found 218.1306 Da; $C_{14}H_{18}O_2$ requires 218.1307 Da) and 4-pent-1-enyltropolone (**62**) (found 190.0991 Da; $C_{12}H_{14}O_2$ requires 190.0994 Da).

4-Hept-1-enyltropolone (**61**) and 4-pent-1-enyltropolone (**62**) were reduced by Pd/C catalysed hydrogenation, in the same manner as 4-non-1-enyltropolone (**59**), to give 4-heptyltropolone (**63**) and 4-pentyltropolone (**64**) in yields of 81% and 83% respectively. The GC/MS spectra of 4-heptyltropolone (**63**) and 4-pentyltropolone (**64**) exhibited molecular ions of *m/z* 220 and *m/z* 192 respectively with base peaks of *m/z* 136. As with 4-nonyltropolone (**60**) this base peak is formed by allylic cleavage with a proton abstracted from the alkyl leaving group as depicted in Figure 4.8. The ^{1}H and ^{13}C NMR spectra were consistent with the alkyltropolone structures (**63**) and (**64**). Overlapping signals were resolved by referring to the 2D ^{1}H-^{1}H and ^{13}C-^{1}H correlation spectra (Appendix E, Tables E2 and E3). 4-Heptyltropolone (**63**) and 4-pentyltropolone (**64**) were further characterised by high resolution MS. Masses of 220.1460

Da ($C_{14}H_{20}O_2$ requires 220.1463 Da) and 192.1152 Da ($C_{12}H_{16}O_2$ requires 192.1150 Da) were recorded for 4-heptyltropolone (**63**) and 4-pentyltropolone (**64**) respectively. 4-Heptyltropolone (**63**) and 4-pentyltropolone (**64**) are novel compounds, this is their first reported synthesis.

4.3 Preparation of 4-Styryltropolones

The anhydride (**52**) of 3-carboxy-4-carboxymethyltropolone (**49**) can undergo condensation with aromatic aldehydes to give 3-carboxy-4-styryltropolones (Figure 4.4). The interesting feature of these compounds is that they are tropolone analogues of stilbenes. A compound with a tropolone ring and partial stilbene structure may have interesting antifungal properties. The synthesis of several styryltropolones is described in this Section.

Tarbell *et al.* (317) synthesised 3-carboxy-4-styryltropolone (**65**) in 59% yield by condensing benzaldehyde with the anhydride (**52**) of 3-carboxy-4-carboxymethyltropolone (**49**) (prepared *in situ* from (**49**) by reaction with acetic anhydride) at 50-55°C in pyridine. This method was followed with the exception that the tropolone anhydride (**52**) was prepared separately from the diacid tropolone (**49**) by dehydration with DCC (Section 4.2.1). 3-Carboxy-4-styryltropolone (**65**) was thus obtained in 31% yield. The IR spectrum of the product contained characteristic carboxylic acid ν O-H and ν C=O vibrations. The mass spectrum exhibited a molecular ion of *m/z* 268, which was also the base peak. An ion of *m/z* 165 (95% relative intensity) can be rationalised in terms of cleavage of the styryl group (MW = 103 Da) from the molecular ion. Other major fragment ions can be explained in terms of losses of CHO and COOH.

The ^{1}H NMR spectrum consisted of a complex mixture of overlapping signals in the δ 7.2-7.7 ppm region. With the assistance of 2D NMR experiments, it was possible to resolve these signals and assign them with confidence (Appendix H). The ^{13}C NMR spectrum (Section 7.2.9) showed olefinic carbon signals at δ 127.6 and 137.6 ppm. The acid carbonyl signal C8 was found to overlap with that of C1. The mechanism for the condensation is analogous to that described for the preparation of 4-non-1-enyltropolone (**59**) (Figures 4.5 and 4.6). The only difference between the two reactions is that at 50-55°C decarboxylation was not observed following condensation with benzaldehyde as it was in the reaction with octanal at

60-65°C. However, when the reaction was carried out at 115°C decarboxylation was observed.

The condensation of the tropolone anhydride (**52**) with benzaldehyde in refluxing pyridine gave 4-styryltropolone (**66**) in 59% yield. The condensation product was characterised from the spectroscopic evidence. The absence of carboxylic acid ν O-H and ν C=O vibrations in the IR spectrum provided evidence of decarboxylation. The GC/MS spectrum of the TMSi derivative exhibited a molecular ion of *m/z* 296 ($C_{15}H_{11}O_2$ + $SiMe_3$) and base peak of *m/z* 281 ($M^{\cdot+}$-CH_3). The positive and negative ion APCI (atmospheric pressure chemical ionisation) mass spectra displayed molecular ions, plus and minus a proton, of *m/z* 225 ($C_{15}H_{12}O_2$ + H) and *m/z* 223 respectively. Due to the complex overlapping nature of the signals in ^1H and ^{13}C NMR spectra, signal assignments were made with the assistance of the 2D ^1H-^1H and ^{13}C-^1H correlation NMR spectra (Table 4.7). The structural identity of the product was confirmed as 4-styryltropolone (**66**) from high resolution MS data (found 224.0838 Da; $C_{15}H_{12}O_2$ requires 224.0837 Da).

Table 4.7: gCOSY, HMQC and HMBC correlations observed for 4-styryltropolone (**66**).

Signal	**δ (ppm)**	**Correlated ^1H signals, δ (ppm)**	**^{13}C, δ (ppm)**	**Correlated ^1H signals**
H7	7.20	H6	C3	H5, H8
H8	7.32	H9	C7	H5
H5	7.35	H3, H6	C5	H3, H7, H8
H3' & H5'	7.43	H2' & H5', H4'	C2' & C6'	H9, H6' & H2', H4'
			C3' & C5'	H5' & H3'
C3	118.7	H3, 7.66	C4'	H2' & H6'
C7	125.0	H7, 7.20	C8	H3, H5, H9
C5	127.5	H5, 7.35	C9	H8, H2' & H6'
C2' & C6'	127.7	H2' & H6', 7.70	C1', 136.9	H8, H3' & H5'
C3' & C5'	129.2	H3' & H5', 7.43	C4, 146.6	H3, H6, H8
C4'	129.2	H4', 7.37	C2, 168.6	H3, H7
C8	130.8	H8, 7.32	C1, 173.9	H3, H6, H7
C9	134.5	H9, 7.52		
C6	137.8	H6, 7.50		

4-Styryltropolone (**66**) is a known compound. Tarbell *et al.* (317) prepared 4-styryltropolone (**66**) from 3-carboxy-4-styryltropolone (**65**) by Cu catalysed decarboxylation in quinoline at

150-160°C. This separate decarboxylation reaction was found to be redundant simply by carrying out the initial condensation at a higher temperature (namely 115°C *cf.* 50-55°C). As in the case of the condensations with aliphatic aldehydes, the β-tropolone carbonyl and α-double bond would facilitate the pyridine catalysed decarboxylation.

Having successfully synthesised 4-styryltropolone (**66**), the utility of the method was investigated by attempting the synthesis of several tropolone stilbene analogues. Heartwood stilbenes are styrylbenzenes with hydroxyl and methoxyl substituents in various positions on the A and B rings (Section 1.5.2.3). Hence styryltropolones containing hydroxyl and methoxyl groups on the benzene ring were identified as a synthetic targets. The attempted pyridine catalysed condensations of the tropolone anhydride (**52**) with 2,4-dihydroxybenzaldehyde and 3,4-dihydroxybenzaldehyde were unsuccessful. In both cases the benzaldehyde was recovered unreacted from the workup mixture. Nozoe *et al.* (183) and Scott *et al.* (318) have reported that oxygenated phenylacetaldehydes (such as 3,4,5-trimethoxyphenylacetaldehyde) fail to condense with the tropolone anhydride (**52**) under base catalysed conditions. Scott *et al.* (318), however, found that by simply heating the tropolone anhydride (**52**) with an excess of 3,4,5-trimethoxyphenylacetaldehyde at 100°C the condensation product could be obtained in good yield (78%).

Based on this precedent, the uncatalysed condensation of the tropolone anhydride (**52**) with 3,4,5-trimethoxybenzaldehyde was attempted. Heating the two reactants together at 100°C gave 3-carboxy-4-(3',4',5'-trimethoxy)styryltropolone (**67**) in 57% yield. The product was characterised from the IR, APCI MS and ^{1}H and ^{13}C NMR spectroscopic evidence including 2D ^{1}H-^{1}H and ^{13}C-^{1}H correlation NMR spectra (Section 7.2.9). The positive ion APCI mass spectrum displayed a molecular ion + H of *m/z* 359 ($C_{19}H_{18}O_7$ + H) and base peak of *m/z* 315 ($(M+H)^+$ -CO_2). The negative ion APCI mass spectrum displayed a molecular ion - H of *m/z* 357 ($C_{19}H_{18}O_7$ - H) and base peak of *m/z* 313 ($(M-H)^-$-CO_2). The synthesis of 3-carboxy-4-(3',4',5'-trimethoxy)styryltropolone (**67**) has been referred to before in a review by Nozoe (183), although no experimental characterisation data was provided.

The decarboxylation of 3-carboxy-4-(3',4',5'-trimethoxy)styryltropolone (**67**) was achieved by the known method of aromatic decarboxylation, namely heating in quinoline with a Cu catalyst (319). The product was obtained in 59% yield and was verified as 4-(3',4',5'-

trimethoxy)styryltropolone (**68**) from spectroscopic evidence and microanalytical data (Section 7.2.9). The IR spectrum lacked the carboxylic acid ν O-H and ν C=O vibrations which were a characteristic feature of the spectrum of the 3-carboxy precursor (**67**). The positive and negative ion APCI mass spectra displayed molecular ions, plus and minus a proton, of *m/z* 315 ($C_{18}H_{18}O_5$ + H) and 313 respectively. The positive ion MS^2 spectrum of the $(M+H)^+$ ion exhibited fragments due to losses of a CH_3 radical, H_2O and OCH_3. The signals observed in the ^1H and ^{13}C NMR spectra of the product were assignable to the 4-(3',4',5'-trimethoxy)styryltropolone (**68**) structure, which was further substantiated by coupling correlations observed in the gCOSY, HMQC and HMBC spectra (Table 4.8). This is the first reported synthesis of 4-(3',4',5'-trimethoxy)styryltropolone (**68**).

Table 4.8: gCOSY, HMQC and HMBC correlations observed for 4-(3',4',5'-trimethoxy)-styryltropolone (**68**).

Signal	**δ (ppm)**	**Correlated ^1H signals, δ (ppm)**	**^{13}C, δ (ppm)**	**Correlated ^1H signals**
H7' & H9'	3.90	H2' & H6'	C2' & C6'	H9, H6' & H2'
H9	7.45	H2' & H6', H8	C3	H5, H8
H6	7.48	H5, H7	C7	H5
H3	7.62	H5	C5	H3, H7, H8
C7' & C9'	56.1	H7' & H9', 3.90	C8	H3, H5, H9
C8'	60.2	H8', 3.80	C1', 132.5	H8, H2' & H6'
C2' & C6'	105.6	H2' & H6', 7.06	C9	H8, H2' & H6'
C3	118.9	H3, 7.62	C4', 140.1	H2' & H6', H8'
C7	124.4	H7, 7.16	C4, 146.9	H3, H6, H8, H9
C5	127.1	H5, 7.33	C3' & C5', 154.2	H2' & H6', H7' & H9'
C8	130.0	H8, 7.28	C2, 168.8	H3, H7
C9	134.8	H9, 7.45	C1, 173.6	H3, H6, H7
C6	137.6	H6, 7.48		

The advantage of the pyridine catalysed condensation over the uncatalysed reaction, discussed above, is that by carrying the reaction out in refluxing pyridine the decarboxylated product is formed, thus eliminating the need for a separate decarboxylation step. As previously mentioned, the pyridine catalysed reaction of the tropolone anhydride (**52**) with 3,4-dihydroxybenzaldehyde was unsuccessful. Tarbell *et al.* (317), however, have reported the successful preparation of 3-carboxy-4-(3',4'-dimethoxy)styryltropolone by condensation with 3,4-dimethoxybenzaldehyde under base-catalysed conditions. With this in mind the pyridine

catalysed condensation of the tropolone anhydride (**52**) with 3,5-dimethoxybenzaldehyde was attempted. The condensation yielded a product in 42% yield which was characterised as 4-(3',5'-dimethoxy)styryltropolone (**69**) from IR, APCI MS, ^{1}H and ^{13}C NMR spectra and microanalytical data (Section 7.2.9). The positive and negative ion APCI mass spectra displayed molecular ions, plus and minus a proton, of *m/z* 285 ($C_{17}H_{16}O_4$ + H) and 283 respectively. The gCOSY, HMQC and HMBC correlations are shown below in Table 4.9. The purity of (**69**) was confirmed by elemental analysis (Section 7.2.9). This is the first reported synthesis of 4-(3',5'-dimethoxy)styryltropolone (**69**).

Table 4.9: gCOSY, HMQC and HMBC correlations observed for 4-(3',5'-dimethoxy)styryl-tropolone (**69**).

Signal	δ (ppm)	Correlated ^{1}H signal, δ (ppm)	^{13}C, δ (ppm)	Correlated ^{1}H signal
H4'	6.45	H2' & H6'	C4'	H2' & H6'
H8	7.01	H9	C2' & C6'	H9, H4', H6' & H2'
H6	7.38	H5, H7	C3	H5, H8
H3	7.59	H5	C7	H5
			C5	H3, H7, H8
C7' & C8'	55.4	H7', H8', 3.83	C8	H3, H5, H9
C4'	101.2	C4', 6.45	C9	H8, H2' & H6'
C2' & C6'	105.2	H2' & H6', 6.66	C1', 137.8	H8
C3	118.7	H3, 7.59	C4, 146.2	H3, H6, H8, H9
C7	125.0	H7, 7.21	C3' & C5', 161.0	H4', H2', H6', H7', H8'
C5	127.4	H5, 7.15	C2, 168.1	H3, H7
C8	130.7	H8, 7.01	C1, 173.6	H3, H6, H7
C9	134.5	H9, 7.17		
C6	137.4	H6, 7.38		

The successful condensation of the tropolone anhydride (**52**) with a tropolone aldehyde would provide a pathway to a tropolone-ethylene-tropolone (**70**), a ditropolone analogue of hydroxystilbenes. It would be interesting to compare the antifungal properties of such a compound with those of natural tropolones and tropolone stilbenes. To explore this synthetic possibility, it was first necessary to prepare 4-formyltropolone (**71**). Tarbell *et al.* (317) discovered that osmium tetraoxide catalysed periodate oxidation of 4-styryltropolone (**66**) gave 4-formyltropolone (**71**) in 84% yield. Following this method 4-formyltropolone (**71**) was obtained in 74% yield after purification by vacuum sublimation. The IR spectrum of

4-formyltropolone (**71**) showed carbonyl stretching vibrations at 1684 cm^{-1} and 1615 cm^{-1}, which were assigned to the aldehyde and tropolone functionalities. The GC/MS spectrum of the 4-formyltropolone (**71**) displayed a molecular ion of *m/z* 150, which was also the base peak. Fragment ions of *m/z* 121 and *m/z* 93, representing losses of CHO and CO + CHO respectively, were also prominent. The ^{1}H and ^{13}C NMR spectra revealed aldehyde proton and carbon signals at δ 9.87 ppm and δ 192.0 ppm respectively. The remaining signals were assigned by referring to the ^{1}H-^{1}H and ^{13}C-^{1}H correlations observed in the 2D gCOSY, HMQC and HMBC NMR spectra (Table 4.10).

Table 4.10: gCOSY, HMQC and HMBC correlations observed for 4-formyltropolone (**71**).

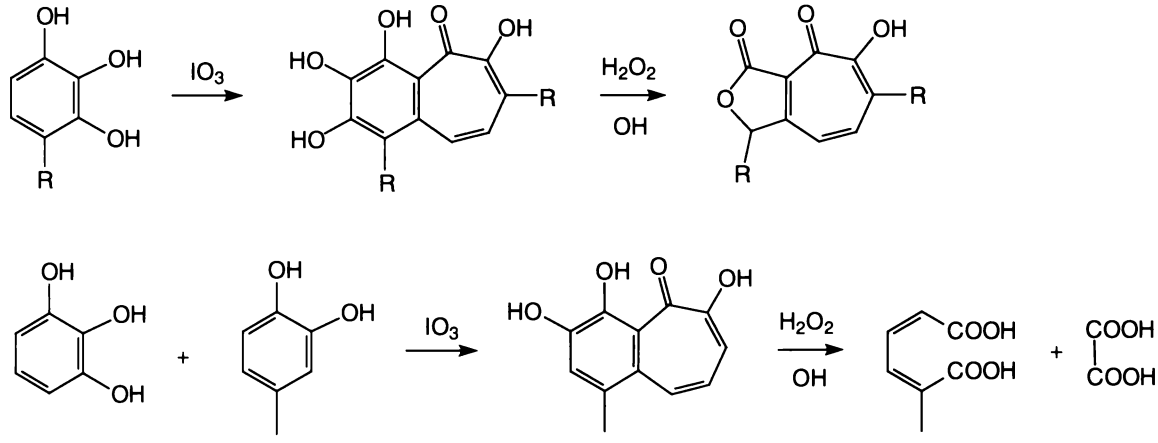
Signal	**δ (ppm)**	**Correlated ^{1}H signals, δ (ppm)**	**^{13}C, δ (ppm)**	**Correlated ^{1}H signals**
H5	7.48	H3	C3	H5, H8
H6	7.64	H5, H7	C5	H3, H8
			C7	H5
C3	115.0	H3, 7.74	C6	H6, H7
C5	132.6	H5, 7.48	C4, 141.1	H3, H6, H8
C7	132.9	H7, 7.49	C2, 166.4	H3, H7, H8
C6	137.4	H6, 7.64	C1, 176.9	H3, H7
C8	192.0	H8, 9.87	C8	H3, H5

In addition to 4-styryltropolone (**66**) the oxidative cleavage was also preformed on 4-pent-1-enyltropolone (**62**). The advantage of this reactant was that the butanal by-product was more easily removed from the 4-formyltropolone (**71**) than the benzaldehyde produced with 4-styryltropolone (**66**). A disadvantage was that a lower yield of (**71**) (50% *cf.* 74%) was obtained with 4-pent-1-enyltropolone (**62**).

The attempted condensation of the tropolone anhydride (**52**) with 4-formyltropolone (**71**) in pyridine at reflux was for the most part unsuccessful. A crude product was obtained in *ca.* 13% yield. This product exhibited signals in the ^{1}H NMR spectrum at δ 3.06 (m), 5.85 (dd) and 7.0-8.0 ppm (numerous overlapping signals). The ^{13}C NMR spectrum displayed signals at δ 43.5 and 79.2 ppm plus a range of signals in the δ 115-147 and 161-179 ppm regions. The chemical shifts and range of signals suggested the crude product was possibly a mixture of lactone and lactone opened condensation products. The poor yield and least than convincing spectroscopic evidence meant that this reaction was not pursued further.

4.4 Towards Alkyltropolones from Alkyltrihydroxybenzocyclohepten-5-ones[2]

It was proposed the synthesis of alkyltropolones might be possible through the oxidation of alkyltrihydroxybenzocyclohept-5-ones[2]. As described in Section 4.2.1 methyltropolone (**51**) can be prepared *via* alkaline hydroxide peroxide oxidation of purpurogallin (**50**) followed by decarboxylation (305). Elaboration of this method would appear to offer a potential pathway to other alkyltropolones. The versatility of this pathway is limited by the availability of suitable purpurogallin derivatives (182). Critchlow *et al.* (320) have reported that iodate oxidation of alkyl pyrogallols gave 1,7-dialkylpurpurogallins (Figure 4.9). Alkaline hydrogen peroxide oxidation of these dialkylpurpurogallins yielded lactone tropolones in 5-10% yield (182) (Figure 4.9). 1-Methyl-3,4,6-trihydroxy-(5*H*)-benzocyclohepten-5-one (**72**) (previously known as 4-methyl-1',2'-dihydroxy-3,4-benzotropolone) has been prepared by Collier (321) by oxidation of an aqueous solution of pyrogallol and methyl catechol with sodium iodate. Alkaline hydrogen peroxide oxidation of 1-methyl-3,4,6-trihydroxy-(5*H*)-benzocyclohepten-5-one (**72**) at 70°C gave small yields of 3-methylmuconic acid (*ca.* 20%) and oxalic acid (*ca.* 15%) (Figure 4.9). Alkaline hydrogen peroxide oxidations of alkylpurpurogallins or alkyltrihydroxy-benzocyclohept-5-ones do not appear to be viable pathways to alkyltropolones.



72

Figure 4.9: Alkaline hydrogen peroxide oxidation products of alkylpurpurogallins and 1-methyl-3,4,6-trihydroxy-(5*H*)-benzocyclohepten-5-one (**72**).

[2] Previous researchers have referred to compounds of this type (*i.e.* **72**) as benzotropolones, however they can also be called hydroxybenzocyclohepten-5-one. For reason of consistency with the tetrahydrobenzocycloheptenone (**46**) discussed in Chapter 3 this alternative name has been used in this thesis.

3,4,6-Trihydroxy-(5*H*)-benzocyclohepten-5-one has been selectively oxidation by tetrachloro-*o*-benzoquinone (*o*-chloranil) to 3,4-dioxo-6-hydroxy-(5*H*)-benzocyclohepten-5-one (also known as 3,4-benzotropolone-1',2'-quinone) (Figure 4.10) (322). 1,2-Naphthaquinones undergo Baeyer-Villiger type oxidations. Karrer and Schneider (323) have reported that 1,2-naphthaquinone reacts with perbenzoic acid to give the cyclic anhydride shown in Figure 4.10. Fernholz (324) has reported that the tropolone ring is insensitive to perbenzoic acid. Based on these observations it was postulated that the synthesis of β-thujaplicin (**47**) from 1-methyl-3,4,6-trihydroxy-(5*H*)-benzocyclohepten-5-one (**72**) may be possible by the strategy proposed in Scheme 4.1.

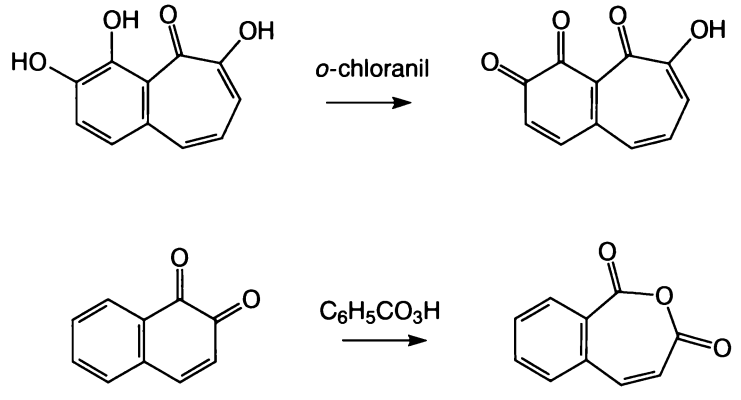
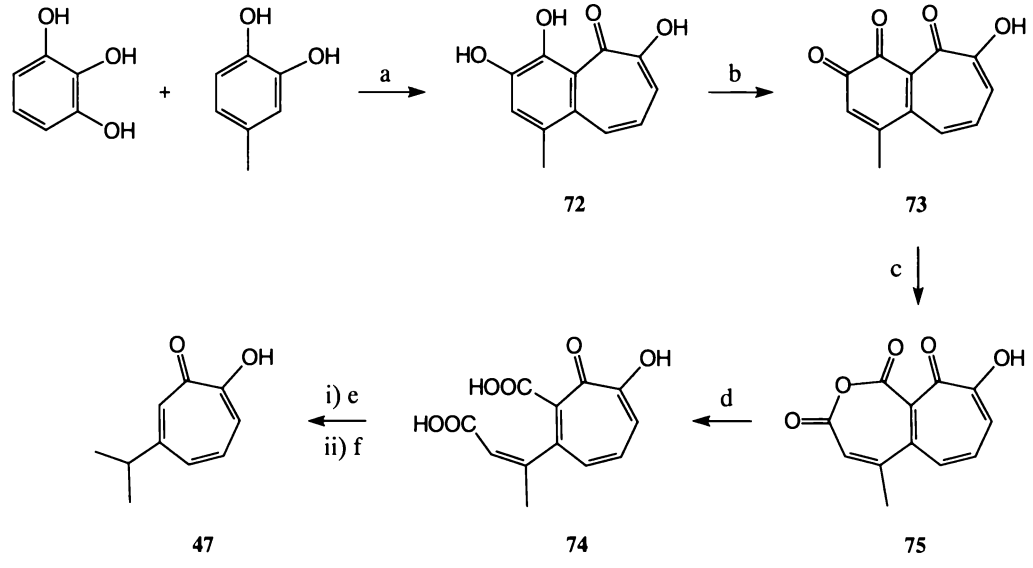


Figure 4.10: Oxidations of 3,4,6-trihydroxy-(5*H*)-benzocyclohepten-5-one with *o*-chloranil and 1,2-naphthaquinone with perbenzoic acid.



Scheme 4.1: Proposed pathway to β-thujaplicin (**47**) from 1-methyl-3,4,6-trihydroxy-(5*H*)-benzocyclohepten-5-one (**72**) (a = $NaIO_3/H_2O$; b = *o*-chloranil; c = *m*-$ClC_6H_5CO_3H$; d = H^+/H_2O; e = Δ, Cu/quinoline; f = H_2, Pd/C).

Exploration of the synthetic strategy outlined in Scheme 4.1 was undertaken with the aim of developing a new pathway to alkyltropolones. Following the method of Collier (321) 1-methyl-3,4,6-trihydroxy-(5*H*)-benzocyclohepten-5-one (**72**) was prepared in 80% crude yield. The IR spectrum possessed both ν O-H and ν C=O stretching vibrations. The MS spectrum exhibited a molecular ion of *m/z* 218, which was also the base peak. Ions of *m/z* 190 (loss of CO) and *m/z* 115 were also prominent. The *m/z* 115 ion is possibly formed through losses of CO, H_2O and CHO from the *m/z* 190 ion.

The ^1H NMR spectrum was characterised by a methyl proton signal at δ 2.61 ppm, four benzocycloheptenone proton signals in the δ 6.9-7.8 ppm region and three hydroxyl proton signals. A signal at δ 14.78 ppm was assigned to the OH proton alpha to C4. The large up-frequency shift is due to strong hydrogen bonding with the C5 carbonyl. The ^{13}C NMR spectrum showed a methyl signal at δ 21.9 ppm (C10), phenolic-like carbon signals at δ 146.1 (C3), 148.9 (C4) and 155.4 ppm (C6) as well as a carbonyl signal at δ 184.7 ppm (C5). Hydroxybenzocycloheptenones (previously known as benzotropolones) do not exist as two separate tautomers like tropolones, hence the phenolic-like (δ 155.4 ppm) and carbonyl-like (δ 184.7 ppm) signals of the tropolone functionality. The gCOSY, HMQC and HMBC correlations facilitated the assignment of the various ^1H and ^{13}C signals of 1-methyl-3,4,6-trihydroxy-(5*H*)-benzocyclohepten-5-one (**72**) (Table 4.11).

Table 4.11: gCOSY, HMQC and HMBC correlations observed for 1-methyl-3,4,6-trihydroxy-(5*H*)-benzocyclohepten-5-one (**72**).

Signal	**δ (ppm)**	**Correlated ^1H signals, δ (ppm)**	**^{13}C**	**δ (ppm)**	**Correlated ^1H signals**
H8	6.91	H7, H9	C4a	121.6	H9
			C8	123.5	H9
C10	21.9	H10, 2.61	C2	124.9	H10
C7	118.2	H7, 7.27	C9a	130.4	H9
C8	123.5	H8, 6.91	C1	130.6	H2, H10
C2	124.9	H2, 7.45	C9	130.9	H7, H8
C9	130.9	H9, 7.73	C3	146.1	H2
			C4	148.9	H2
C10	21.9	H2	C6	155.4	H7, H8
C7	118.2	H9	C5	184.7	H7

Following the method of Collier (321) oxidation of 1-methyl-3,4,6-trihydroxy-(5*H*)-benzocyclohepten-5-one (**72**) with *o*-chloranil gave 1-methyl-3,4-dioxo-6-hydroxy-(5*H*)-benzocyclohepten-5-one (**73**) in 82% yield. The product was characterised from its spectroscopic data. The IR spectra displayed absorbance maxima at 1630 cm^{-1} and 1600 cm^{-1} representing carbonyl stretching vibrations from the quinone and cycloheptenone functional groups. The GC/MS spectra of the TMSi derivative exhibited a molecular ion of *m/z* 434 ($C_{12}H_7O_4$ + $3SiMe_3$) and base peak of 419 ($M^{\bullet+}$- 15). The ^1H NMR spectrum showed a methyl proton signal at δ 2.45 ppm and benzocycloheptenone proton signals in the δ 6.64-7.27 ppm region, but lacked any hydroxyl proton signals. The ^{13}C NMR spectrum exhibited four carbon signals in the δ 177.7-183.6 ppm region, attributable to the carbonyls in the tropolone and *o*-quinone functionalities, suggesting this compound can exist in tautomeric forms. The 2D ^1H-^1H and ^{13}C-^1H NMR correlations provided further confirmation of the 1-methyl-3,4-dioxo-6-hydroxy-(5*H*)-benzocyclohepten-5-one (**73**) structure (Table 4.12).

Table 4.12: gCOSY, HMQC and HMBC correlations observed for 1-methyl-3,4-dioxo-6-hydroxy-(5*H*)-benzocyclohepten-5-one (**73**).

Signal	**δ (ppm)**	**Correlated ^1H signal, δ (ppm)**	**^{13}C**	**δ (ppm)**	**Correlated ^1H signal**
H10	2.45	H2	C10	22.9	H2
H8	7.27	H7, H9	C4a	113.6	H9
			C9	127.9	H7
C10	22.9	H10, 2.45	C2	129.4	H10
C9	127.9	H9, 7.08	C7	133.1	H9
C2	129.4	H2, 6.64	C9a	135.1	H2, H10
C7	133.1	H7, 6.88	C8	137.0	H9
C8	137.0	H8, 7.27	C1	154.7	H9, H10
			C5, C6	177.7, 178.3	H7
			C3, C4	183.1, 183.6	H2

The next step required the selective oxidisation of the *o*-quinone functionality of 1-methyl-3,4-dioxo-6-hydroxy-(5*H*)-benzocyclohepten-5-one (**73**) to an anhydride while maintaining the tropolone ring intact. The attempted oxidation of 1-methyl-3,4-dioxo-6-hydroxy-(5*H*)-benzocyclohepten-5-one (**73**) with *m*-chloroperbenzoic acid gave a crude product in 28% yield, which precipitated from the reaction solvent. The product was characterised from its IR, ES(electrospray)/MS, APCI/MS and ^1H and ^{13}C NMR spectra (Section 7.2.10). The IR

spectrum exhibited broad ν O-H vibrations in the 3000-3500 cm^{-1} region and carbonyl stretching vibrations at 1760 and 1734 cm^{-1}. The electrospray positive and negative ion spectra exhibited $(M+H)^+$ and $(M-H)^-$ ions of *m/z* 251.1 ($C_{12}H_{10}O_6$+H) and *m/z* 248.9 respectively. The MS^2 spectra on the $(M-H)^-$ ion displayed a base peak of m/z 205 representing loss of CO_2. Fragments representing losses of CO_2 + H_2O and CO_2 + CO were also evident. This evidence points towards 3-carboxy-4-(1-methyl-2-carboxyethenyl)tropolone (**74**) as a potential product of the oxidation.

The APCI/MS positive and negative ion spectra exhibited $(M+H)^+$ and $(M-H)^-$ ions of *m/z* 233.0 and *m/z* 231.1 respectively. In ES/MS, ionisation occurs under 'wet' conditions (molecules of solvent and water present). In APCI/MS, ionisation occurs under 'dry' conditions. For APCI/MS the vaporiser temperature is set at 500°C. It is possible that if the oxidation product were 3-carboxy-4-(1-methyl-2-carboxyethenyl)tropolone (**74**) then under these conditions it could undergo dehydration prior to ionisation thus giving the observed $(M+H)^+$ and $(M-H)^-$ ions.

The ^1H NMR spectrum of the oxidation product exhibited signals for the methyl protons at δ 2.19 ppm, the olefinic proton at δ 6.15 ppm and tropolonic protons in the δ 7.1-7.3 ppm region (overlapping signals were resolved in the HMQC spectrum). The ^{13}C NMR spectra exhibited three carbon signals in the carbonyl region, when four would be expected if the product were 3-carboxy-4-(1-methyl-2-carboxyethenyl)tropolone (**74**). Additionally, two signals at δ 151.0 and 93.8 ppm in the carbon spectrum are not readily assignable to the 3-carboxy-4-(1-methyl-2-carboxyethenyl)tropolone (**74**) structure. They could possibly be assigned to an enol carbon (δ 151.0 ppm) and C3 (δ 93.8 ppm) in the tropolone anhydride (**75**) if this were to exist in an enolic form (as observed for **52**). The ^{13}C NMR spectrum raised questions over the identity of the oxidation product.

The spectroscopic evidence offered conflicting interpretations as to the structure of the oxidation product. If the product was the tropolone anhydride (**75**) then aqueous hydrolysis should yield 3-carboxy-4-(1-methyl-2-carboxyethenyl)tropolone (**74**). An attempted acid catalysed aqueous hydrolysis reaction left the oxidation product unchanged. If 3-carboxy-4-(1-methyl-2-carboxyethenyl)tropolone (**74**) was the structure of the oxidation product then

decarboxylation could possibly yield β-dolabrin (Scheme 4.1). Attempts to decarboxylate the oxidation product by heating in quinoline to 150-160°C in the presence of Cu metal resulted only in polymerisation of the oxidation product. The aliphatic acid chain of the possible product 3-carboxy-4-(1-methyl-2-carboxyethenyl)tropolone (**74**) is analogous in structure to crotonic acid. Crotonic acid has been reported to undergo polymerisation on heating (325). These results suggested that the proposed pathway to β-thujaplicin (**47**) (Scheme 4.1) was untenable and further work was abandoned.

4.5 Direct Alkylation of Tropolone with Diethyl Acetals

Substitution of hydrogen on the tropolone ring with alkyl electrophiles offers a potential pathway to alkyltropolones. Tropolones are reactive towards electrophilic reagents, resembling both benzenoid compounds and β-diketones in their reactivity (326). Tropolones, however, do not undergo Friedel-Crafts alkylation or acylation due to their tendency to form complexes with the metal catalysts, which are resistant to electrophilic attack (326). Few methods exist for the direct alkylation of tropolones. Certain 3-allyl and 3-benzyltropolones can be prepared by Claisen rearrangement of 2-allyloxy- and 2-benzyloxytropones (327, 328). Yamato *et al.* (329, 330) discovered a method for the direct alkylation of tropolones with diethyl acetals. They found that 1-ethoxyisochroman will reacts with β-thujaplicin (**47**) at 150-160°C to give 3-isochroman-1-yl-6-isopropyltropolone (329) (Figure 4.11). Having made this discovery Yamato and colleagues (330) went on to react β-thujaplicin (**47**) with diethyl acetals including crotonaldehyde diethyl acetal and cinnamaldehyde diethyl acetal to give 3-isopropyl-6-(1-ethoxy-2-butenyl)tropolone[3] and 3-isopropyl-6-(1-ethoxy-2-phenylpropenyl)tropolone[3] (330) (Figure 4.11). The alkylation of tropolones with diethyl acetals offers a potential route to 3-alkyltropolones and related compounds. This was explored with the objective of synthesising analogues of α-thujaplicin. The preparation of tropolone (**48**) and suitable diethyl acetals was the first step towards this objective.

[3] As named by Yamato *et al.* (330).

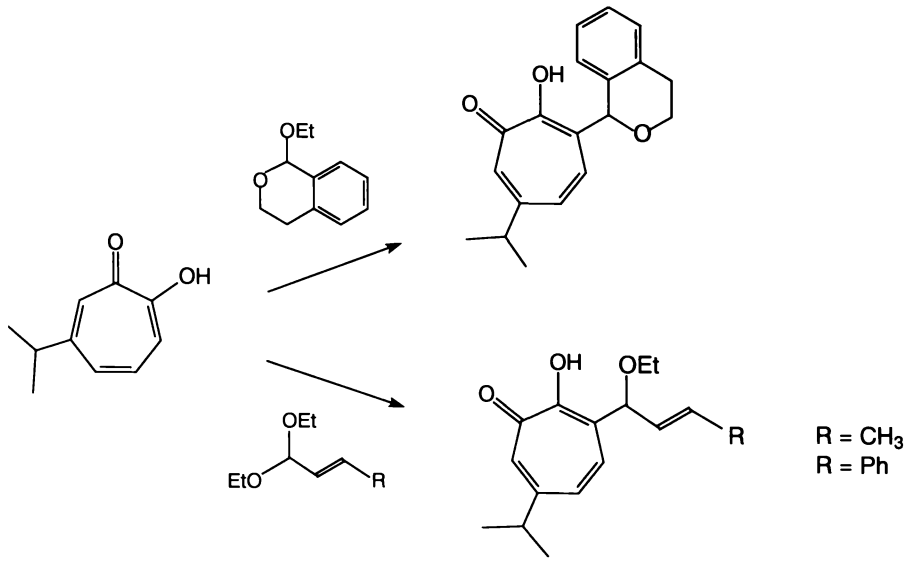


Figure 4.11: Reaction of β-thujaplicin (**47**) with 1-ethoxyisochrom and alkenyl diethyl acetals.

4.5.1 Preparation of Tropolone (48) and Alkyl Diethyl Acetals

The simplest, high-yielding multistep synthesis of tropolone was first discovered by Steven *et al.* (331) and involves the [2+2] cycloaddition of cyclopentadiene with dichloroketene to produce 7,7-dichlorobicyclo(3.2.0)hept-2-en-6-one (**76**), which on solvolysis gives tropolone (**48**). Several modifications of the original method have been reported. Here the method of Minns (332) was followed. Cyclopentadiene, obtained by the thermal cracking of dicyclopentadiene, was reacted with dichloroketene generated *in situ* from dichloroacetyl chloride and triethylamine to give 7,7-dichlorobicyclo(3.2.0)hept-2-en-6-one (**76**) in 79% yield. The mass spectrum of the product featured molecular ions of *m/z* 180 (0.3%), 178 (2%) and 176 (3%), which is consistent with the natural abundances of the two Cl isotopes. Significant fragment ions were observed of *m/z* 113 (89%), *m/z* 77 (100%) and *m/z* 66 (61%). These fragmentations were accounted for as follows. Loss of Cl followed by ejection of CO could give the *m/z* 113 ion. Loss of HCl from the 113 fragment would give the 77 ion. The *m/z* 66 ion could be formed by loss of $CCl_2C{=}O$ (MW = 110 Da) from the molecular ion via a retro-Diels-Alder reaction within the cyclobutanone ring. The ^{1}H and ^{13}C NMR spectra of the product were consistent with the 7,7-dichlorobicyclo(3.2.0)hept-2-en-6-one (**76**) structure (Section 7.2.11). The 2D ^{1}H-^{1}H and ^{13}C-^{1}H correlation NMR spectra provided confirmation of signal assignments.

7,7-Dichlorobicyclo(3.2.0)hept-2-en-6-one (**76**) was converted to tropolone (**48**) in 71% yield by solvolysis with sodium acetate in glacial acetic acid. The product was confirmed as tropolone (**48**) and its purity verified from melting point and spectroscopic evidence. The GC/MS spectrum of tropolone (**48**) exhibited a molecular ion of *m/z* 122, which is also the base peak. Losses of CO to give ions of *m/z* 94 and *m/z* 66 were the main MS fragmentations. The GC/MS chromatogram of the TMSi derivative consisted of a single peak with a molecular ion of *m/z* 194 ($C_7H_5O_2$ + $SiMe_3$) and base peak of *m/z* 179 ($M^{\cdot+}$- CH_3). The ^1H and ^{13}C NMR spectra were identical to those of authentic commercial tropolone (**48**).

Alkyl diethyl acetals were obtained either by chemical synthesis or by purchase. Following the method of Zhu *et al.* (333) the reaction of octanal with triethyl orthoformate in the presence of NH_3NO_3 gave octanal diethyl acetal (**77**) in 76% yield. The GC/MS spectrum of the reaction product showed a tiny molecular ion of *m/z* 204, a base peak of *m/z* 103 and a fragment of *m/z* 157. These fragment ions were formed by losses of C_7H_{15} and OC_2H_5 radicals respectively from the molecular ion. The ^1H and ^{13}C NMR spectra (Section 7.2.11) indicated the product was octanal diethyl acetal (**77**). The methylene protons in the ethoxy group (H9, H9') gave separate signals at δ 3.49 and 3.63 ppm in the ^1H NMR spectrum on account of the prochiral centre at C1.

Klein and Bergmann (334) reported a 95% yield of *trans*-cinnamaldehyde diethyl acetal (**78**) by NH_4NO_3 catalysed transacetalation of *trans*-cinnamaldehyde with triethyl orthoformate. Using this method, *trans*-cinnamaldehyde diethyl acetal (**78**) was obtained in 68% yield. The GC/MS spectrum of the distilled product exhibited a molecular ion of *m/z* 206, a base peak of *m/z* 161 and a prominent ion of *m/z* 133. The base peak represents allylic cleavage with loss of a OCH_2CH_3 radical from the molecular ion. The *m/z* 133 ion can be rationalised in terms of proton abstraction by the conjugated double bond to give loss of a CH_2=CH_2 radical from the *m/z* 161 ion. An interesting feature of the ^1H NMR spectrum is the presence of four resolvable quartets at δ 3.54, 3.56, 3.68 and 3.70 ppm due to the non-equivalence of the methylene protons in the ethoxy groups as a result of the three prochiral centres at the C9, C10 and C10' carbons. The ^1H and ^{13}C NMR spectra were otherwise consistent with the *trans*-cinnamaldehyde diethyl acetal (**78**) structure.

Technical grade commercial hex-2-enyl diethyl acetal (**79**) (94%) was used in the subsequent alkylation reactions after an attempted synthesis of pure oct-2-enyl diethyl acetal (**80**) from technical grade *trans*-octenal and triethyl orthoformate was found to give unsatisfactory levels of by-products. Transacetalation of octenal with triethyl orthoformate gave a product in 88% yield which GC/MS evidence suggested was octenal diethyl acetal (**80**) in 90% purity. GC/MS analysis suggested that the 10% impurity consisted of two isomers of possibly 1-ethoxy-1,3-octadiene (**81**). As these compounds were only identified from their mass spectra only tentative identifications were possible. An attempted purification of the crude octenal diethyl acetal (**80**) by distillation resulted in increased levels of the 1-ethoxy-1,3-octadiene (**81**) impurities (up to *ca.* 30%), possibly as a result of thermal expulsion of ethanol.

To determine whether alkenyl ethyl ethers could also alkylate tropolone in an analogous manner to alkenyl diethyl acetals, 1-ethoxy-2-hexene (**82**) was prepared by a Williamson ether synthesis. The Na salt of 2-hexen-1-ol was prepared and reacted with ethyl bromide to give 1-ethoxy-2-hexene (**82**) in 27% yield as determined from its GC/MS and ^{1}H and ^{13}C NMR spectra (Section 7.2.11).

4.5.2 Alkylations of Tropolone (48) and β-Thujaplicin (47) with Diethyl Acetals

Yamato *et. al* (330) reported that crotonaldehyde diethyl acetal gave 3-isopropyl-6-(1-ethoxy-2-butenyl)tropolone[4] (Figure 4.11) in 55% when heated with β-thujaplicin (**47**) at 150-180 °C. The attempted analogous alkylation of tropolone (**48**) with octanal diethyl acetal (**77**) under similar conditions was unsuccessful. Work-up of the reaction mixture gave only unreacted tropolone (**48**), octanal diethyl acetal (**77**) and a by-product which was possibly 1-ethoxy-1-octene formed by thermal expulsion of ethanol (Section 7.2.11). Yamato *et al.* (330) reported that heating β-thujaplicin (**47**) with a 2 mol excess of cinnamaldehyde diethyl acetal (**78**) at 180°C for 6 hours gave a 38% yield of 3-isopropyl-6-(1-ethoxy-3-phenylpropenyl)tropolone[5] (**83**). Attempting this reaction with tropolone (**48**) in place of β-thujaplicin (**47**) resulted in the formation of a black tar after 1 hour from which *trans*-cinnamaldehyde was isolated but no

[4] Named according to the system used by Yamato *et al.* (330). This differs from the system used in this thesis (which gives the name 3-(1-ethoxy)but-2-enyl-6-isopropyltropolone).

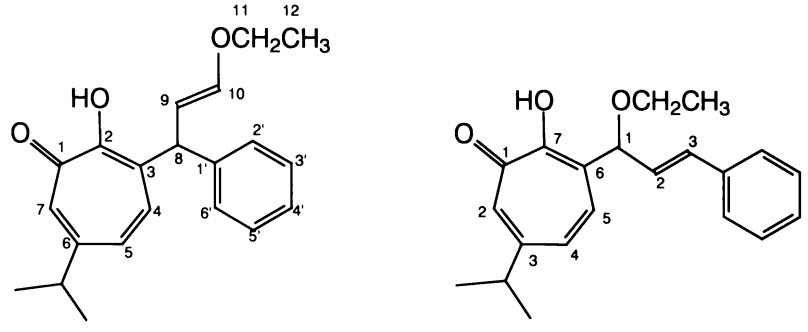
[5] As footnote 4. The system used in this thesis would give the name 3-(1-ethoxy-3-phenyl)prop-2-enyl-6-isopropyltropolone.

alkylation product. Reducing the temperature to 150°C gave a similar result after 2 hours. Heating the reactants at 110°C for 28 hrs gave no reaction.

Discouraged by these results an attempt to replicate the result of Yamato *et al.* (330) was undertaken. Reaction of *trans*-cinnamaldehyde diethyl acetal (**78**) with β-thujaplicin (**47**) at 150-160°C for 6 hrs gave, after chromatographic purification, a mixture of two compounds in 24% yield. The spectroscopic evidence suggested these two compounds were the *cis* and *trans* isomers of 3-(1-phenyl-3-ethoxy)prop-2-enyl-6-isopropyltropolone (**84**, **85**) (Figure 4.12). The product differed from that reported by Yamato *et al.* (330) (*i.e.* **83**) (Figure 4.12). The spectroscopic evidence for the 3-(1-phenyl-3-ethoxy)prop-2-enyl-6-isopropyltropolone (**84**, **85**) structure was as follows. The GC/MS spectrum of the product exhibited a molecular ion of *m/z* 324 ($C_{21}H_{24}O_3$), a base peak of 223 ($M^{\cdot+}$-101) and significant fragment ions of *m/z* 279 and *m/z* 265. The *m/z* 279 fragment can be explained in terms of loss of an ethoxy radical. The GC/MS spectrum of the TMSi derivative of the product consisted of two overlapping peaks with identical mass spectra. Each contained a molecular ion of *m/z* 396 ($C_{21}H_{23}O_3$ + $SiMe_3$) and a base peak of *m/z* 337. The GC/MS evidence confirmed the success of the alkylation but was unsuitable for distinguishing between the possible products (**83**) and (**84**, **85**) (Figure 4.12).

The ^1H and ^{13}C NMR spectra provided crucial evidence for the assigned structure (**84**, **85**). The spectra were a compilation of signals from the two diastereomers. Fortunately, the *cis* isomer (**84**) was present in *ca.* 28% and the *trans* isomer (**85**) in *ca.* 72% and this coupled with the 2D correlations made it possible to accurately assign all signals of the two isomers (Section 7.2.11). The most informative features of the ^1H NMR spectrum were a pair of doublet of doublets at δ 4.86 and 5.18 ppm (H9) and a pair of doublets at δ 6.13 and 6.29 ppm (H10). These signals were assigned to H9 and H10 in the *cis* (**84**) and *trans* (**85**) diastereomers respectively. The coupling constants between these signals, J = 6.2 Hz and J = 12.5 Hz respectively, enabled the isomers to be assigned the *cis* and *trans* stereochemistry. The alkylation reaction, as proposed by Yamato *et al.* (330), would lead only to the *trans* isomer of 3-isopropyl-6-(1-ethoxy-3-phenylpropenyl)tropolone (**83**) as the double bond remains intact throughout the reaction and there is no opportunity for isomerisation. The presence of the *cis* and *trans* isomers casts doubt on the structure proposed by Yamato *et al.* (330). Further evidence for the (**84**, **85**) structure can be found in the ^{13}C NMR spectrum of the product.

Carbon signals at δ 104.9 and 148.8 ppm (*trans* isomer) and δ 106.8 and 146.0 ppm (*cis* isomer) are indicative of olefinic carbons in an enol ether functionality (*i.e.* C9 and C10 respectively) rather than either of the two olefinic carbons in the proposed structure (**83**).



85 and **84** (cis-9,10)

83

Figure 4.12: Proposed products of the reaction of cinnamaldehyde diethyl acetal (**78**) with β-thujaplicin (**47**) as determined by this research and by Yamato *et al.* (330). Note: the numbering for IUPAC naming differs from the numbering system used for NMR assignment (shown for **85** above). The numbering of (**83**) follows the system used by Yamato *et al.* (330), which differs from the system used in this thesis.

Conclusive evidence for the 3-(1-phenyl-3-ethoxy)prop-2-enyl structure (**84**, **85**) over the 6-(1-ethoxy-3-phenylpropenyl) structure (**83**) was found in the 2D ^{1}H-^{1}H and ^{13}C-^{1}H correlation NMR spectra (Appendix G, Tables G1 and G2). Those correlations that were crucial to structural elucidation are depicted in Figure 4.13. They included coupling of **C11** to **H10**, coupling of C8 to H4, H9, H10, **H2'**, **H6'** and the coupling of numerous carbons to H8, including C2, C3, C4, C9, C10, **C1'**, **C2'** and **C6'**. If the product were (**83**) then the couplings in bold would not be observed. Additionally it would be reasonable to expect couplings between the methylene carbon of OCH_2CH_3 and H1 and between C3, H3 and the aromatic protons and carbons *alpha* and *beta* to C3, H3 (see (**83**) Figure 4.12). No such correlations were observed. The NMR evidence strongly points towards 3-(1-phenyl-3-ethoxy)prop-2-enyl-6-isopropyltropolone (**84**, **85**) and not 3-isopropyl-6-(1-ethoxy-3-phenylpropenyl)-tropolone (**83**) as the condensation product of *trans*-cinnamaldehyde diethyl acetal (**78**) and β-thujaplicin (**47**).

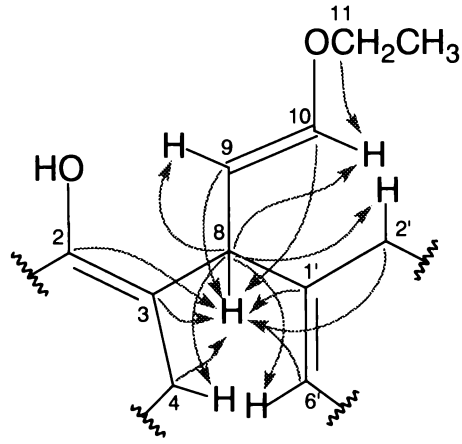


Figure 4.13: Structurally informative HMBC correlations for *trans*-3-(1-phenyl-3-ethoxy)prop-2-enyl-6-isopropyltropolone (**85**).

There are two possibilities as to why a different product was observed to that reported by Yamato *et al.* (330). Firstly 3-isopropyl-6-(1-ethoxy-3-phenylpropenyl)tropolone (**83**) could have formed initially in the reaction and then undergone rearrangement to 3-(1-phenyl-3-ethoxy)prop-2-enyl-6-isopropyltropolone (**84**, **85**), a rearrangement which was not observed by Yamato *et al.* (330). The second alternative is that Yamato and colleagues incorrectly characterised their product. 3-Isopropyl-6-(1-ethoxy-3-phenylpropenyl)tropolone (**83**) was assigned based on evidence from the ^{1}H NMR spectrum, the mass spectrum (only $M^{\cdot+}$ reported) and elemental analysis as well as the observation that 1-ethoxyisochrom reacts with β-thujaplicin (**47**) to give 3-isochroman-1-yl-6-isopropyltropolone (329) (Figure 4.11). The product of this reaction was characterised from ^{1}H and ^{13}C NMR spectroscopic evidence and X-ray crystallography of the methyl ether derivative (329). Therefore, there can be no doubt that this reaction yields 3-isochroman-1-yl-6-isopropyltropolone (Figure 4.11).

Although it follows that cinnamaldehyde diethyl acetal (**78**) would undergo an analogous reaction to 1-ethoxyisochrom to give 3-isopropyl-6-(1-ethoxy-3-phenylpropenyl)tropolone (**83**) the evidence presented by Yamato *et al.* (330) is less than convincing. The MS molecular ion and microanalytical data would of course be identical for both products (**83**) and (**84**, **85**). The quoted chemical shifts of the ^{1}H NMR spectrum were only broadly defined (*e.g.* 5.08-5.58, m, 2H, H2 and H3 (Figure 4.11), 6.83-7.76, m, 8H, tropolone and aromatic protons) in the paper by Yamato *et al.* (330). Before either of the two aforementioned reasons, for the different structural characterisations, can be proposed with confidence it was necessary to

consider the results of reactions between β-thujaplicin (**47**) and hex-2-enyl diethyl acetal (**79**) and tropolone (**48**) and hex-2-enyl diethyl acetal (**79**).

trans-Hex-2-enyl diethyl acetal (**79**) heated with β-thujaplicin (**47**) at 160°C for 8 hrs gave, after chromatographic purification, a mixture of two compounds in 59% yield. The spectroscopic evidence indicated these products were the *cis* and *trans* isomers of 3-(1-propyl-3-ethoxy)prop-2-enyl-6-isopropyltropolone (**86**, **87**). As in the case of the reaction with cinnamaldehyde diethyl acetal (**78**), the alkyl enol ether thujaplicin (**86**, **87**) was formed as opposed to the ethoxy alkenyltropolone (**88**) as proposed by Yamato *et al.* (330) for the analogous reaction of crotonaldehyde with β-thujaplicin (**47**) (Figure 4.11). Evidence for the 3-(1-propyl-3-ethoxy)prop-2-enyl-6-isopropyltropolone (**86**, **87**) structure was found in the HMBC ^{1}H-^{13}C NMR correlations (Appendix G, Table G3). The ^{1}H NMR spectrum exhibited olefinic proton signals at δ 4.92 (H9), 6.34 (H10) ppm (J = 12.5 Hz) and δ 4.62 (H9), 5.97 (H10) ppm (J = 6.1 Hz) for the *trans* (**87**) and *cis* (**86**) isomers respectively. The mass spectra of the TMSi derivatives of the two isomers were identical, both exhibiting molecular ions of *m/z* 362 ($C_{18}H_{25}O_3$ + $SiMe_3$) and base peaks of *m/z* 333 ($M^{\cdot+}$-CH_2CH_3). Although the neat reaction of tropolone (**48**) with cinnamaldehyde diethyl acetal (**78**) was unsuccessful the moderate yield (56%) obtained in this reaction suggested that the reaction of tropolone (**48**) with *trans*-hex-2-enyl diethyl ether (**79**) under similar conditions should be achievable.

The reaction of *trans*-hex-2-enyl diethyl ether (**79**) with tropolone (**48**) gave a mixture of products, the composition of which changed with time as the result of rearrangement and hydrolysis. Initially a product isolated in *ca.* 14% yield following chromatography appeared to be a mixture of compounds of which 3-(1-ethoxy)hex-2-enyltropolone (**89**) was a possible major component based on signals observed in the ^{1}H NMR spectrum (Section 7.2.11). However upon standing this product mixture rearranged to a different mixture of compounds. This mixture was tentatively identified as a mixture of 3-(1-propyl-3-ethoxy)prop-2-enyltropolone (**90**, **91**) and 3-(1-formylmethyl)butyltropolone (**92**) based on probe MS and ^{1}H and ^{13}C NMR spectroscopic evidence. Eventually the original product was found to consist solely of 3-(1-formylmethyl)butyltropolone (**92**) (see characterisation data below); it formation brought about by possible rearrangement and hydrolysis reactions. The reaction outcome and changes observed in the product on standing are summarised in Figure 4.14.

Hydrolysis of the enol ether of (**90**, **91**) to give the aldehyde of (**92**) was possibly brought about by atmospheric moisture with (**92**) possibly involved in acid catalysis of the reaction.

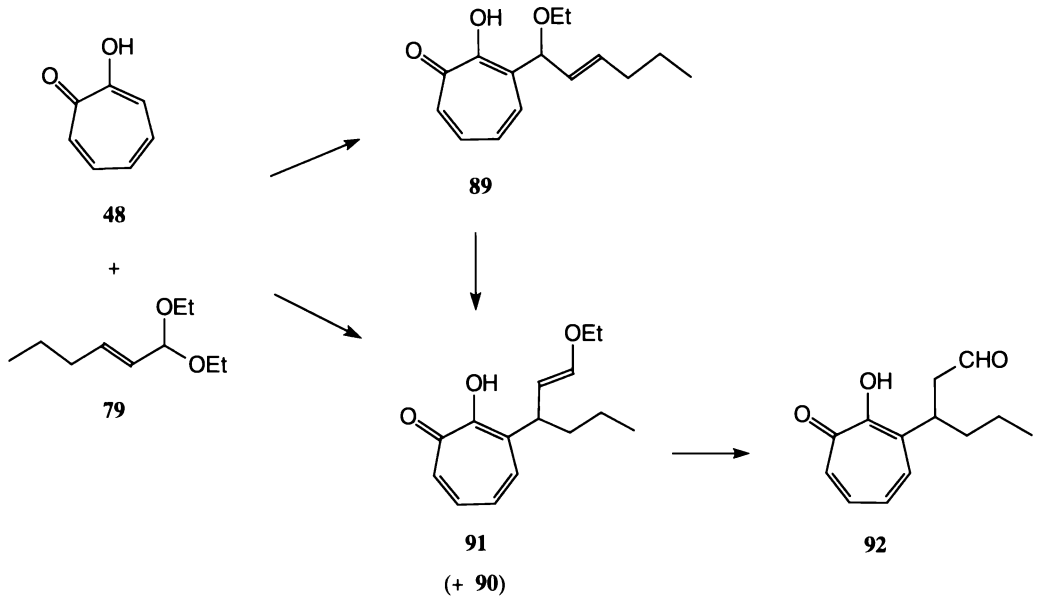


Figure 4.14: Proposed pathway to 3-(1-formylmethyl)butyltropolone (**92**) following alkylation of tropolone (**48**) with *trans*-hex-2-enyl diethyl acetal (**79**).

Since 3-(1-formylmethyl)butyltropolone (**92**) appeared to be the ultimate product of the alkylation of tropolone (**48**) with *trans*-hex-2-enyl diethyl acetal (**79**) aqueous acid hydrolysis in an acetone cosolvent was added to the reaction workup in an attempt to improve the preparation of 3-(1-formylmethyl)butyltropolone (**92**). Using this modified method 3-(1-formylmethyl)butyltropolone (**92**) was obtained in 33% yield. Spectroscopic analysis and microanalytical data (Section 7.2.11) confirmed the structural identity and purity of the product. The infrared spectrum displayed carbonyl stretching vibrations at 1723 cm^{-1} and 1615 cm^{-1} which are characteristic of the aldehyde and tropolone functional groups. The GC/MS spectrum exhibited a *m/z* 220 $M^{\bullet+}$, a base peak of *m/z* 149 and significant fragment ions of *m/z* 191, 177 and 163. The *m/z* 191 and *m/z* 163 fragments are formed by losses of CHO and CO + CHO respectively. The *m/z* 177 and *m/z* 149 fragments are possibly the result of allylic cleavage of a $CH_2CH_2CH_3$ or CH_2CHO radical with ejection of CO in the case of the base peak. The ^1H and ^{13}C NMR spectra were consistent with 3-(1-formylmethyl)butyltropolone (**92**) as the structure of the product. The aldehyde proton and carbon signals were found at δ 9.78 and 201.5 ppm in the respective spectra. Correlations observed in the 2D gCOSY, HMQC and HMBC NMR spectra provide critical evidence for the structure (**92**) (Table 4.13).

Structurally informative coupling correlations included C8 to H4, H9 and H12 and C2, C3, C4, C9, C10, C12 and C13 to H8. This is the first reported synthesis of 3-(1-formylmethyl)butyltropolone (**92**).

Table 4.13: gCOSY, HMQC and HMBC correlations observed for 3-(1-formylmethyl)-butyltropolone (**92**).

Signal	δ (ppm)	Correlated ^{1}H signals, δ (ppm)	^{13}C, δ (ppm)	Correlated ^{1}H signals
H10	1.22	H9, H11	C11	H9, H10
H9	1.70	H8	C10	H8, H9, H11
H12	2.78	H5, H8, H13	C9	H8, H11
H5	7.01	H4, H6	C8	H4, H9, H12
H6	7.29	H7	C12	H8, H9, H13
			C7	H5
C11	14.0	H11, 0.87	C5	H7
C10	20.6	H10, 1.22	C6	H4, H7
C9	36.4	H9, 1.70	C4	H6, H8
C8	37.1	H8, 3.94	C3, 142.2	H5, H8, H12
C12	49.2	H12, 2.78	C1, 168.4	H6, H7
C7	121.1	H7, 7.33	C2, 173.8	H4, H7, H8
C5	127.4	H5, 7.01	C13, 201.5	H8, H12
C6	136.4	H6, 7.29		
C4	138.0	H4, 7.42		

A postulated mechanistic pathway for the formation of 3-(1-formylmethyl)butyltropolone (**92**) from tropolone (**48**) and *trans*-hex-2-enyl diethyl acetal (**79**) is depicted in Figure 4.15. Tropolone (**48**) is postulated to react with *trans*-hex-2-enyl diethyl acetal (**79**) in two ways. Electrophilic attack on the acetal carbon with expulsion of an ethoxy ion (**A**), followed by loss of a proton from the addition product (pathway **i**), would yield 3-(1-ethoxy)hex-2-enyltropolone (**89**) (Figure 4.15). Alternatively tropolone could attack the acetal at the alkene carbon, C3, in a vinylogous attack (**B**). This would result in double bond migration, expulsion of an ethoxy ion and lead directly to 3-(1-propyl-3-ethoxy)prop-2-enyltropolone (**90**, **91**), after loss of a proton from the addition product (Figure 4.15). The situation is analogous to 1,2 *vs* 1,4 Michael addition to an α,β-unsaturated aldehyde. The enol ether group of (**90**, **91**) is readily hydrolysed with aqueous acid to the aldehyde generating 3-(1-formylmethyl)butyl-tropolone (**92**). Any 3-(1-ethoxy)hex-2-enyltropolone (**89**) produced in the reaction evidently

ends up as 3-(1-formylmethyl)butyltropolone (**92**). It is proposed that 3-(1-ethoxy)hex-2-enyltropolone (**89**) can rearrange to 3-(1-propyl-3-ethoxy)prop-2-enyltropolone (**90**, **91**) (Figure 4.15). In order for this to occur 3-(1-ethoxy)hex-2-enyltropolone (**89**), upon gaining a proton, must lose the ethoxyalkenyl group as a carbocation (pathway **ii**), whereby enabling reattachment at C3 *via* electrophilic attack. It is difficult to rationalise a driving force for such a rearrangement.

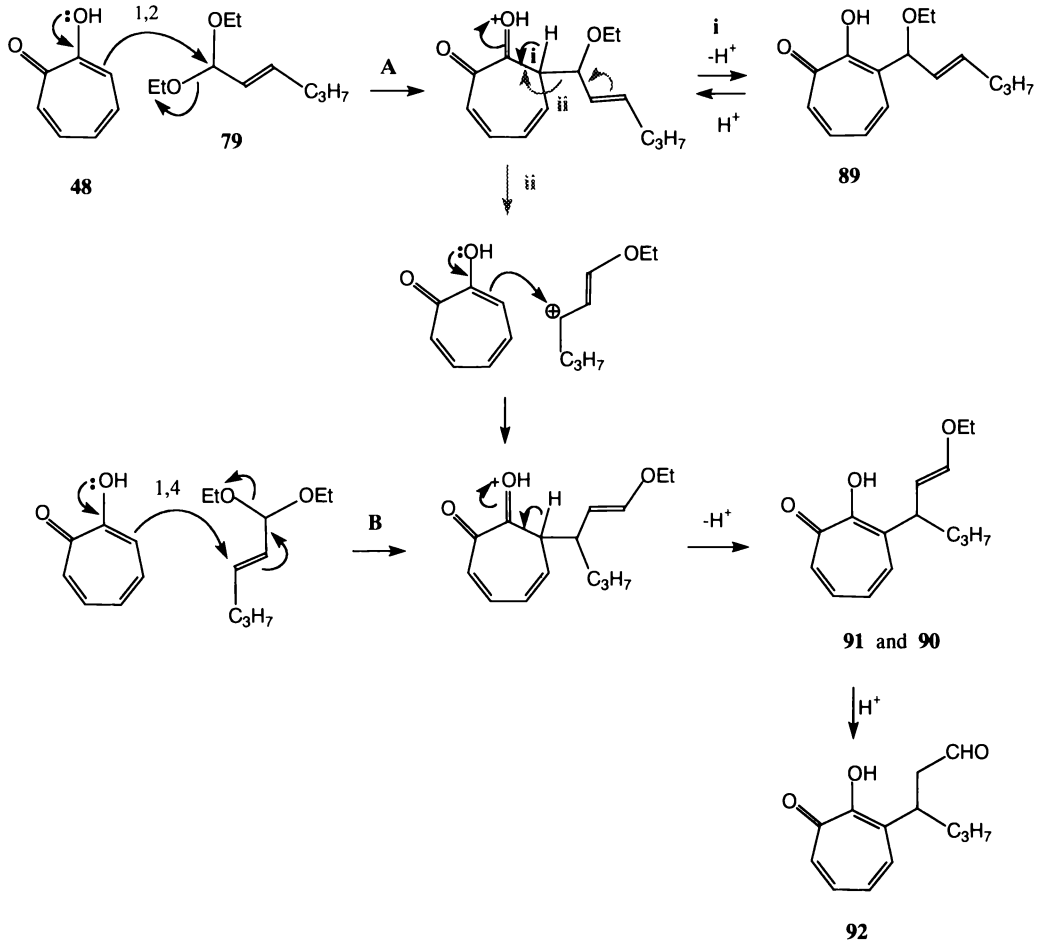


Figure 4.15: Proposed mechanistic pathway to 3-(1-formylmethyl)butyltropolone (**92**) from tropolone (**48**) and *trans*-hex-2-enyl diethyl acetal (**79**).

Formation of 3-(1-propyl-3-ethoxy)prop-2-enyltropolone (**90**, **91**) directly by the proposed electrophilic attack of tropolone (**48**) on the C3 carbon of *trans*-hexenyl diethyl acetal (**79**) raises the question of whether the analogous reaction would occur with 1-ethoxy-2-hexene (**82**). Heating 1-ethoxy-2-hexene (**82**) with tropolone (**48**) at 150°C for 7 hrs gave only unreacted starting materials.

The neat reaction between cinnamaldehyde diethyl acetal (**78**) and tropolone (**48**) failed to yield any alkylated product. Refluxing tropolone (**48**) and cinnamaldehyde diethyl acetal (**78**) in pyridine for 64 hrs gave possibly 3-(1-phenyl-3-ethoxy)prop-2-enyltropolone (**93, 94**) in 49% yield. The GC/MS spectrum of the product showed two peaks with molecular ions of *m/z* 282 ($C_{18}H_{18}O_3$) and base peaks of *m/z* 181. The base peak ions were possibly formed by losses of 2 CO molecules and a OCH_2CH_3 radical. The ^1H and ^{13}C NMR spectra, in conjunction with the 2D ^1H-^1H and ^{13}C-^1H correlation NMR spectra, indicated the product consisted of *cis* and *trans*-3-(1-phenyl-3-ethoxy)prop-2-enyltropolone (**93, 94**) (Section 7.2.11). When the reaction between hexenyl diethyl acetal (**79**) and tropolone (**48**) was carried out in refluxing pyridine a product with spectroscopic evidence consistent with *cis* and *trans*-3-(1-propyl-3-ethoxy)prop-2-enyltropolone (**90, 91**) was obtained in 18% yield (Section 7.2.11). A lack of evidence for the 3-(1-ethoxy)hex-2-enyltropolone (**89**) product suggested the 1,4-electrophilic attack, discussed above, was favoured in pyridine.

4.5.3 Derivatives of 3-(1-Formylmethyl)butyltropolone (92)

The aldehyde group of 3-(1-formylmethyl)butyltropolone (**92**) provides the opportunity for introducing other functional groups into the structure that could potentially influence the antifungal properties of the tropolone. The aldehyde group of 3-(1-formylmethyl)butyl-tropolone (**92**) was successful oxidised with freshly prepared Ag_2O to give 3-(1-carboxymethyl)butyltropolone (**95**) in 71% yield. The IR spectrum exhibited acid hydroxyl and carbonyl stretching vibrations at 3200-3600 cm^{-1}, 2500-2700 cm^{-1} and 1712 cm^{-1} respectively. The GC/MS spectrum of the TMSi derivative displayed at molecular ion of *m/z* 380 ($C_{13}H_{14}O_4$ + 2 $SiMe_3$) and base peak of *m/z* 360 ($M^{\cdot+}$-CH_3). The ^1H and ^{13}C NMR spectra of the product supported the assigned structure (**95**). A feature of the ^1H NMR spectrum was the non-equivalence of the methylene protons *alpha* to the carboxylic acid (two doublet of doublets at δ 2.68 and 2.76 ppm) resulting from the chiral centre at C8. A signal at δ 177.4 ppm in the ^{13}C NMR spectrum was attributed to the acid carbonyl. High resolution MS analysis verified the product as 3-(1-carboxymethyl)butyltropolone (**95**) (found 236.1045 Da; $C_{13}H_{16}O_4$ requires 236.1049 Da). This is the first reported synthesis of 3-(1-carboxy-methyl)butyltropolone (**95**).

Reduction of 3-(1-formylmethyl)butyltropolone (**92**) with $NaBH_4$ gave 3-(1-hydroxyethyl)-butyltropolone (**96**) in 78% yield. The GC/MS spectrum of the TMSi derivative consisted of a single peak with a molecular ion of *m/z* 366 ($C_{13}H_{16}O_3$ + 2 $SiMe_3$), a base peak of *m/z* 351 and fragments of *m/z* 250 (25%) and 205 (41%). Possible fragmentation pathways to these ions are depicted in Figure 4.16. Loss of CH_3 from the $SiMe_3$ group would give the base peak ion. This ion could then undergo a McLafferty rearrangement within the alkyl chain to eject ethane and CH_2=CHOSiMe_3 and yield the *m/z* 205 ion (Figure 4.16). The molecular ion could undergo allylic cleavage through the C8-C12 bond with proton abstraction by the tropolone carbonyl to give the *m/z* 250 fragment as illustrated.

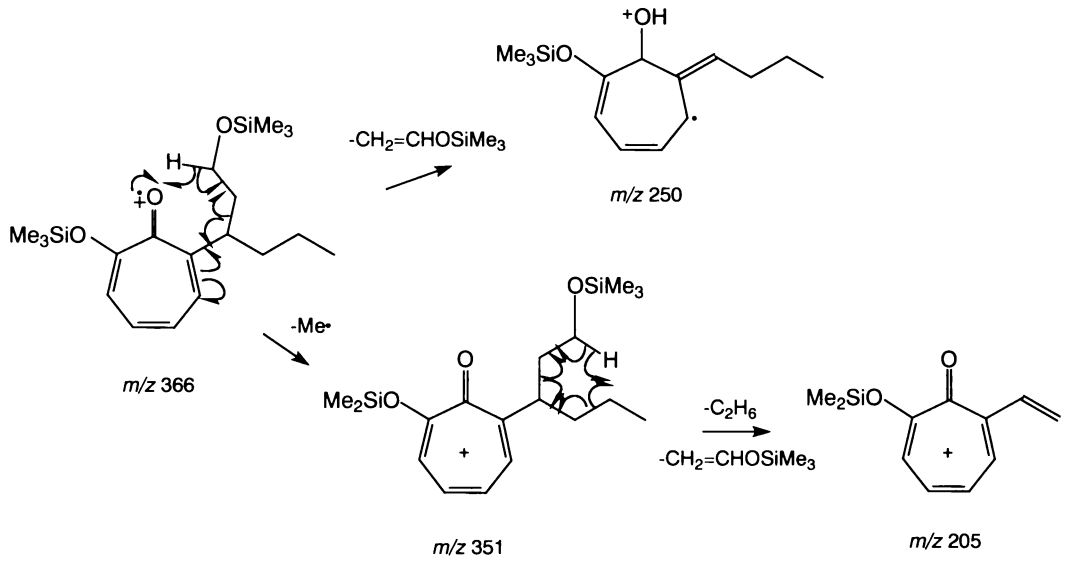


Figure 4.16: Proposed MS fragmentation of 3-(1-trimethylsilyloxyethyl)butyltropolone trimethylsilyl ether.

The ^1H and ^{13}C NMR spectra were consistent with the designated structure (**96**) (Section 7.2.12). Signal assignments were verified from ^1H-^1H and ^{13}C-^1H NMR correlations observed in the gCOSY, HMQC and HMBC spectra. As with 3-(1-carboxymethyl)butyltropolone (**95**) the ^1H NMR spectrum of 3-(1-hydroxyethyl)butyltropolone (**96**) exhibited non-equivalent methylene protons due to the chiral centre at C8. The product was verified as 3-(1-hydroxy-ethyl)butyltropolone (**96**) by high resolution MS analysis (found 222.1257 Da; $C_{13}H_{18}O_3$ requires 222.1256 Da). This is the first reported synthesis of 3-(1-hydroxyethyl)butyltropolone (**96**).

Reduction of the aldehyde group of 3-(1-formylmethyl)butyltropolone (**92**) was required in order to achieve the objective of a α-thujaplicin analogue. A Wolff-Kishner reduction appeared feasible, however the resistance of the tropolone functionality to reduction under such conditions was uncertain. Reduction of 3-(1-formylmethyl)butyltropolone (**92**) with hydrazine hydrate and KOH in triethylamine gave 3-(1-ethyl)butyltropolone (**97**) in 11% yield after chromatographic purification. The mass spectrum exhibited a molecular ion of *m/z* 206 ($C_{13}H_{18}O_2$) and a base peak ion of *m/z* 177 resulting from cleavage of a CH_2CH_3 radical. Other fragment ions of *m/z* 163 and 149 were formed by losses of a $CH_2CH_2CH_3$ radical and a CH_2CH_3 radical + CO respectively. The presence of two methyl proton signals at δ 0.78 and 0.84 ppm and two methyl carbon signals at δ 11.8 and 14.2 ppm in the ^1H and ^{13}C NMR spectra confirmed the successful reduction of the aldehyde. The ^1H-^1H and ^{13}C-^1H NMR correlations observed in the gCOSY, HMQC and HMBC experiments provided further evidence for 3-(1-ethyl)butyltropolone (**97**) as the product (Appendix G, Table G4). High resolution MS data (found 206.1301 Da; $C_{13}H_{18}O_2$ requires 206.1307 Da) was consistent with identification of the product as 3-(1-ethyl)butyltropolone (**97**). This is the first reported synthesis of 3-(1-ethyl)butyltropolone (**97**).

...man will occasionally stumble over the truth, but usually manages to pick himself up, walk over or around it and carry on.

Winston Churchill

Chapter 5: Antifungal Activity Assays

5.1 Introduction

This chapter discusses antifungal activity assay results of the synthetic terpenoid and tropolone compounds described in Chapters 2, 3 and 4. As a means of assessing their wood protection potential these analogues were assayed against representative species from each of the major types of wood deteriorating fungi. The test organisms included brown and white rot basidiomycetes, *Coniophora puteana* and *Trametes versicolor*, the soft rot ascomycete, *Chaetomium globosum* and the sapstain deuteromycete, *Sphaeropsis sapinea* (formerly *Diplodia pinea*). The differences in the modes by which these fungi deteriorate wood were discussed in Section 1.2.2. Various intermediate compounds synthesised on the way to the target synthetic terpenoid and tropolone analogues were also tested for activity. The natural lead compounds upon which the analogues were based were included for comparative purposes where possible. As a measure of wood protection potential, the compound activities were compared against the wood preservatives and sapstain control agents TBTN (**98**), IPBC (**99**) and PCP (**100**) on the basis of minimum inhibitory concentrations (MICs). Compounds were initially screened against the basidiomycetes, with only the target terpenoid analogues and the more active of the tropolone analogues tested against the soft rot and sapstain fungi.

5.2 Terpenoid Analogues

5.2.1 β-Terpineol Analogue, 1-Methyl-4-nonylcyclohexan-1-ol (5)

5.2.1.1 Brown Rot Fungus, *Coniophora puteana*

Brown rot fungi are generally the most important deteriorators of softwood timbers in countries of temperate climate. *Coniophora puteana* is found in temperate regions throughout Europe, UK, USA, Australia and New Zealand and generally attacks softwoods rather than hardwoods (8). As a degrader of cellulose and hemicelluloses, *Coniophora puteana* is well suited to the test method employed, which utilised cellulose filter paper as a wood substitute.

The assay method (Section 7.3.1.1) was developed using this fungus and found to give reproducible results (178). Additionally *Coniophora puteana* is a commonly used decay fungus in laboratory trials and shows some tolerance to certain preservatives (8).

The activities of β-terpineol analogue, 1-methyl-4-nonylcyclohexan-1-ol (**5**) and related compounds at a variety of concentration loadings are presented in Table 5.1. Activities are presented in terms of the average extent of fungal growth at various treatment concentrations (% mass of compound/mass of filter paper (% m/m)). The minimum concentration required to completely inhibit the growth of the fungus (MIC) provides a relative measure of antifungal efficacy. The maximum growth possible by the fungus on the filter papers (*i.e.* 8.5 cm) was observed in the water and dichloromethane controls (Table 5.1).

Table 5.1: Mean colony diameter (cm) of *Coniophora puteana* grown on filter papers treated with the β-terpineol analogue (**5**), 4-nonylcyclohexanone (**20**), TBTN (**98**) and controls.

Compound (with estimated purity[1])	**Calculated treatment loading (% m/m)**	**Av. radial growth of *C. puteana* (cm)**	**Minimum inhibitory concentration (MIC)**
β-terpineol analogue (**5**) (*cis* and *trans*) (*ca.* 98%)	2	1.6[2]	
	0.6	1.6[2]	
	0.2	2.4	>0.2%≤0.6%
	0.1	3.4	
	0.02	8.0	
β-terpineol analogue (**21**) (*cis*) (*ca.* 100%)	2	1.9	>2%
	0.6	2.7	
	0.2	2.5	
β-terpineol analogue (**22**) (*trans*) (*ca.* 100%)	2	0.8[2]	
	0.6	1.5[2]	>0.2%≤0.6%
	0.2	1.7	
4-nonylcyclohexanone (**20**) (*ca.* 98%)	2	2.6	>2%
	0.6	3.3	
	0.2	5.0	
	0.1	6.1	
TBTN (**98**)	0.2	0	
	0.1	0	
	0.02	0	
	0.01	0	
	0.002	1.6	>0.002%≤0.01%
	0.001	2.5	
	0.0002	6.5	
Water		8.5	
Dichloromethane		8.5	

[1] Estimate of purity based on GC/MS or microanalytical analysis; [2] Fungal growth confined to inoculated AA disc.

The β-terpineol analogue (**5**) (mixture of both isomers) showed moderate fungistatic activity against *Coniophora puteana*. Fungal growth at a treatment loading of 0.6% was confined to the inoculated AA disc and did not encroach onto the treated papers. Partial growth inhibition was observed down to the 0.1% loading. Complete control required a concentration of >0.2%≤0.6% (Table 5.1). The antifungal activities of the *cis* and *trans* isomers (**21**, **22**) were compared to assess whether the tertiary alcohol stereochemistry influences the antifungal effect. The *trans* isomer (**22**) showed superior inhibitory activity to that of the *cis* isomer (**21**) (Table 5.1). Although the differences were relatively small, this trend was consistent at all three test concentrations. 4-Nonylcyclohexanone (**20**) was weakly fungistatic against the brown rot fungus at the higher treatment loadings (Table 5.1). The β-terpineol analogue (**5**) was only weakly active relative to TBTN (**98**) which was extremely potent against *C. puteana* with a MIC of >0.002%≤0.01%.

5.2.1.2 White Rot Fungus, *Trametes versicolor*

White rot fungi differ from brown rot fungi in that they degrade both carbohydrates and lignin polymers. Typically, white rot fungi are more problematic degraders of hardwoods than softwoods, for the reason discussed in Section 1.2.3.5. *Trametes versicolor* (formerly *Coriolus versicolor*) is a commonly used laboratory assay white rot fungus. It is widespread throughout temperate countries and is known to attack softwoods (8). *Trametes versicolor* degrades wood components in a simultaneous fashion (Section 1.2.2.1) and therefore requires lignin and cellulose substrates to fully utilise its degradative enzymatic capabilities. Growth rates of this fungus on cellulose filter paper were inadequate to enable assessment within a suitable timeframe. Consequenctly antifungal assays were initially carried out on thermo-mechanical pulp (TMP) handsheets, which are rich in cellulose and lignin. Satisfactory growth rates and consistent results were achieved on this medium. However, due to the time and labour intensive requirements of preparing large numbers of handsheets from TMP, this medium was subsequently replaced by commercial newsprint paper. (Tables 5.15, 5.19, 5.22). Newsprint paper, which is chemically very similar to TMP, proved a suitable substitute, the growth rate of the fungus was not adversely effected and appropriate treatment levels were readily achievable. The diameter of both the TMP and newsprint samples was 8.0 cm, hence the maximum growth attainable for *T. versicolor* was set at this limit.

The β-terpineol analogue (**5**) was only weakly fungistatic against the white rot fungus *Trametes versicolor* (Table 5.2). Although significant impediment of growth was observed at a 2% loading, little improvement in activity was achieved at double this concentration and complete control was not achieved. 4-Nonylcyclohexanone (**20**) was weakly inhibitory with fungal growth appreciably retarded only at concentrations of 3% and above. TBTN (**98**), on the other hand, showed a MIC in the order of 0.5%. The efficacy of TBTN (**98**) against *T. versicolor* was approximately an order of magnitude superior to that of the β-terpineol analogue, 1-methyl-4-nonylcyclohexan-1-ol (**5**).

Table 5.2: Mean colony diameter (cm) of *Trametes versicolor* grown on mechanical pulp handsheets treated with the β-terpineol analogue (**5**), 4-nonylcyclohexanone (**20**), TBTN (**98**) and controls.

Compound	**Calculated treatment loading (% m/m)**	**Av. radial growth of *T. versicolor* (cm)**	**Minimum inhibitory concentration (MIC)**
β-terpineol analogue (**5**) (*cis* and *trans*)	4	1.9	>4%
	3	1.7	
	2	2.1	
	0.6	3.1	
	0.2	5.6	
	0.1	6.2	
4-nonylcyclohexanone (**20**)	4	1.5	>4%
	3	1.7	
	2	3.9	
	0.6	6.1	
TBTN (**98**)	0.5	1.3[1]	
	0.2	1.9	>0.2%≤0.5%
	0.1	2.0	
	0.02	7.2	
Water		7.3	
Dichloromethane		7.8	

[1] Fungal growth confined to inoculated AA disc.

5.2.1.3 Soft Rot Fungus, *Chaetomium globosum*

In general soft rot fungi are more resilient to wood preservatives than brown rot fungi and, in some cases, white rot fungi (7). Soft rot fungi are competitively less aggressive than basidiomycetes, however they become important in wood decay where marginal preservative concentrations (or other conditions) are sufficient to prevent infestation by basidiomycetes but are inadequate to deter soft rot species (7, 8). Therefore, it was important to test the activities of the synthetic terpenoid and tropolone analogues against an important soft rot decay fungus. *Chaetomium globosum* causes terrestrial soft rot in softwoods and is regarded as a good

organism for laboratory testing (8). *Chaetomium globosum* is a degrader of cellulose, hemicellulose and lignin and was found to grow especially well on newsprint paper. *Chaetomium globosum* exhibited a greater tolerance for TBTN (**98**) compared to the two basidiomycetes. At the 0.1% loading, TBTN (**98**) prevented the growth of *C. puteana* and strongly inhibited the growth of *T. versicolor*, but was ineffective against *C. globosum* (Tables 5.1-5.3).

The β-terpineol analogues (*cis* (**21**) and *trans* (**22**)) were only weakly active against *C. globosum*. The *cis* isomer (**21**) was slightly more effective, showing good inhibitory action at a 2.0% loading, while the *trans* isomer (**22**) retarded fungal growth by *ca.* 50% at this concentration (Table 5.3). This was the opposite trend to that observed for the *cis* and *trans* isomers against *C. puteana*. Complete coverage of the water and solvent treated controls was achieved in one week in the case of *C. globosum*, compared to two weeks for the basidiomycetes (Table 5.3).

Table 5.3: Mean colony diameter (cm) of *Chaetomium globosum* grown on newsprint papers treated with the β-terpineol analogues (**21**, **22**) and TBTN (**98**).

Compound	Calculated treatment loading (% m/m)	Av. radial growth of *C. globosum* (cm)	Minimum inhibitory concentration (MIC)
β-terpineol analogue (**21**) (*cis*)	2	1.6	>2%
	0.5	7.4	
	0.1	8.0	
β-terpineol analogue (**22**) (*trans*)	2	3.5	>2%
	0.5	6.5	
	0.1	8.0	
TBTN (**98**)	2	0	
	1	0.9[1]	1%
	0.1	7.3	
	0.01	8.0	
Water		8.0[2]	
Dichloromethane		8.0[2]	

[1] Fungal growth confined to inoculated AA disc; [2] Complete coverage of newsprint samples after 1 week of incubation.

5.2.1.4 Sapstain Fungus, *Sphaeropsis sapinea*

Staining fungi deteriorate wood through discolouration rather than decay. *Sphaeropsis sapinea* was selected as a suitable test organism for the newsprint paper based method after preliminary trials with three staining fungi. *Ophiostoma piliferum* and *Alternaria alternata*

were the other two organisms (Section 7.3.2). The β-terpineol analogues (**21**, **22**) were essentially inactive against the staining fungus, *Sphaeropsis sapinea*. The *trans* isomers (**22**) slightly hindered fungal growth at the 2% loading (Table 5.4). In contrast, IPBC (**99**) exhibited excellent activity in this assay with a MIC of >0.01%≤0.1% (Table 5.4). PCP (**100**) was somewhat less active, but still considerably more effective than 1-methyl-4-nonylcyclohexan-1-ol (**21, 22**).

Table 5.4: Mean colony diameter (cm) of *Sphaeropsis sapinea* grown on newsprint papers treated with the β-terpineol analogues (**21, 22**), PCP (**100**) and IPBC (**99**).

Compound	**Calculated treatment loading (% m/m)**	**Av. radial growth of *S. sapinea* (cm)**	**Minimum inhibitory concentration (MIC)**
β-terpineol analogue (**21**) (*cis*)	2	8.0	
	0.5	8.0[1]	
	0.1	8.0[1]	
β-terpineol analogue (**22**) (*trans*)	2	4.7	>2%
	0.5	8.0	
	0.1	8.0[1]	
PCP (**100**)	1	0	
	0.1	1.9	>0.1%≤1.0%
	0.01	7.7	
IPBC (**99**)	1	0	
	0.1	0	
	0.01	2.2	>0.01%≤0.1%
Water		8.0[1]	
Dichloromethane		8.0[1]	

[1] Complete coverage of newsprint sample after 1 week of incubation.

5.2.2 Thymol Analogue, 2-Isopropyl-4-nonylphenol (4)

5.2.2.1 Brown Rot Fungus, *Coniophora puteana*

The results of the bioassays of the thymol analogue, 2-isopropyl-4-nonylphenol (**4**) and associated compounds against *C. puteana* are summarised in Table 5.5. The thymol analogue (**4**) was moderately toxic to this fungus, displaying a MIC of 0.2%≤0.6% and substantial inhibitory action at concentrations as low as 0.1%. It should be noted that 2-isopropyl-4-nonylphenol (**4**) was estimated at 91% pure by GC/MS analysis. The minor di-isopropylphenol impurites could have contributed to the observed antifungal activity. Thymol (**1**) was significantly more effective with a MIC of >0.02%≤0.1% (Table 5.5). The extra alkyl

character considered important for persistence in wood (Section 2.1) has led to a lower efficacy against *C. puteana*. The inactivity of the di-nonylphenol, possibly 2,4-dinonylphenol (**30**) reinforces this trend of lower activity with increased alkyl substitution. This assay method does not accommodate any possible benefits due to enhanced water repellency (as would be observed in natural exposure or laboratory leaching trials).

Table 5.5: Mean colony diameter (cm) of *Coniophora puteana* grown on filter papers treated with the thymol analogue (**4**) and related compounds.

Compound (with estimated purity[1])	**Calculated treatment loading (% m/m)**	**Av. radial growth of *C. puteana* (cm)**	**Minimum inhibitory concentration (MIC)**
thymol analogue (**4**) (*ca.* 91%)	2	0	
	0.6	0	
	0.2	1.7	>0.2%≤0.6%
	0.1	2.0	
	0.02	5.8	
	0.002	8.5	
2,4-di-nonylphenol (**30**) (*ca.* 95%)	2	4.0	>2%
	0.6	4.8	
p-n-nonylphenol (**9**) (*ca.* 100%)	2	0	
	0.6	0	
	0.2	1.7	>0.2%≤0.6%
	0.1	2.1	
	0.02	2.3	
	0.002	7.0	
commercial nonylphenol	1	0	
	0.2	0	
	0.1	0	≤0.1%
p-n-nonylanisole (**7**) (*ca.* 100%)	2	4.3	>2%
	0.6	7.4	
thymol (**1**)	2	0	
	0.6	0	
	0.2	0	
	0.1	0	
	0.02	8.5	>0.02%≤0.1%

[1] Estimate of purity based on GC/MS or microanalytical analysis.

The activity of the *p-n*-nonylphenol (**9**) (MIC >0.2%≤0.6%) was equivalent to that of the thymol analogue (**4**) indicating no enhancement or decline in activity due to *o*-isopropylation. The antifungal activities of (**4**), (**9**) and the di-nonylphenol (possibly **30**) suggest that there may be an optimum level of alkylation to achieve both antifungal activity and preservative retention in wood (*i.e.* water repellency). The phenol group was a necessary element for toxicity as *p-n*-nonylanisole (**7**) was largely inactive (Table 5.5). It is interesting to note that commercial nonylphenol, which consists of a mixture of isomers with various branchings of

the alkyl chain, showed enhanced efficacy over the straight chained *p-n*-nonylphenol (**9**) against *C. puteana*. The thymol analogue (**4**) was less effective than TBTN by at least an order of magnitude on the basis of MIC values (Tables 5.1 and 5.5).

5.2.2.2 White Rot Fungus, *Trametes versicolor*

The thymol analogue, 2-isopropyl-4-nonylphenol (**4**), was only weakly fungistatic against *T. versicolor*. At the 2.0% loading approximately a 50% inhibition of fungal growth was observed (Table 5.6). The di-isopropylphenol impurities presence in the 2-isopropyl-4-nonylphenol (**4**) sample may have contributed to this weak activity. By comparison thymol (**1**) was relatively active, demonstrating a MIC of >0.5≤1.0%. The efficacy of thymol was approximately half that of TBTN (**98**) (Tables 5.2 and 5.6). As with the *C. puteana* bioassay the addition of an inert alkyl chain has lead to a reduction in efficacy.

Table 5.6: Mean colony diameter (cm) of *Trametes versicolor* grown on mechanical pulp handsheets treated with the thymol analogue (**4**) and related compounds.

Compound	**Calculated treatment loading (% m/m)**	**Av. radial growth of *T. versicolor* (cm)**	**Minimum inhibitory concentration (MIC)**
thymol analogue (**4**)	2	3.5	
	1	3.8	
	0.6	5.7	
	0.2	6.9	
	0.1	6.5	
thymol (**1**)	2	0	
	1	0	
	0.5	2.6	>0.5%≤1.0%
	0.2	4.6	
	0.06	6.8	
	0.02	7.0	
p-n-nonylphenol (**9**)	2	2.0	>2%
	1	1.9	
	0.6	2.3	
	0.2	4.0	
	0.1	5.5	
	0.02	7.3	
commercial nonylphenol	2	1.3	>2%
	1	2.2	
	0.5	2.4	
p-n-nonylanisole (**7**)	4	6.0	
	2	7.0	
2,4-dinonylphenol (**30**)	2	7.9	

p-n-Nonylphenol (**9**), although more active than its isopropylated derivative, could only be classed as weakly fungistatic against *T. versicolor*. Commercial branched nonylphenol was of comparable activity to the straight chain version (**9**). The di-nonylphenol, possibly 2,4-dinonylphenol (**30**) and *p-n*-nonylanisole (**7**) were inactive (Table 5.6).

5.2.2.3 *Chaetomium globosum* and *Sphaeropsis sapinea*

Only the thymol analogue, 2-isopropyl-4-nonylphenol (**4**), thymol (**1**) and *p-n*-nonylphenol (**9**) were assayed against the soft rot fungus *Chaetomium globosum* and sapstain fungi *Sphaeropsis sapinea*. The anti-basidiomycetes activity of the other compounds did not justify their inclusion in these assays. The thymol analogue (**4**) was inactive against both *C. globosum* and *S. sapinea*; very little inhibitory action was observed at the highest loading of 2% (Table 5.7). Conversely, thymol (**1**) showed moderate activity, completely preventing and strongly impeding the growth of the two fungi at the 2% and 0.5% levels respectively. *p-n*-Nonylphenol (**9**) showed slightly superior antifungal activity to that of the thymol analogue (**4**) against both fungi (Table 5.7). *p-n*-Nonylphenol (**9**) was marginally more effective against the soft rot fungus than the sapstain fungus. Little differentiation in efficacy between the two species was seen with the other two phenols (**4**) and (**1**). Although the synthetic thymol analogue was ineffectual, the toxicities of thymol (**1**) and TBTN (**98**) against *C. globosum* were similar (Tables 5.3 and 5.7).

Table 5.7: Mean colony diameter (cm) of *Chaetomium globosum and Sphaeropsis sapinea* grown on newsprint papers treated with the thymol analogue (**4**), thymol (**1**) and *p-n*-nonylphenol (**9**).

Compound	**Treatment**	**Av. radial fungal growth (cm)**		**MIC**	
	loading (% m/m)	***C. globosum***	***S. sapinea***	***C. globosum***	***S. sapinea***
thymol analogue (**4**)	2	6.6	5.8		
	0.5	6.0	6.6		
	0.1	7.8	8.0[1]		
thymol (**1**)	2	0	0		
	0.5	3.3	3.4	>0.5%≤2.0%	>0.5%≤2.0%
	0.1	8.0	8.0		
p-n-nonylphenol (**9**)	2	2.6	4.1	>2.0%	>2.0%
	0.5	3.9	6.3		
	0.1	5.3	8.0		

[1] Complete coverage of newsprint sample after 1 week of incubation.

5.2.3 Totarol Analogue, 1-Isopropyl-2-naphthol (6)

5.2.3.1 Brown Rot Fungus, *Coniophora puteana*

The bioassay results of the simplified totarol analogue, 1-isopropyl-2-naphthol (**6**), its autoxidation product, 1-hydroperoxy-1-isopropyl-2-(1*H*)-naphthalenone (**33**) and totarol (**3**) against *C. puteana* are collated in Table 5.8. Treatment solutions of 1-isopropyl-2-naphthol (**6**) and 1-hydroperoxy-1-isopropyl-2-(1*H*)-naphthalenone (**33**) were prepared in MeOH, hence the inclusion of this solvent as a control (Table 5.8). 1-Isopropyl-2-naphthol (**6**) exhibited significant fungicidal activity against *C. puteana* with a MIC of *ca.* 0.1% and fungistatic activity at a 0.05% loading. Totarol (**3**) was not fungitoxic at any of the test concentrations, although strong impediment of fungal growth was observed at concentrations as low as 0.02% (Table 5.8). 2-Naphthol (**31**), like 1-isopropyl-2-naphthol (**6**), showed a MIC of *ca.* 0.1%, indicating that *o*-isopropylation did not improve the toxicity of the naphthol to *C. puteana*.

Table 5.8: Mean colony diameter (cm) of *Coniophora puteana* grown on filter papers treated with totarol analogue, 1-isopropyl-2-naphthol (**6**) and related compounds.

Compound (with estimated purity[1])	**Calculated treatment loading (% m/m)**	**Av. radial growth of *C. puteana* (cm)**	**Minimum inhibitory concentration (MIC)**
1-isopropyl-2-naphthol (**6**) (*ca.* 98%)	2	0	
	0.6	0	
	0.2	0	
	0.1	0	
	0.05	2.3	>0.05≤0.1%
	0.02	5.6	
totarol (**3**) (*ca.* 100%)	2	2.2	>2%
	0.2	2.1	
	0.1	1.8	
	0.02	2.5	
	0.002	6.3	
2-naphthol (**31**)	2	0	
	0.6	0	
	0.2	0	
	0.1	0	
	0.02	3.7	>0.02≤0.1%
	0.002	8.5	
1-hydroperoxy-1-isopropyl--2-(1*H*)-naphthalenone (**33**) (*ca.* 100%)	0.2	0	
	0.1	0	
	0.02	5.1	>0.02≤0.1%
	0.002	7.7	
MeOH		8.5	

[1] Estimate of purity based on GC/MS analysis.

The autoxidation product, 1-hydroperoxy-1-isopropyl-2-(1*H*)-naphthalenone (**33**), also exhibited a MIC of *ca.* 0.1%. Evidently, the hydroperoxy α-β-unsaturated ketone functional group is comparable in its activity to that of the phenolic group in the case of *C. puteana*. The fungitoxicities of these compounds were still substantially less than TBTN (**98**); 1-isopropyl-2-naphthol (**6**) was approximately 5-fold less effective (Tables 5.1 and 5.8).

5.2.3.2 White Rot Fungus, *Trametes versicolor*

The totarol analogue, 1-isopropyl-2-naphthol (**6**), showed only weak fungistatic activity against *T. versicolor*. Significant growth inhibition was observed at the 2% loadings (Table 5.9), yet complete prevention was not achieved. In contrast, totarol (**3**) was ineffective with very little inhibitory action observed for treatment concentrations up to 4% (Table 5.9). 2-Naphthol (**31**) was of similar activity to the isopropylated derivative with a MIC in excess of 2%. A MIC against *T. versicolor* of between 1% and 2% was obtained for 1-hydroperoxy-1-isopropyl-2-(1*H*)-naphthalenone (**33**).

Table 5.9: Mean colony diameter (cm) of *Trametes versicolor* grown on mechanical pulp handsheets treated with the totarol analogue, 1-isopropyl-2-naphthol (**6**) and related compounds.

Compound	**Calculated treatment loading (% m/m)**	**Av. radial growth of *T. versicolor* (cm)**	**Minimum inhibitory concentration (MIC)**
1-isopropyl-2-naphthol (**6**)	2	1.3	>2%
	1	3.0	
	0.6	5.9	
	0.2	7.5	
	0.1	7.7	
totarol (**3**)	4	6.3	>4%
	3	5.9	
	2	5.1	
	0.2	6.0	
	0.1	7.4	
2-naphthol (**31**)	2	1.9	>2%
	1	2.7	
	0.2	7.6	
	0.06	7.4	
	0.02	7.6	
1-hydroperoxy-1-isopropyl-	2	0	
-2-(1*H*)-naphthalenone (**33**)	1	2.1	>1%≤2%
	0.2	6.8	
	0.06	7.9	
	0.02	8.0	
MeOH		7.7	

5.2.3.3 *Chaetomium globosum and Sphaeropsis sapinea*

The totarol analogue, 1-isopropyl-2-naphthol (**6**) was the most active of the synthetic terpenoid compounds against *C. globosum* and *S. sapinea*. While totarol was inactive against both fungi, 1-isopropyl-2-naphthol (**6**) exhibited MICs of >0.1%≤0.5% and >0.5%≤2% against the soft rot and sapstain fungi respectively (Table 5.10). Isopropylation of 2-naphthol (**31**) appeared to have no effect on activity against *C. globosum* and yet led to a measurable decline in efficacy against *S. sapinea*. MICs of >0.1%≤0.5% against both fungi were recorded for 2-naphthol (**31**). 1-Hydroperoxy-1-isopropyl-2-(*1H*)-naphthalenone (**33**) was less active than 1-isopropyl-2-naphthol (**6**) with MICs of >0.5%≤2% observed against both fungi (Table 5.10).

Table 5.10: Mean colony diameter (cm) of *Chaetomium globosum* and *Sphaeropsis sapinea* grown on newsprint papers treated with the totarol analogue, 1-isopropyl-2-naphthol (**6**) and related compounds.

Compound	Treatment loading (% m/m)	Av. radial fungal growth (cm)		MIC	
		C. globosum	*S. sapinea*	*C. globosum*	*S. sapinea*
1-isopropyl-2-naphthol (**6**)	2	0	0		
	0.5	0	2.5		>0.5%≤2%
	0.1	5.3	8.0	>0.1%≤0.5%	
totarol (**3**)	2	6.3	6.9		
	0.5	5.1	7.4		
	0.1	6.9	8.0[1]		
2-naphthol (**31**)	2	0	0		
	0.5	0	0		
	0.1	6.7	5.6	>0.1%≤0.5%	>0.1%≤0.5%
1-hydroperoxy-1-isopropyl-2-(1*H*)-naphthalenone (**33**)	2	0	0		
	0.5	1.8	5.5	>0.5%≤2%	>0.5%≤2%
	0.1	8.0	8.0		

[1] Complete coverage of newsprint sample after 1 week of incubation.

5.2.4 4-Nonylcyclohexane-1,2-epoxide (39) and Simplified Analogues of α-Cadinol and Himachalol

5.2.4.1 *Coniophora puteana* and *Trametes versicolor*

The results of antifungal assays of 4-nonylcyclohexane-1,2-epoxide (**39**), 4-ethyl-1-methyl-1,2,3,4-tetrahydronaphth-1-ol (**40**) and 5,9-dimethyl-6,7,8,9-tetrahydrobenzocyclohepten-5-ol (**44**) against the basidomycetes *Coniophora puteana* and *Trametes versicolor* are tabulated in

Table 5.11. The nonylcyclohexane epoxide (**39**) completely inhibited the growth of the brown rot fungus at the 0.2% and 0.6% loadings. A MIC of >0.1≤0.2% was recorded against *C. puteana*. The epoxide (**39**) was considerably less effective against *T. versicolor*, with only weak fungistatic activity observed at the 2% loading (Table 5.11). 4-Nonylcyclohexane-1,2-epoxide (**39**) exhibited superior efficacy to that of the sesquiterpenoid epoxide rich totara oil fraction, TF8f2 (see Chapter 3) against both fungi.

Table 5.11: Mean colony diameter (cm) of *Coniophora puteana* and *Trametes versicolor* grown on newsprint papers treated with synthetic epoxide and tertiary alcohol compounds.

Compound	**Treatment**	**Av. radial fungal growth (cm)**		**MIC**	
(with estimated purity[1])	**loading (% m/m)**	***C. puteana***	***T. versicolor***	***C. puteana***	***T. versicolor***
4-nonylcyclohexane-	2		4.2		
1,2-epoxide (**39**)	0.6	0	7.6		
(*ca.* 97%)	0.2	0	8.0		
	0.1	7.4		>0.1%≤0.2%	
TF8f2	2		7.3		
	0.6	0	8.0		
	0.2	8.5	8.0	>0.2%≤0.6%	
	0.1	8.5			
50:50 mixture of (**45**)	2		0		
and (**46**)[2]	0.6	0	8.0		>0.6%≤2%
	0.2	0			
	0.1	7.8		>0.1%≤0.2%	
85:15 mixture of (**40**)	2		0		
and (**44**)[2]	0.6	0	3.5		>0.6%≤2%
	0.2	0	8.0		
	0.1	0			
	0.05	8.5		>0.05%≤0.1%	
79:21 mixture of (**44**)	2		0		
and (**40**)[2]	0.6	0	7.3		>0.6%≤2%
	0.2	0	8.0		
	0.1	0			
	0.05	8.2		>0.05%≤0.1%	
α-cadinol (**38**)	2		1.4		>2%
(*ca.* 100%)	0.6	0	3.8		
	0.2	0	7.3		
	0.1	4.4	8.0	>0.1%≤0.2%	
Kf6	2		1.6		>2%
	0.6	0	5.2		
	0.2	0	7.6		
	0.1	0	7.8		
	0.05	8.0		>0.05%≤0.1%	

[1] Estimate of purity based on GC/MS analysis; [2] Estimated composition of mixture based on GC/MS analysis.

The mixture which contained mainly 4-ethyl-1-methyl-1,2,3,4-tetrahydronaphth-1-ol (**40**) (*ca.* 85%), a simplified analogue of α-cadinol (**38**), exhibited good anti-basidomycetes properties. This mixture completely preventing the growth of *C. puteana* and *T. versicolor* at 0.1% and 2.0% loadings respectively (Table 5.11). The MIC of >0.05≤0.1% recorded for this mixture against *C. puteana* was superior to pure α-cadinol (**38**). The mixture which contained mainly 5,9-dimethyl-6,7,8,9-tetrahydrobenzocyclohepten-5-ol (**44**) (*ca.* 79%), a simplified analogue of himachalol, was equally as effective as the α-cadinol analogue (**40**) rich mixture against both fungi. MICs of >0.05≤0.1% and >0.6%≤2.0% against *C. puteana* and *T. versicolor* respectively were recorded for this mixture (Table 5.11). Both tertiary alcohol mixtures (mainly **40**) and (mainly **44**) were equivalent in efficacy to that of the kahikatea foliage oil fraction 6 (Kf6) which contained several sesquiterpenoid tertiary alcohols including α-cadinol (**36**) and globulol (**37**). The mixture of 4-ethyl-α-tetralone (**45**) and 9-methyl-6,7,8,9-tetrahydrobenzocyclohepten-5-one (**46**) was also active against the decay fungi, completely inhibiting growth of *C. puteana* and *T. versicolor* at 0.2% and 2.0% loadings respectively.

5.2.4.2 *Chaetomium globosum* and *Sphaeropsis sapinea*

The results of antifungal assays of 4-nonylcyclohexane-1,2-epoxide (**39**), 4-ethyl-1-methyl-1,2,3,4-tetrahydronaphth-1-ol (**40**) and 5,9-dimethyl-6,7,8,9-tetrahydrobenzocyclohepten-5-ol (**44**) against wood deteriorating fungi *Chaetomium globosum* and *Sphaeropsis sapinea* are tabulated in Table 5.12. The nonylcyclohexane epoxide (**39**) was completely ineffectual against both fungi at the range of concentrations tested up to a loading of 2%. The mixture of the ketones 4-ethyl-α-tetralone (**45**) and 9-methyl-6,7,8,9-tetrahydrobenzocyclohepten-5-one (**46**) was ineffective against *S. sapinea* but showed good inhibitory activity against *C. globosum* at the 2% loading. The mixture containing mainly 4-ethyl-1-methyl-1,2,3,4-tetrahydronaphth-1-ol (**40**) and the mixture containing mainly 5,9-dimethyl-6,7,8,9-tetrahydrobenzocyclohepten-5-ol (**44**) exhibited MICs of >0.5%≤2.0% against *C. globosum.* They were equivalent in activity to α-cadinol (**38**) (Table 5.12). The mixture containing mainly 4-ethyl-1-methyl-1,2,3,4-tetrahydronaphth-1-ol (**40**) showed a strong inhibitory effect against *S. sapinea* at the 2% loading, but was inactive at lower concentrations. The mixture containing mainly 5,9-dimethyl-6,7,8,9-tetrahydrobenzocyclohepten-5-ol (**44**) and α-cadinol (**38**) were virtually inactive against *S. sapinea*. Some weak fungistatic activity at the 2% level was apparent (Table 5.12).

Table 5.12: Mean colony diameter (cm) of *Chaetomium globosum* and *Sphaeropsis sapinea* grown on papers treated with synthetic epoxide and tertiary alcohol compounds.

Compound	Treatment loading (% m/m)	Av. radial fungal growth (cm) *C. globosum*	*S. sapinea*	MIC *C. globosum*
4-nonylcyclohexane-1,2-epoxide (**39**)	2	8.0	7.5	
	0.5	8.0	8.0	
	0.1	8.0	8.0	
50:50 mixture of (**45**) and (**46**)	2	1.9	7.0	>2%
	0.6	8.0	7.8	
	0.1	8.0	8.0	
85:15 mixture of (**40**) and (**44**)	2	0	1.8	
	0.5	4.8	6.4	>0.5%≤2%
	0.1	8.0	8.0	
79:21 mixture of (**44**) and (**40**)	2	0	4.3	
	0.5	4.0	7.0	>0.5%≤2%
	0.1	8.0	8.0	
α-cadinol (**38**)	2	0	4.2	
	0.5	5.3	7.6	>0.5%≤2%
	0.1	8.0	8.0	

5.2.5 Wood Protection Potential

Antifungal activities of the terpenoid analogues, which were the primary synthetic targets, along with those of the wood preservative, TBTN, and current and historical antistaining agents, IPBC and PCP are summarised in Table 5.13. In general, the terpenoid analogues were most effective against the brown rot fungus *C. puteana* against which they could be rated as moderately fungitoxic. Although minimum inhibitory concentrations in the order of 0.1% to 0.6% were observed for the six terpenoid target compounds these efficacies were considerably less than that observed for TBTN (**98**), which showed a MIC of at least 0.01%. With the exception of the simplified α-cadinol and himachalol analogues (**40**) and (**44**) the synthetic terpenoid analogues were largely inactive against *T. versicolor*. The mixtures of (**40**) and (**44**) were however significantly less effective than TBTN (**98**) against this white rot fungus (Table 5.13). The totarol analogue, 1-isopropyl-2-naphthol (**6**) showed good fungitoxicity against the soft rot fungus *C. globosum*. This activity was superior to that of TBTN (**98**) (Table 5.13). Overall the results suggest the synthetic terpenoid analogues lack sufficient toxicity and a spectrum of activity to be considered viable candidates for wood preservation with the possible exception of the mixtures of 4-ethyl-1-methyl-1,2,3,4-tetrahydronaphth-1-ol (**40**) and 5,9-dimethyl-6,7,8,9-tetrahydrobenzocyclohepten-5-ol (**44**).

Table 5.13: MICs of synthetic terpenoid analogues and the wood protection agents, TBTN (**98**), IPBC (**99**) and PCP (**100**).

Compound	**Minimum inhibitory concentration**			
	C. puteana	***T. versicolor***	***C. globosum***	***S. sapinea***
1-methyl-4-nonylcyclo-hexan-1-ol (**5**)	>0.2%≤0.6%	>4%	>2%	-
2-isopropyl-4-nonyl-phenol (**4**)	>0.2%≤0.6%	>2%	-	-
1-isopropyl-2-naphthol (**6**)	>0.05%≤0.1%	>2%	>0.1≤0.5%	>0.5%≤2%
4-nonylcyclohexane-1,2-epoxide (**39**)	>0.1%≤0.2%	-	-	-
85:15 mixture of (**40**) and (**44**)	>0.05%≤0.1%	>0.6%≤2%	>0.5%≤2%	-
79:21 mixture of (**44**) and (**40**)	>0.05%≤0.1%	>0.6%≤2%	>0.5%≤2%	-
TBTN (**98**)	>0.002%≤0.01%	>0.2%≤0.5%	1%	
IPBC (**99**)				>0.01%≤0.1%
PCP (**100**)				>0.1%≤1.0%

With the exception of 1-isopropyl-2-naphthol (**6**) the terpenoid analogues were essentially inactive against the sapstain fungus *S. sapinea* (Table 5.13). The fungitoxicity of 1-isopropyl-2-naphthol (**6**) was poor compared to IPBC (**99**) indicating little potential for its use in sapstain control formulations. The synthetic tropolones, on the other hand, appeared to show greater promise as novel wood protection agents.

5.3 Tropolone Analogues

The various synthetic tropolones described in Chapter 4, along with tropolone (**48**), the ditropolone copper chelate (**101**) and β-thujaplicin (**47**) were tested against the four test fungi with the aims of identifying potential wood protection candidates and gaining insights into structure/antifungal activity relationships. These relationships are discussed in reference to activity against the brown rot fungus *Coniophora puteana* as this was the most sensitive of the four fungi to the fungicidal effects of the tropolones. In general the relationships between the structure of the tropolone and the activity against *Coniophora puteana* were reflected in the other three fungi, although specific activities varied considerably.

5.3.1 3-Carboxy-4-carboxymethyltropolone (49) and Derivatives

3-Carboxy-4-carboxymethyltropolone (**49**) was an important intermediate in the synthesis of tropolone analogues. The anti-basidiomycetes properties of this compound, its precursor purpurogallin (**50**) and decarboxylated and esterified derivatives are discussed herein. The antifungal activities of tropolone (**48**) and Cu(tropolone)$_2$ (**101**) are introduced here for the purposes of comparison.

5.3.1.1 Brown Rot Fungus, *Coniophora puteana*

The diacid tropolone, 3-carboxy-4-methylcarboxytropolone (**49**) showed a MIC against *C. puteana* of >0.2≤0.6% (Table 5.14). This compared with a MIC of >0.002≤0.02% for 4-methyltropolone (**51**). Hence removal of the carboxyl groups resulted in a considerable enhancement in antifungal activity. This enhancement is possible due to the reduced polarity and hydrogen bonding capability of 4-methyltropolone (**51**) compared to the diacid tropolone (**49**). The diacid tropolone (**49**) may have been less available to inhibit oxidative phosphorylation processes within the fungal cell due to hydrogen bonding between its carboxyl groups and the chitin-β-glycan composed cell wall (335) of the fungus.

Esterification of the aliphatic acid group of the diacid tropolone (**49**) with a long alkyl chain resulted in a significant activity enhancement. The MIC of 3-carboxy-4-octylcarboxy-methyltropolone (**53**) was in the order of 6-fold lower (>0.02%≤0.1%) than that of the diacid tropolone (**49**). Removal of the 3-carboxyl group appeared to result in a further improvement in activity. 4-Octylcarboxymethyltropolone (**56**) was strongly fungistatic at a concentration of 0.02% and completely toxic at 0.05%, while 3-carboxy-4-octylcarboxymethyltropolone (**53**) was only marginally inhibitory at the 0.02% level (Table 5.14). Esterification of the 3-carboxyl group and decarboxylation of the aliphatic carboxyl group similarly improved antifungal activity; 3-octylcarboxy-4-methyltropolone (**55**) exhibited a MIC of >0.02%≤0.2% against *C. puteana* (Table 5.14). Esterification and decarboxylation reduced the hydrogen bonding capabilities and enhanced the lipophilicity of the tropolone. This may have resulted in a more appropriate balance of lipophilic and hydrophilic character to facilitate permeability through the fungal cell membrane.

Table 5.14: Mean colony diameter (cm) of *Coniophora puteana* grown on filter papers treated with tropolone compounds and TBTN (**98**).

Compound	**Calculated treatment loading (% m/m)**	**Av. radial growth of *C. puteana* (cm)**	**Minimum inhibitory concentration (MIC)**
3-carboxy-4-carboxy-methyltropolone (**49**)	2.6	0	
	0.6	0	
	0.2	2.3	>0.2%≤0.6%
	0.1	3.4	
	0.026	8.0	
4-methyltropolone (**51**)	2	0	
	0.2	0	
	0.02	0	
	0.002	1.6	>0.002%≤0.02%
	0.001	6.1	
	0.0002	7.8	
3-carboxy-4-octylcarboxy-methyltropolone (**53**)	2	0	
	0.2	0	
	0.1	0	
	0.02	4.3	>0.02%≤0.1%
4-octylcarboxymethyl-tropolone (**56**)	0.1	0	
	0.05	0	
	0.02	1.6	>0.02%≤0.05%
3-octylcarboxy-4-methyl-tropolone (**55**)	2	0	
	0.2	0	
	0.02	1.5	>0.02%≤0.2%
	0.01	7.4	
tropolone (**48**)	2.6	0	
	0.26	0	
	0.13	0	
	0.026	0	
	0.0026	1.3	>0.0026%≤0.026%
	0.001	7.4	
	0.0002	7.6	
$Cu(tropolone)_2$ (**101**)	0.6	0	
	0.26	0	
	0.026	0	
	0.002	3.8	>0.002%≤0.026%
	0.001	7.7	
purpurogallin (**50**)	2.6	2.9	
	0.78	2.6	
	0.2	0	
	0.1	1.3	>0.1%≤0.2%
	0.02	5.4	
TBTN (**98**)	0.02	0	
	0.002	1.6	>0.002%≤0.02%
	0.001	2.5	
Water		8.5	
Dichloromethane		8.5	
Methanol		8.5	

The efficacy of the ester tropolones (**53**), (**55**) and (**56**) was inferior to that of 4-methyltropolone (**51**). The long alkyl chain has lead to a lower specific activity, as a larger part of the structure does not contribute any fungicidal character. The ester groups are possibly susceptible to hydrolysis by H_2O at wood pH or as a result of fungal action and hence are unlikely to be suitable as long-term preservatives as saponification would liberate water soluble tropolone acids. The non-labile alkylated tropolones (Section 5.3.2) would appear more suitable candidates for wood preservation.

Tropolone (**48**) and Cu(tropolone)$_2$ (**101**) were equivalent to 4-methyltropolone (**51**) and TBTN (**98**) in their activities against *C. puteana* with MICs of >0.002≤0.026% (Table 5.14). The ester tropolones, 4-octylcarboxymethyltropolone (**56**) and 3-octylcarboxy-4-methyl-tropolone (**55**) were approximately an order of magnitude less effective than TBTN (**98**) if the degree of fungal growth at the 0.02% and 0.002% levels is used as a measure (Table 5.14). Purpurogallin (**50**), completely inhibited the fungal growth of *C. puteana* at a loading of 0.2%, making it more effective than its oxidised derivative, 3-carboxy-4-carboxymethyltropolone (**49**). At higher concentrations only partial inhibition of the fungus was observed, possibly due to crystallisation of purpurogallin (**50**) from the paper test medium.

5.3.1.2 White Rot Fungus, *Trametes versicolor*

3-Carboxy-4-carboxymethyltropolone (**49**) showed weak fungistatic activity against the white rot fungus, *T. versicolor*. A 2% loading was inhibitory, while concentrations below 2% were ineffective (Table 5.15). 4-Methyltropolone (**51**) was again significantly more active than the diacid tropolone (**49**), with strong fungistatic action at a loading as low as 0.06% and a MIC of 0.2%. 3-Carboxy-4-methyloctylcarboxytropolone (**53**) showed moderate fungistatic activity at 0.6% and 1% loadings (Table 5.15). Although MICs were not determined it was evident from the degree of fungal growth observed for (**49**) and (**53**) at loadings of 1% and 0.6% that esterification improved the activity against *T. versicolor*. This apparent enhancement in activity was less significant than that observed against *C. puteana*. 4-Octylcarboxy-methyltropolone (**56**) was not tested against *T. versicolor* due to insufficient compound. The ester tropolone, 3-octylcarboxy-4-methyltropolone (**55**), weakly hindered fungal growth at the 2% loading (Table 5.15). Both ester tropolones (**53**) and (**55**) were considerably less effective than TBTN (**98**). Cu(tropolone)$_2$ (**101**) exhibited an efficacy equivalent to that of 4-methyl-

tropolone (**51**) and superior to TBTN (**98**). Tropolone (**48**) with a MIC of 0.06% was the most potent (Table 5.15). Purpurogallin (**50**) was inactive at loadings ranging from 0.1% to 2%. Overall these tropolone compounds were less active against the white rot fungus *T. versicolor* than the brown rot fungus *C. puteana* by at least an order of magnitude.

Table 5.15: Mean colony diameter (cm) of *Trametes versicolor* grown on mechanical pulp handsheets treated with tropolone compounds and TBTN (**98**).

Compound	**Calculated treatment loading (% m/m)**	**Av. radial growth of *T. versicolor* (cm)**	**Minimum inhibitory concentration (MIC)**
3-carboxy-4-carboxy-methyltropolone (**49**)	2	2.3	>2%
	1	5.9	
	0.6	6.7	
	0.2	7.1	
	0.1	7.9	
4-methyltropolone (**51**)	0.2	0	
	0.1	1.5	>0.1%≤0.2%
	0.06	1.5	
	0.02	4.4	
	0.002	7.4	
3-carboxy-4-octylcarboxy-methyltropolone (**53**)	1	2.6	>1%
	0.6	2.7	
	0.2	7.2	
	0.1	7.5	
3-octylcarboxy-4-methyl-tropolone (**55**)	2	3.5	>2%
	1	5.3	
	0.6	4.5	
	0.2	6.9	
	0.1	6.6	
tropolone (**48**)	0.06	0	*ca.* 0.06%
	0.04	1.5	
	0.02	2.0	
	0.002	7.6	
$Cu(tropolone)_2$ (**101**)	0.2	0	
	0.1	1.8	>0.1%≤0.2%
	0.06	1.2	
	0.02	2.8	
	0.002	7.7	
purpurogallin (**50**)	2	6.5	
	0.6	7.9	
	0.2	8.0	
	0.1	8.0	
TBTN (**98**)	0.5	1.3[1]	>0.2%≤0.5%
	0.2	1.9	
	0.02	7.2	
Water		8.0	
Dichloromethane		8.0	
Methanol		8.0	

[1] Fungal growth confined to inoculated AA disc.

5.3.2 4-Alkyltropolones, Analogues of β-Thujaplicin (47)

Bioassay results for the synthetic 4-alkyltropolones and precursor compounds described in Section 4.2.3 are discussed in this section. The antifungal activities of these tropolones were compared against the wood extractive, β-thujaplicin (**47**).

5.3.2.1 Brown Rot Fungus, *Coniophora puteana*

4-(2-Hydroxynonyl)-3-carboxylic acid δ-lactone tropolone (**57**) exhibited a MIC against *C. puteana* of >0.2%≤2% and considerable inhibitory action at the 0.1% level (Table 5.16). The lactone tropolone (**57**) was less active than 3-carboxy-4-octylcarboxymethyltropolone (**53**) (Tables 5.14 and 5.16). The rigidity of the structure of (**57**) may have adversely impacted on its mode of action. The lactone tropolone (**57**) was strongly water repellent, more so that the other alkyltropolones. Even at low loadings (*i.e.* 0.2%) the salt solution used to moisten the samples had difficulty permeating into the paper. High hydrophobicity may have limited the assimilation of the lactone tropolone (**57**) by the fungus.

The 4-alkenyltropolones and 4-alkyltropolones were highly fungitoxic against *C. puteana*. Minimum inhibitory concentrations of 0.02% were recorded for 4-non-1-enyltropolone (**59**), 4-nonyltropolone (**60**), 4-hept-1-enyltropolone (**61**) and 4-heptyltropolone (**63**) (Table 5.16). No significant difference in activity was observed between the 4-alkenyl and 4-alkyl structures. There was a detectable relationship between antifungal efficacy and length of the alkyl chain. 4-Pent-1-enyltropolone (**62**) and 4-pentyltropolone (**64**), with MICs of >0.004%≤0.01%, were notably more active than 4-non-1-enyltropolone (**59**) and 4-nonyltropolones (**60**) and were of comparable activity to β-thujaplicin (**47**). Based on the extent of fungal growth at the lower concentrations 4-hept-1-enyltropolone (**61**) and 4-heptyl-tropolone (**63**) could be said to be marginally more effective than 4-non-1-enyltropolone (**59**) and 4-nonyltropolones (**60**) (Table 5.16). The decline in activity with the progressively longer hydrocarbon chain, although significant, was not excessive considering the potential benefits in treatment longevity (Section 2.1) as a result of increasing hydrophobicity. The decline in activity with increasing alkyl chain length is possibly a factor of the increasing molecular weight relative to the antifungal tropolone group. Additionally there may be an effect of steric interference of enzyme inhibition with increase in hydrocarbon chain length.

Table 5.16: Mean colony diameter (cm) of *Coniophora puteana* grown on filter papers treated with 4-alkyltropolones and related compounds.

Compound	Calculated treatment loading (% m/m)	Av. radial growth of *C. puteana* (cm)	Minimum inhibitory concentration (MIC)
4-(2-hydroxynonyl)-3-carboxylic acid δ-lactone tropolone (**57**)	2	0	>0.2%≤2%
	0.2	1.8	
	0.1	2.1	
	0.02	7.2	
	0.002	8.5	
4-non-1-enyltropolone (**59**)	0.1	0	0.02%
	0.02	0	
	0.01	3.1	
	0.004	6.5	
4-nonyltropolone (**60**)	0.1	0	0.02%
	0.05	0	
	0.02	0	
	0.01	2.7	
	0.004	4.8	
4-hept-1-enyltropolone (**61**)	0.1	0	0.02%
	0.02	0	
	0.01	1.2	
	0.004	2.9	
4-heptyltropolone (**63**)	0.1	0	0.02%
	0.02	0	
	0.01	1.4	
	0.004	3.4	
4-pent-1-enyltropolone (**62**)	0.1	0	>0.004%≤0.01%
	0.02	0	
	0.01	0	
	0.004	2.0	
4-pentyltropolone (**64**)	0.1	0	>0.004%≤0.01%
	0.02	0	
	0.01	0	
	0.004	1.1	
β-thujaplicin (**47**)	0.2	0	>0.002%≤0.02%
	0.02	0	
	0.002	2.8	
	0.001	5.3	

5.3.2.2 White Rot Fungus, *Trametes versicolor*

The lactone tropolone (**57**) was largely inactive against *T. versicolor*, showing only moderate growth inhibition at the 2% loading (Table 5.17). 4-Non-1-enyltropolone (**59**) prevented fungal growth at a 1% concentration and was strongly inhibitory at the 0.2% level. Similarly 4-nonyltropolone (**60**) gave a MIC of >0.2%≤1.0% against *T. versicolor* (Table 5.17). 4-Heptyltropolone (**63**) and 4-hept-1-enyltropolone (**61**) were strongly active, displaying MICs of 0.2% and significant growth inhibition at half this concentration (Table 5.17). Minimum

inhibitory concentrations of >0.02%≤0.1% and 0.2% were observed for 4-pentyltropolone (**64**) and 4-pent-1-enyltropolone (**62**) suggesting the former was of greater efficacy. With only three duplicates it is impossible to assess whether this rather subtle difference in fungal growth was truly significant. 4-Pentyltropolone (**64**) was similar in activity to β-thujaplicin (**47**). A reduction in antifungal activity with increasing hydrocarbon chain length was evident against *T. versicolor* as it was against *C. puteana* (Table 5.17). Where no fungal growth was observed the antifungal activity was due to fungicidal rather that fungistatic properties.

Table 5.17: Mean colony diameter (cm) of *Trametes versicolor* grown on newsprint papers treated with 4-alkyltropolones and related compounds.

Compound	**Calculated treatment loading (% m/m)**	**Av. radial growth of *T. versicolor* (cm)**	**Minimum inhibitory concentration (MIC)**
4-(2-hydroxynonyl)-3-carboxylic acid δ-lactone tropolone (**57**)	2	2.8	>2.8%
	0.6	6.7	
	0.2	7.8	
4-non-1-enyltropolone (**59**)	1	0	
	0.2	1.6	>0.2%≤1.0%
	0.1	3.3	
	0.02	8.0	
4-nonyltropolone (**60**)	1	0	
	0.2	1.6	>0.2%≤1.0%
	0.1	2.6	
	0.02	8.0	
4-hept-1-enyltropolone (**61**)	1	0	
	0.2	0	0.2%
	0.1	1.9	
	0.02	8.0	
4-heptyltropolone (**63**)	1	0	
	0.2	0	0.2%
	0.1	1.9	
	0.02	8.0	
4-pent-1-enyltropolone (**62**)	1	0	
	0.2	0	0.2%
	0.1	1.6	
	0.02	7.5	
4-pentyltropolone (**64**)	1	0	
	0.2	0	
	0.1	0	
	0.02	5.7	>0.02%≤0.1%
β-thujaplicin (**47**)	0.2	0	
	0.1	0	
	0.06	2.1	>0.06%≤0.1%
	0.02	2.7	
	0.002	7.4	

5.3.2.3 Soft Rot Fungus, *Chaetomium globosum*

The three 4-alkyltropolones (**60**), (**63**) and (**64**), but not the corresponding 4-alkenyltropolones (**59**), (**61**) and (**62**), were tested against the soft rot and sapstain fungi, *Chaetomium globosum* and *Sphaeropsis sapinea*. This was because matching 4-alkyltropolones and 4-alkenyl-tropolones showed equivalent activities against the two basidiomycetes. The lactone tropolone (**57**) was also omitted on account of its lack of activity against *C. puteana* and *T. versicolor* relative to the 4-alkyltropolones. 4-Nonyltropolone (**60**) and 4-heptyltropolone (**63**) demonstrated MICs of >0.3%≤1.0% and >0.1%≤1.0% respectively against *C. globosum*. 4-Heptyltropolone appeared to be marginally more active based on the lower degree of fungal growth at the 0.1% loading (Table 5.18).

Table 5.18: Mean colony diameter (cm) of *Chaetomium globosum* grown on newsprint papers treated with 4-alkyltropolones and related compounds and TBTN (**98**).

Compound	**Calculated treatment loading (% m/m)**	**Av. radial growth of *C. globosum* (cm)**	**Minimum inhibitory concentration (MIC)**
4-nonyltropolone (**60**)	1	0	
	0.3	1.8	>0.3%≤1%
	0.1	6.9	
	0.01	8.0[1]	
4-heptyltropolone (**63**)	1	0	
	0.1	3.6	>0.1%≤1%
	0.01	8.0[1]	
4-pentyltropolone (**64**)	1	0	
	0.1	0	*ca.* 0.1%
	0.05	5.8	
	0.01	8.0[1]	
β-thujaplicin (**47**)	1	0	
	0.1	0	*ca.* 0.1%
	0.01	8.0[1]	
tropolone (**48**)	1	0	
	0.1	0	*ca.* 0.1%
	0.01	7.4	
$Cu(tropolone)_2$ (**101**)	1	0	
	0.1	0	*ca.* 0.1%
	0.01	8.0	
TBTN (**98**)	2	0	
	1	0.9[2]	1%
	0.1	7.3	
Water		8.0[1]	
Dichloromethane		8.0[1]	

[1] Complete coverage of newsprint sample after 1 week of incubation; [2] Fungal growth confined to inoculated AA disc.

4-Pentyltropolone (**64**) exhibited a MIC of *ca.* 0.1%, which was equivalent to that of tropolone (**48**), β-thujaplicin (**47**) and Cu(tropolone)$_2$ (**101**). The efficacy of the 4-alkyl-tropolones (**63**) and (**64**) compared favourably to that of TBTN (**98**) which was ineffectual at the 0.1% loading (Table 5.18).

5.3.2.4 Sapstain Fungus, *Sphaeropsis sapinea*

A minimum inhibitory concentration of *ca.* 0.1% was recorded for 4-nonyltropolone (**60**) and 4-heptyltropolone (**63**) against the staining fungus, *Sphaeropsis sapinea* (Table 5.19). Surprisingly 4-pentyltropolone (**64**) appeared to be slightly less active. These compounds were fungistatic at the 0.1% level and fungicidal at the 1% loading.

Table 5.19: Mean colony diameter (cm) of *Sphaeropsis sapinea* grown on newsprint papers treated with 4-alkyltropolones and related compounds, PCP (**100**) and IPBC (**99**).

Compound	**Calculated treatment loading (% m/m)**	**Av. radial growth of *S. sapinea* (cm)**	**Minimum inhibitory concentration (MIC)**
4-nonyltropolone (**60**)	1	0	
	0.1	0	*ca.* 0.1%
	0.05	2.0	
	0.01	8.0[1]	
4-heptyltropolone (**63**)	1	0	
	0.1	0	*ca.* 0.1%
	0.01	8.0[1]	
4-pentyltropolone (**64**)	1	0	
	0.1	2.3	>0.1%≤1%
	0.01	8.0[1]	
β-thujaplicin (**47**)	1	0	
	0.1	0	
	0.05	0	*ca.* 0.05%
	0.01	8.0[1]	
tropolone (**48**)	1	0	
	0.1	0	
	0.01	2.0	>0.01%≤0.1%
Cu(tropolone)$_2$ (**101**)	1	0	
	0.1	0	
	0.01	1.4	>0.01%≤0.1%
PCP (**100**)	1	0	
	0.1	1.9	>0.1%≤1%
	0.01	7.7	
IPBC (**99**)	1	0	
	0.1	0	
	0.01	2.2	>0.01%≤0.1%
Water		8.0	
Dichloromethane		8.0	

[1] Complete coverage of newsprint sample after 1 week of incubation.

β-Thujaplicin (**47**) displayed fungicidal properties with a MIC of *ca.* 0.05%. The antifungal activities of the 4-alkyltropolones were equal, if not superior, to that of PCP (**100**) (Table 5.17). Tropolone (**48**), Cu(tropolone)$_2$ (**101**) and IPBC (**99**) were all highly effective with MICs of >0.01%≤0.1%. Activity was of a fungicidal nature for all three compounds at both the 0.1% and 1% loadings.

5.3.3 4-Styryltropolones, Analogues of Stilbene-tropolones

The concept behind the 4-styryltropolones, which can be viewed as stilbene-tropolone analogues, was the merging of two structure elements of known antifungal character, with the aim of improving fungitoxicity and water repellency characteristics. In the case of the 4-alkyltropolones (Section 5.3.2), different degrees of hydrophobicity were attained by adding functionally inert alkyl chains of differing lengths. In this case, the structures added for hydrophobic character could potentially possess their own antifungal properties.

5.3.3.1 Brown Rot Fungus, *Coniophora puteana*

4-Styryltropolone (**66**) showed potent activity against *C. puteana* with a MIC of 0.02% and a strong fungistatic effect at the 0.01% level (Table 5.20). The more hydrophilic 3-carboxy-4-styryltropolone (**65**), although moderately effective with a MIC of *ca.* 0.1%, was at least 5-fold less potent. A similar enhancement in activity against *C. puteana* on decarboxylation was observed with 3-carboxy-4-octylcarboxymethyltropolone (Table 5.14). The addition of methoxyl groups to the benzene ring of the styryl group lead to a decline in activity against *C. puteana* in the cases of 4-(3',4',5'-trimethoxy)styryltropolone (**68**) and 4-(3',5'-dimethoxy)-styryltropolone (**69**). Both exhibited MICs of >0.02%≤0.1% compared to 0.02% for 4-styryltropolone (**66**) (Table 5.20). This trend was not observed in the case of the 3-carboxystyryltropolones (**65**) and (**67**). The trimethoxystyryltropolone (**67**) was equivalent, if not superior, in activity to the styryltropolone (**65**) (Table 5.20). No detectable difference in antifungal activity was observed between the trimethoxystyryltropolones (**67**) and (**68**) indicating, in this case at least, decarboxylation had no impact on the antifungal activity.

Table 5.20: Mean colony diameter (cm) of *Coniophora puteana* grown on filter papers treated with 4-styryltropolones.

Compound	Calculated treatment loading (% m/m)	Av. radial growth of *C. puteana* (cm)	Minimum inhibitory concentration (MIC)
3-carboxy-4-styryl-tropolone (**65**)	2	0	
	0.2	0	
	0.1	0	*ca.* 0.1%
	0.02	7.2	
	0.002	8.5	
4-styryltropolone (**66**)	0.2	0	
	0.1	0	
	0.02	0	0.02%
	0.01	2.2	
3-carboxy-4-(3',4',5'-tri-methoxy)styryltropolone (**67**)	0.2	0	
	0.1	0	
	0.02	3.2	>0.02%≤0.1%
4-(3',4',5'-trimethoxy)-styryltropolone (**68**)	0.1	0	
	0.02	3.2	>0.02%≤0.1%
	0.01	7.2	
4-(3',5'-dimethoxy)-styryltropolone (**69**)	0.2	0	
	0.1	0	
	0.02	4.3	>0.02%≤0.1%
	0.01	2.0	

5.3.3.2 White Rot Fungus, *Trametes versicolor*

4-Styryltropolone (**66**) was the most active of the styryltropolones against the white rot fungus, *T. versicolor*. The MIC was in the range 0.2% to 1.0% placing it in the same class as 4-nonyltropolone (**60**), yet inferior to β-thujaplicin (**47**) (Table 5.21 and 5.17). Antifungal activity was of a fungistatic nature. 3-Carboxy-4-styryltropolone (**65**) showed good fungistatic activity against *T. versicolor* at 1% and 2% loadings although complete control was not achieved (Table 5.21). As was observed against *C. puteana*, 3-carboxy-4-styryltropolone (**65**) was significantly less effective than its decarboxylated derivative (**66**). 3-Carboxy-4-(3',4',5'-trimethoxy)styryltropolone (**67**) marginally hindered the growth of *T. versicolor* at the 1% loading. Its decarboxylated derivative, 4-(3',4',5'-trimethoxy)styryltropolone (**68**) showed improved activity with strong growth inhibition at the 1% level. 4-(3',5'-Dimethoxy)-styryltropolone (**69**) exhibited a similar level of fungistatic activity. The degree of fungal growth observed for the methoxystyryltropolones (**68**) and (**69**) at the 1% loading was comparable to that observed for 4-styryltropolone (**66**) at the 0.2% level suggesting a 5-fold difference in activity.

Table 5.21: Mean colony diameter (cm) of *Trametes versicolor* grown on newsprint papers treated with 4-styryltropolones.

Compound	Calculated treatment loading (% m/m)	Av. radial growth of *T. versicolor* (cm)	Minimum inhibitory concentration (MIC)
3-carboxy-4-styryl-tropolone (**65**)	2	1.3	>2%
	1	1.5	
	0.6	4.2	
	0.2	7.2	
	0.1	7.8	
4-styryltropolone (**66**)	1	0	
	0.2	1.9	>0.2%≤1%
	0.1	2.5	
3-carboxy-4-(3',4',5'-tri-methoxy)styryltropolone (**67**)	1	4.7	>1%
	0.2	7.6	
	0.1	8.0	
4-(3',4',5'-trimethoxy)-styryltropolone (**68**)	1	1.9	>1%
	0.2	7.3	
	0.1	8.0	
4-(3',5'-dimethoxy)-styryltropolone (**69**)	1	1.7	>1%
	0.2	6.4	
	0.1	8.0	

5.3.3.3 *Chaetomium globosum* and *Sphaeropsis sapinea*

In light of the inferior anti-basidiomycetes activity of 3-carboxy-4-styryltropolone (**65**) compared to 4-styryltropolone (**66**) and the similar activities of the dimethoxy and trimethoxystyryltropolones (**69**) and (**68**), only two styryltropolones (**66**) and (**69**) were tested against *C. globosum* and *S. sapinea* (Table 5.22). As was observed against the basidiomycetes, 4-styryltropolone (**66**) was the most effective with a MIC of *ca.* 1% and >0.1%≤1% against the soft rot and sapstain fungi respectively. The activity of 4-styryltropolone (**66**) against these two fungi was notably inferior to that of β-thujaplicin (**47**) and, in the case of *S. sapinea*, was inferior to that of 4-nonyltropolone (**60**) (Table 5.22, 5.18 and 5.19). The control of fungal growth by 4-styryltropolone (**66**) was of a fungicidal nature in the case of *S. sapinea* and fungistatic nature in the case of *C. globosum*. 4-(3',5'-Dimethoxy)styryltropolone (**69**) displayed good fungistatic activity against *S. sapinea* at the 1% and 0.1% levels, but was only weakly active against *C. globosum*, with only slightly better than 50% growth inhibition at the 1% loading (Table 5.22).

Table 5.22: Mean colony diameter (cm) of *Chaetomium globosum and Sphaeropsis sapinea* grown on newsprint papers treated with 4-styryltropolones.

Compound	Treatment loading (% m/m)	Av. radial fungal growth (cm)		MIC	
		C. globosum	*S. sapinea*	*C. globosum*	*S. sapinea*
4-styryltropolone (**66**)	1	0	0	*ca.* 1%	
	0.1	8.0	2.0		>0.1%≤1%
	0.01	8.0[1]	8.0[1]		
4-(3',5'-dimethoxy)-styryltropolone (**69**)	1	3.3	1.6	>1%	>1%
	0.1	8.0	1.9		
	0.01	8.0[1]	8.0[1]		

[1] Complete coverage of newsprint sample after 1 week of incubation.

In general substitution of the styryl ring with methoxyl groups lead to decreases rather than increases in antifungal activity. The concept of producing a compound with enhanced antifungal activity by combining tropolone and methoxystilbene structures would appear to be invalid, based on the few examples described.

5.3.4 3-Alkyltropolones, Analogues of α-Thujaplicin

Alkylation of tropolone (**48**) with hexenal diethyl acetal (**79**) with subsequent oxidations or reductions yielded four analogues of α-thujaplicin with formylmethyl (**92**), carboxymethyl acid (**95**), hydroxyethyl (**96**) and ethyl (**97**) groups attached to the butyl chain of 3-butyltropolone (Section 4.5). The results of antifungal testing of these compounds against wood deteriorating fungi are discussed below, with reference to the impact of each of the functionalities of tropolone efficacy. Activities were compared against β-thujaplicin (**47**) (Tables 5.16-5.19) as α-thujaplicin was commercially unavailable.

5.3.4.1 Brown Rot Fungus, *Coniophora puteana*

3-(1-Ethyl)butyltropolone (**97**) showed the greatest activity against *C. puteana* of the four 3-substituted synthetic tropolones. Although a MIC was not established, 3-(1-ethyl)-butyltropolone (**97**) was extremely potent, with zero fungal growth observed at a concentration of 0.005% (Table 5.23). The aldehyde tropolone 3-(1-formylmethyl)-butyltropolone (**92**) was the next most effective; a MIC of 0.02% was recorded and partial fungistatic properties were observed at the 0.01% level. The aldehyde group either adversely affects the tropolone's mode of fungitoxicity or provides a site from which the fungus can

initiate detoxification. 3-(1-Carboxymethyl)butyltropolone (**95**) was significantly less active than the aldehyde tropolone (**92**). Very little fungal growth inhibition was observed at the 0.02% loading for this tropolone, although it was fungicidal at the 0.1% level (Table 5.23). 3-(1-Hydroxyethyl)butyltropolone (**96**) exhibited a MIC of >0.02%≤0.1% making it marginally less active than the aldehyde tropolone (**92**).

From a structure *vs* activity standpoint a pattern of decreasing antifungal activity against *C. puteana* (<0.005% → 0.02% → >0.02%≤0.1% → *ca.* 0.1%) with increasing polarity of the substituted alkyl chain is evident in these results. 3-(1-Ethyl)butyltropolone (**97**) showed comparable efficacy to that of β-thujaplicin (**47**) and 4-pentyltropolone (**64**), the most active of the synthetic 4-alkyltropolone (Tables 5.16, 5.23). The antifungal effects, at zero growth, of all four tropolones (**92**), (**95**), (**96**) and (**97**) were the result of fungicidal rather that fungistatic properties.

Table 5.23: Mean colony diameter (cm) of *Coniophora puteana* grown on filter papers treated with 3-alkyltropolones.

Compound	**Calculated treatment loading (% m/m)**	**Av. radial growth of *C. puteana* (cm)**	**Minimum inhibitory concentration (MIC)**
3-(1-formylmethyl)butyl-tropolone (**92**)	0.2	0	
	0.1	0	
	0.02	0	0.02%
	0.01	3.1	
3-(1-carboxymethyl)butyl-tropolone (**95**)	0.2	0	
	0.1	0	*ca.* 0.1%
	0.02	6.2	
	0.01	6.4	
3-(1-hydroxyethyl)butyl-tropolone (**96**)	0.2	0	
	0.1	0	
	0.02	2.6	>0.02%≤0.1%
	0.01	3.8	
3-(1-ethyl)butyltropolone (**97**)	0.2	0	
	0.1	0	
	0.02	0	
	0.01	0	
	0.005	0	≤0.005%

5.3.4.2 White Rot Fungus, *Trametes versicolor*

3-(1-Formylmethyl)butyltropolone (**92**) showed good antifungal activity against *T. versicolor*. A MIC of >0.2%≤1% was recorded with significant growth inhibition at the 0.1% concentration (Table 5.24). 3-(1-Hydroxyethyl)butyltropolone (**96**) displayed a comparable MIC, although the degree of fungal growth inhibition at the 0.1% level suggested it was slightly less active. In contrast to the results observed with *C. puteana*, 3-(1-carboxy-methyl)butyltropolone (**95**) showed enhanced fungitoxicity over the aldehyde tropolone (**92**) and alcohol tropolone (**96**) with complete prevention of fungal growth at the 0.2% concentration (Table 5.24). This result was confirmed by repetition of the assay. At the lower loading of 0.1% the inhibitory action of the acid tropolone (**95**) appeared inferior to that of the aldehyde tropolone (**92**). As was observed against *C. puteana*, 3-(1-ethyl)butyltropolone (**97**) was the most potent of this group of synthetic tropolones against *T. versicolor*. 3-(1-Ethyl)butyltropolone (**97**) exhibited a MIC value of *ca.* 0.1% and was equivalent in activity to β-thujaplicin (**47**) and 4-pentyltropolone (**64**) (Table 5.17, Table 5.24).

Table 5.24: Mean colony diameter (cm) of *Trametes versicolor* grown on newsprint papers treated with 3-alkyltropolones.

Compound	**Calculated treatment loading (% m/m)**	**Av. radial growth of *T. versicolor* (cm)**	**Minimum inhibitory concentration (MIC)**
3-(1-formylmethyl)butyl-tropolone (**92**)	1	0	>0.2%≤1%
	0.2	2.0	
	0.1	2.3	
	0.02	8.0	
3-(1-carboxymethyl)butyl-tropolone (**95**)	1	0	>0.1%≤0.2%
	0.2	0	
	0.1	4.9	
	0.02	8.0	
3-(1-hydroxyethyl)butyl-tropolone (**96**)	1	0	>0.2%≤1%
	0.2	2.9	
	0.1	5.1	
	0.02	8.0	
3-(1-ethyl)butyltropolone (**97**)	1	0	*ca.* 0.1%
	0.2	0	
	0.1	0	
	0.02	8.0	

5.3.4.3 *Chaetomium globosum* and *Sphaeropsis sapinea*

The aldehyde tropolone 3-(1-formylmethyl)butyltropolone (**92**) possessed good antifungal activity against *C. globosum* and excellent activity against *S. sapinea*; MICs of >0.1≤1% and 0.05% were observed against the respective fungi (Table 5.25). 3-(1-Hydroxyethyl)-butyltropolone (**96**) was of similar activity to the aldehyde tropolone (**92**) against the sapstain fungus and slightly more effective against the soft rot species. 3-(1-Ethyl)butyltropolone (**97**) displayed a MIC of 0.05% against both fungi and hence was the most effective of the four 3-alkyltropolones (Table 5.25). It should be noted, however, that the alcohol tropolone (**96**) was not tested at the 0.05% loading. The most apparent difference in fungitoxicity of the four 3-alkyltropolones against *C. globosum* and *S. sapinea* was the comparatively poor efficacy of 3-(1-carboxymethyl)butyltropolone (**95**). This tropolone displayed only weak inhibitory effects at a loading of 0.1% against both fungi (Table 5.25). As with the brown and white rot fungi, the nature of the antifungal activity at zero growth was fungicidal rather than fungistatic properties.

Table 5.25: Mean colony diameter (cm) of *Chaetomium globosum and Sphaeropsis sapinea* grown on newsprint papers treated with 3-alkyltropolones.

Compound	**Treatment**	**Av. radial fungal growth (cm)**		**MIC**	
	loading (% m/m)	***C. globosum***	***S. sapinea***	***C. globosum***	***S. sapinea***
3-(1-formylmethyl)-butyltropolone (**92**)	1	0	0		
	0.1	1.9	0	>0.1%≤1%	
	0.05	-	0		*ca.* 0.05%
	0.01	8.0[1]	8.0[1]		
3-(1-carboxymethyl)-butyltropolone (**95**)	1	0	0		
	0.1	4.8	3.6	>0.1%≤1%	>0.1%≤1%
	0.01	8.0[1]	8.0[1]		
3-(1-hydroxyethyl)-butyltropolone (**96**)	1	0	0		
	0.1	0	0	*ca.* 0.1%	*ca.* 0.1%
	0.01	8.0[1]	8.0[1]		
3-(1-ethyl)butyl-tropolone (**97**)	1	0	0		
	0.1	0	0		
	0.05	0	0	*ca.* 0.05%	*ca.* 0.05%
	0.01	8.0[1]	8.0[1]		

[1] Complete coverage of newsprint sample after 1 week of incubation.

The relative antifungal activities for these four 3-alkyltropolones (**92**), (**95**), (**96**) and (**97**) differed with the fungal species under investigation. If any general pattern can be elucidating from these results it is that a secondary functional group (aldehyde, alcohol or acid) had, in

most cases, a negative effect on the fungitoxicity. Overall, 3-(1-ethyl)butyltropolone (**97**) showed the highest specific activities against all four test organisms.

5.3.5 Wood Protection Potential

Minimum inhibitory concentrations of the more active of the synthetic tropolones against all four test fungi are compared against those of TBTN (**98**), IPBC (**99**) and PCP (**100**) in Table 5.26. These summarised results provide a means of assessing the potential of these compounds for wood protection. In terms of susceptibility of the fungi to the tropolones, the brown rot fungus *C. puteana* was the most vulnerable followed by the white rot fungus *T. versicolor*. Overall *C. globosum* was the most resilient although, in certain cases, equal or higher tropolone concentrations were required to control *S. sapinea* (Table 5.26). The activities of selected tropolones (Table 5.26) against *C. puteana* were comparable with that of TBTN (**98**), if perhaps slightly inferior. The activities against *T. versicolor* were in all cases equal to, if not superior to, TBTN (**98**). 3-(1-Ethyl)butyltropolone (**97**) and 4-pentyltropolone (**64**) appeared to be significantly more potent than TBTN (**98**) (Table 5.26).

Table 5.26: MICs of synthetic tropolone compounds and the wood protection agents, TBTN (**98**), IPBC (**99**) and PCP (**100**).

Compound	**Minimum inhibitory concentration**			
	C. puteana	***T. versicolor***	***C. globosum***	***S. sapinea***
4-nonyltropolone (**60**)	0.02%	>0.2%≤1.0%	>0.1%≤1%	0.1%
4-heptyltropolone (**63**)	0.02%	0.2%	>0.1%≤1%	0.1%
4-pentyltropolone (**64**)	>0.004%≤0.01%	>0.02%≤0.1%	0.1%	>0.1%≤1%
3-(1-formylmethyl)butyl-tropolone (**92**)	0.02%	>0.2%≤1%	>0.1%≤1%	0.05%
3(1-ethyl)butyltropolone (**97**)	≤0.005%	>0.02%≤0.1%	0.05%	0.05%
4-styryltropolone (**66**)	0.02%	>0.2%≤1.0%	1%	>0.1%≤1%
β-thujaplicin (**47**)	>0.002%≤0.02%	>0.06%≤0.1%	0.1%	0.05%
TBTN (**98**)	>0.002%≤0.01%	>0.2%≤0.5%	1%	
IPBC (**99**)				>0.01%≤0.1%
PCP (**100**)				>0.1%≤1%

Many of the synthetic tropolones showed greater efficacy against the soft rot fungus *C. globosum* than TBTN (**98**) (Table 5.26). One of the weaknesses of TBTN (**98**) as a wood preservative is its lower efficacy against white and soft rot decay fungi compared to brown rot

fungi (54). The synthetic tropolones, in particular 4-pentyltropolone (**64**) and 3-(1-ethyl)butyl-tropolone (**97**), exhibited good fungicidal properties across the spectrum of decay types. The alkyltropolones and 4-styryltropolone (**66**) displayed antifungal activities against the sapstain fungus *S. sapinea* equal to that of PCP (**100**), although inferior to that of IPBC (**99**) (Table 5.26).

On the basis of the fungitoxicity results alone the above alkylated tropolones showed excellent potential for further development as novel wood preservation agents. Their potential for use in sapstain control is less promising. Only in the case of 3-(1-formylmethyl)butyltropolone (**92**) and 3-(1-ethyl)butyltropolone (**97**) were activities approaching that of IPBC (**99**) achieved. Interestingly, Cu(tropolone)$_2$ (**101**) and tropolone (**48**) were of equivalent activity to that of IPBC (**99**) as assessed by this assay method (Table 5.19). Overall the synthetic tropolone that exhibited the greatest specific activities against all four fungi was 3-(1-ethyl)butyltropolone (**97**). Its toxicity was equivalent to that of β-thujaplicin (**47**) across the range of fungi. The longer hydrocarbon chain of (**97**) should give it superior water repellence properties to that of β-thujaplicin (**47**) which would be advantageous from the point of view of wood preservation.

The important thing is not to stop questioning

Albert Einstein

Chapter 6: Summary and Conclusions

Protecting wood against biological attack encompasses the fields of wood preservation and protection against fungal stain. In New Zealand and other countries of temperate climate, decay fungi are the primary deteriorators of timber in service, while sapstain fungi cause the greatest economic losses on freshly felled or unseasoned timber. Currently, methods of controlling decay and staining fungi involve treating with broadly active biocides. Concerns over occupational health and safety and environmental safety have led to strict regulations, and in some cases outright bans, on certain chemicals traditionally used in these applications.

Opportunities exist for the development of novel, less toxic, more environmentally friendly, alternatives. One avenue of research under exploration is the use of natural products, in particular antifungal wood extractives from naturally durable timbers, as a source of new wood protection agents. In this thesis the use of known antifungal terpenoid and tropolone wood extractives or essential oil components as synthetic leads to novel compounds with potential for wood protection was explored. The overall objective was to evaluate the validity and practicality of this approach and identify relationships between activity against decay and sapstain fungi and chemical structure.

In Chapter 2 analogues of the antifungal terpenoids thymol (**1**), β-terpineol (**2**) and totarol (**3**) were synthesised. Analogues of thymol and β-terpineol (**4**, **5**) were synthesised from *p-n*-nonylanisole (**7**) in multi-step syntheses. The reasons for synthesising analogues of these antifungal monoterpenoids with a long alkyl chain included enhancement of water insolubility (and hence persistence in wood), the possibility for durability enhancements due to water repellency effects and a possible lowering of mammalian toxicity. A single step synthesis of 1-isopropyl-2-naphthol (**6**), a simplified analogue of totarol (**3**), was achieved from 2-naphthol (**31**) based on the propensity of β-naphthoxide to undergo C-alkylation under certain conditions.

The antifungal assays on these terpenoid analogues indicated desirable levels of fungitoxicity were only achieved against the brown rot fungus, *Coniophora puteana*, with the exception of

1-isopropyl-2-naphthol (**6**) against *Chaetomium globosum*. This selective toxicity severely limits the potential of such compounds for use in wood preservation. A spectrum of activity across a range of decay types is usually desirable. The terpenoid analogues (**4**), (**5**) and (**6**) showed little promise for use in antisapstain formulations.

The β-terpineol analogue (**5**) showed moderate and weak fungistatic effects against *Coniophora puteana* and *Trametes versicolor* respectively. The tertiary alcohol functional group of (**5**) appeared less active than the phenolic group of (**9**), yet more effective than the ketone of (**20**). The stereochemistry of the tertiary alcohol group in (**5**) had a small but detectable effect on activity against *C. puteana*, with the *trans*-configuration showing slightly superior fungistatic properties. The thymol analogue (**4**) was less active than thymol (**1**) across the range of fungi tested. Alkylation of phenols has been shown to improve antifungal activity for the series phenol to creosol to ethylphenol to thymol (**1**) (156). The nonyl group, in this case, has severely compromised fungitoxicity. A further decline in activity is observed in the case of a di-nonylphenol, possibly 2,4-di-nonylphenol (**30**). Hence, there appears to be an optimum level to which lipophilicity can be improved before it starts to adversely affect antifungal activity. The partially sterically crowded phenolic group was proposed as an important feature in antifungal activity. This was proved incorrect. Although the phenolic group was essential for activity, *p-n*-nonylphenol (**9**) and the thymol analogue (**4**) showed similar levels of toxicity, as did 1-isopropyl-2-naphthol (**6**) and 2-naphthol (**31**). Branching of the alkyl chain was a structural feature that was identified as potentially important in fungitoxicity of alkylated phenols. Commercial nonylphenol, which is comprised of isomers with different branchings of the nonyl chain, was more active against *C. puteana* than pure *p-n*-nonylphenol (**9**). This observation is worthy of future investigation.

In Chapter 3, a bio-activity led investigation of oxygenated sesquiterpenes from the foliage oils of *Podocarpus totara*, *Dacrycarpus dacrydioides* and *Dacrydium cupressinum* identified caryophyllene oxide (**35**), humulene epoxide II (**36**), globulol (**37**) and α-cadinol (**38**) as major components of essential oil fractions with anti-basidiomycetes activity. The oxygenated sesquiterpenoids were more effective against the brown rot fungus, *C. puteana* than the white rot *T. versicolor*. With the exception of α-cadinol (**38**), the antifungal activity of these sesquiterpenoids against wood destroying basidiomycetes has not been reported in the literature. Simple synthetic compounds with epoxide and tertiary alcohol functional groups, namely 4-nonylcyclohexan-1,2-epoxide (**39**), 4-ethyl-1-methyl-1,2,3,4-tetrahydronaphth-1-ol

(**40**) and 5,9-dimethyl-6,7,8,9-tetrahydrobenzocyclohepten-5-ol (**44**) were prepared by multi-step syntheses.

The synthetic epoxide (**39**) showed good fungitoxicity against *C. puteana*, but was largely ineffective against the other three test fungi. The synthetic tertiary alcohols (**40**) and (**44**) were active against all three decay fungi, in particular *C. puteana*, but were only weakly active against the sapstain fungi, *Sphaeropsis sapinea*. Antifungal activities for the synthetic compounds were equal to, and in some cases superior to, that of the mixtures of natural sesquiterpenoids. Although this investigation was limited in scope, the use of natural oxygenated sesquiterpenoids as leads to novel compounds with antifungal activity proved a valid approach.

In the case of tertiary alcohol compounds (**40**) and (**44**) the basic molecular shape and functional groups of the natural product leads were preserved in the analogue, while other structural features were sacrificed for reasons of synthesis simplicity. This resulted in equivalent, and in certain cases enhanced, antifungal activities compared to the sesquiterpenoid lead compounds. These results illustrated one of the major advantages the synthetic approach has over exploring the natural products alone. By manipulation of the structure of the lead compound, analogues with enhanced activity can be discovered. The development of the synthetic pyrethroids by Elliott and colleagues (336, 337), based on natural pyrethrum compounds found in chrysanthemum flowers, is the most pertinent wood preservation example of this approach (337). The synthetic pyrethroids are now widely used as insecticides in wood preservation.

In Chapter 4, alkyltropolones and styryltropolones were synthesised by several multi-step pathways. The anhydride (**52**) of 3-carboxy-4-carboxymethyltropolone (**49**) was a key intermediate, which upon reaction with aliphatic alcohols and aldehydes and aromatic aldehydes gave 4-alkylcarboxytropolones, an alkyl δ-lactone tropolone, 4-alkenyltropolones and 4-styryltropolones. 4-Alkyltropolones were prepared by reduction of 4-alkenyltropolones. 3-Alkyltropolones were successfully prepared directly by electrophilic substitutions on tropolone (**48**) and β-thujaplicin (**47**) with alkenyl diethyl acetals. In contrast, however, a proposed new synthetic pathway to β-thujaplicin (**47**) from 1-methyl-3,4,6-trihydroxy-(5*H*)-benzocyclohepten-5-one (**72**) proved unsuccessful.

Researchers have shown that the keto-enolic structure of tropolones is critical for antifungal activity (92, 193, 210). Hence, it was essential to retain the tropolone functionality in the syntheses undertaken. Using the thujaplicins as models and directing syntheses toward novel tropolones with other potentially desirable structural features proved successful in producing a range of tropolone compounds with varying degrees of activity against decay and sapstain fungi. From the point of view of fungitoxicity, several of these compounds have potential for development as novel wood protection agents. In general, the synthetic tropolones displayed good to excellent antifungal properties against the four test fungi. Antifungal activities were superior to those of the terpenoid analogues.

The brown rot fungus, *Coniophora puteana*, was the most susceptible of the test fungi to the tropolone analogues. In the case of 3-(1-ethyl)butyltropolone (**97**), the most active of the synthetic tropolones, a concentration as low at 0.005% was effective in completely inhibiting fungal growth. The metabolisms of the white and soft rot fungi are possibly less vulnerable to the phenolic-ketonic properties of the tropolones due to their ability to degrade and detoxify phenolic lignin degradation products.

This study revealed certain insights into the structure/activity relationships of substituted tropolones. For the most part, conclusions based on bioassay results against the most susceptible fungus *Coniophora puteana* were reflected in the assay results against the other fungal species. Tropolone compounds with additional polar functional groups possessed lower antifungal activity than their less polar counterparts. For example, 3-carboxy-4-carboxymethyltropolone (**49**) was considerably less fungitoxic that methyltropolone (**51**). The fungitoxicity of the 3-alkyltropolones followed a pattern of decreasing activity in the series 3-(1-ethyl)butyltropolone (**97**) to 3-(1-formylmethyl)butyltropolone (**92**) to 3-(1-hydroxy-ethyl)butyltropolone (**96**) to 3-(1-carboxymethyl)butyltropolone (**95**). Reductions in polarity of the tropolone compound through esterification and decarboxylation of attached carboxyl groups similarly improved fungitoxicity.

The addition of alkyl groups to the tropolone ring was undertaken with the aim of improving water insolubility and lowering mammalian toxicity, without compromising fungitoxicity. Addition of a five carbon hydrocarbon chain appeared to be the optimum level to which lipophilic character could be enhanced without an appreciable decline in fungitoxicity. Beyond this level fungitoxicity declined, although not too severely. 4-Pentyltropolone (**64**)

was the most active of the 4-alkyltropolones against the three decay fungi. Antifungal activities were equivalent to that of TBTN (**98**) against *C. puteana* and vastly superior to TBTN (**98**) against *T. versicolor* and *C. globosum*. 3-(1-Ethyl)butyltropolone (**97**) was the most fungitoxic of the 3-alkyltropolones studied. Although 3-(1-ethyl)butyltropolone (**97**) possessed an additional alkyl carbon to that of 4-pentyltropolone (**64**), its activity against *C. puteana*, *C. globosum* and *S. sapinea* was superior. As with the nonylphenol example, branching of the alkyl chain of alkyltropolones appears to lead to fungitoxicity improvements.

4-Styryltropolones were investigated to assess whether the merging of the known fungitoxic structure elements of tropolones and methoxystilbenes would lead to compounds with improved fungitoxicity over the alkylated tropolones. This approach proved unsuccessful, for although 4-styryltropolone (**66**) exhibited similar antifungal activities to 4-nonyltropolone (**60**), styryltropolones with methoxyl substituents on the benzene ring were significantly less active.

The research thus described is illustrative of the direction in which research into bioactive wood extractives is now focused. Historically, research in the field of antifungal substances from woody plants was primarily concerned with identifying new active compounds or evaluating the role of extractives in the natural durability of timbers. This direction eventually led to a decline in this field as few researchers took the next step of attempting the development of novel bioactive compounds by molecular modification of the natural products. One example of this approach is the work of Bultman and Parrish (95), who synthesised and bioassayed analogues of the microbiocide obtusastyrene (Figure 1.17, page 39), which occurs in the durable timber *Dalbergia retusa*, in search of novel antifungal, antiborer and insecticidal agents. Although to date no commercial biocides have been developed from durable timber extractives, the potential still exists and in today's environmentally conscious times there are now opportunities to redirect research into wood natural product chemistry along these lines. Examples of such research include the work of Schultz and coworkers on structure/antifungal activity relationships of modified hydrostilbenes (79) and Laks and colleague's endeavours to develop novel biocides for wood from tannins and flavonoids (92, 93, 121, 338).

The synthetic tropolones developed in this study have proved promising from the point of view of fungitoxicity. The greatest obstacle to their use is a simple, inexpensive method of

production. At present the costs of chemical syntheses are prohibitive. With many methods for tropolone synthesis well established and the majority of known natural tropolones having been synthesised, research into tropolone synthesis has waned. A commercial demand for tropolone compounds could see a resurgence in research into novel methods of synthesis. In terms of alternative options, sourcing a supply of natural tropolones for use in wood protection is not practicable as the only natural source of alkyltropolones are durable timbers of the Cupressaceace family. Biosynthesis in cell cultures has potential. Haluk and Roussel (98) have reported the biosynthesis of tropolones from *Thuja plicata* cell cultures and the use of the crude extract, thus obtained, in impregnation trials for wood preservation. Biotechnological synthesis is in its infancy. Yields at present are poor and the technology is a long way from commercialisation. A fourth, longer-term option, which is only at the conceptual stage, is the possibility of genetic engineering of timber durability. The biosynthetic pathways to tropolones are now being elucidated (197) and the identification of the genes responsible for expression of tropolones in durable timbers will eventually follow. Synthetic organic chemistry will undoubtedly have a role to play in these emerging fields of biotechnology.

Experimental confirmation of a prediction is merely a measurement. An experiment disproving a prediction is a discovery.

Enrico Fermi

Chapter 7: Experimental

7.1 Materials and General Methods

7.1.1 Materials

General use solvents and reagents were AR grade and were used as provided by the supplier, without prior purification, unless otherwise stated. Solvents used for column chromatography were purified, prior to use, by distillation or double distillation in the case of hexane. Solvents used for NMR spectroscopy were used as supplied by Sigma-Aldrich, with the exception that in certain cases, $CDCl_3$ was freed of HCl by passage through a column of basic Al_2O_3 I (Brockman activity 1). Solvents used for synthesis were dried and purified prior to use in the following ways. CS_2, CH_2Cl_2, toluene, xylene, pyridine, THF were dried by heating under reflux with CaH_2 and distilling under a N_2 atmosphere. Diethyl ether, 1,4-dioxane and *tert*-butyl alcohol were dried by heating under reflux with Na metal prior to distillation under a N_2 atmosphere. CH_3NO_2 and acetone were dried by standing over $CaSO_4$ overnight and distilled under a N_2 atmosphere. Benzene was freed of thiophen by shaking with concentrated H_2SO_4, water, 5% aqueous NaOH, and finally water. Heating under reflux over CaH_2 and distilling under a N_2 atmosphere gave dry benzene. Quinoline was dried with Na_2SO_4 and distilled from zinc dust under reduced pressure.

Reactants used in synthesis were used as supplied by the supplier, unless otherwise stated, with the following exceptions. Octanal was purified by distillation. Benzaldehyde was purified by distillation under reduced pressure. Cinnamaldehyde was freed of cinnamic acid by dissolving in ether and washing with 5% aqueous $NaHCO_3$, and then water. Following drying over $MgSO_4$, filtration and removal of the ether, cinnamaldehyde was purified by vacuum distillation (oil pump). *Trans*-hexenal diethyl acetal (98%) as sourced from Sigma-Aldrich was shown by GC/MS analysis to consist of *ca.* 90% *trans*-hexenal diethyl acetal, *ca.* 8% *cis*-hexenal diethyl acetal and *ca.* 2% hexenal.

Mid-range steam distillation fractions of foliage essential oils of totara, kahikatea, and rimu used in Chapter 3 were provided courtesy of Professor R. C. Cambie (formerly of the University of Auckland). Details of the sampling and distillation of the totara essential oil have been reported elsewhere (177). Only those distillation fractions containing sesquiterpenoids (fraction 8-11 (TF8-TF11)) were used in this study. The boiling points and masses of these fractions have been reproduced in Table 7.1 with the permission of Franich and Cambie (177). Unfortunately the sampling history and distillation details of kahikatea and rimu oils were unknown. Crude α-cadinol from foliage essential oil of *Araucaria imbricata* was also obtained courtesy of Professor Cambie and purified by column chromatography on silica gel eluting with 3:1 hexane:ether.

Table 7.1: Fractions from the distillation of the essential oil of *Podocarpus totara* leaves.

Fraction	b.p. °C/20 mm Hg	Mass (g)
TF8	86-90 (3 mm)	34.88
TF9	84-91	23.84
TF10	84-90	43.45
TF11	85-90	88.03

7.1.2 Analytical Techniques

Analytical normal phase thin layer chromatography (tlc) was performed on 0.2 mm thick sheets of Merck Kieselgel 70 F_{254} on aluminium backing. Eluted compounds were visualised by UV fluorescence quenching at 254 nm or I_2 staining. Preparative column chromatography was performed on Al_2O_3 and silica gel stationary phases including BDH and ICN neutral Al_2O_3 (5% deactivated with water), ICN basic Al_2O_3 (5% deactivated with 5% aqueous acetic acid) or BDH and ICN silica gel (particle size 73-200 μm). Column pack weight varied with mass of eluant. In a typical separation a column of length 200 mm and internal diameter 25 mm was packed with 70 g Al_2O_3 or 35 g silica gel onto which 1-2 g of the compound for purification was loaded. Vacuum distillations were performed using an Edwards dual oil pump vacuum system (E2M2 rotary pump and EO50/70 diffusion pump) in series with a liquid N_2 trap.

Melting point were determined using a Reichert Thermopan melting point microscope and were uncorrected. Elemental analysis was performed by The Campbell Microanalytical Laboratory, Department of Chemistry, University of Otago, Dunedin, New Zealand.

7.1.3 Spectroscopic Techniques

7.1.3.1 Fourier Transform Infrared Spectroscopy

Infrared spectra were obtained on either a Digilab FTS60 fourier transform infrared spectrometer or a Bruker Vector 33 fourier transform infrared spectrometer. Samples were run either as neat films between KBr or NaCl discs or as dispersed solids in KBr discs.

7.1.3.2 Integrated Gas Chromatography/Mass Spectroscopy

Two instruments were used to obtain GC/MS spectra during the course of this research. One of these was a Hewlett-Packard 5985 integrated GC/MS equipped with a 25 m × 0.2 mm × 0.33 μm (film thickness) HP-5 ultra 2 (cross linked 5% PhMe silicone) column. Helium was the carrier gas with a mean linear velocity of 30 cm/s and the GC temperature was raised from 40 to 300°C at 5°C/minute. The purged splitless injection was held for 1 minute after which the split ratio was 15:1. Mass spectra were recorded under electron ionisation (70 eV).

The other instrument used was a Hewlett-Packard 5890 GC with Hewlett-Packard 5971A mass spectra detector equipped with either a 50 m × 0.2 mm × 0.33 μm (film thickness) HP-5 ultra 2 (cross linked 5% PhMe silicone) column or a 30 m × 0.25 mm × 0.25 μm (film thickness) Alltech EC^{TM}-Wax column. The GC conditions were the same for the HP-5 column as those described above. In the case of the EC^{TM}-Wax column the GC temperature was raised from 40 to 260°C at 5°C/minute. A purged splitless injection was used and mass spectra were recorded under electron ionisation (70 eV). High resolution GC/MS and Probe MS analysis was performed by Daryl Rowan and John Allen of HortResearch, Palmerston North, New Zealand.

For pure compounds MS data are provided in terms of the *m/z* and relative abundances of the molecular ion and the five most abundant ions. For mixtures GC retention times (Rt), relative GC peak area, MS data and the proposed compound is provided for each of the components.

GC/MS was used to provide an estimate of product purity and percentages of compounds in a product mixture. In doing so unit response factors were assumed for reaction products and by-products. This was deemed a reasonable assumption as reaction products and by-products were, in most cases, closely related structures.

7.1.3.3 Direction Insert Probe (DIP) Mass Spectroscopy

Probe MS spectra were obtained on a Hewlett-Packard 5985 GC/MS with a direct insertion probe inlet. The probe temperature was raised from 40 to 300°C at 30°C/minute with the ion source set at 200°C. Spectra were recorded under electron ionisation with ionising energy set at 70 eV.

7.1.3.4 Direct Infusion Electrospray Mass Spectroscopy

Direct infusion ES/MS and APCI/MS spectra were obtained on a Thermofinnigan LCQ DECA XP mass spectrometer operating in electrospray ionisation (ESI) and atmosphere pressure chemical ionisation (APCI) modes. Analytes (0.1 mg/ml in methanol) were introduced to the ES/MS or APCI/MS interface directly by syringe pump at a flow rate of 40 µl/minute. MS data were collected using Xcalibur software. The spectra obtained were an average of at least 20 scans. Spectra were collected over a window of *m/z* 50-500 every 1 s with the electron multiplier voltage set at -870.4 V. For ESI the spray voltage was 4.99 kV, heated capillary temperature was 252°C and sheath gas flow was 26.9 psi. For APCI the vaporiser temperature was 500°C, the heated capillary temperature was 206°C and the sheath gas flow was 39.0 psi. The MS data were presented in terms of positive and negative molecular ions and the *m/z* and relative abundance of ions created by MS^2 on the positive and negative molecular ions at 40% collision energy.

7.1.3.5 Nuclear Magnetic Resonance Spectroscopy

Two NMR spectrometers were used during the course of this research. Initially one dimensional NMR spectra were acquired on a Bruker AC-200 spectrometer at 4.7 T and 298 K, operating at 200.13 MHz and 50.33 MHz for ^{1}H and ^{13}C respectively, using a 5 mm standard dual $^{1}H/^{13}C$ probe. Subsequently one and two dimensional ^{1}H and ^{13}C NMR spectra were acquired on a Bruker Avance 400 spectrometer at 9.4 T and 300 K, operating at 400.13

MHz and 100.63 MHz for ^{1}H and ^{13}C respectively, using a 5 mm ^{1}H/broadband inverse detection probe with z gradient. A variety of deuterated solvents were used including $CDCl_3$, acetone-d_6, D_2O, MeOD, MeOH-d_4, THF-d_8, pyridine-d_6 and DMSO-d_6. Spectra were processed used standard Bruker software and chemical shifts were referenced against the solvent signal or internal TMS. The operating parameters for both instruments were as follows:

Bruker AC-200

^{1}H Spectra

^{1}H spectra were acquired using a standard single 90° pulse of 7.9 μs and relaxation delay of 1 sec. Spectra were collected over a 10 ppm window with 16K data points giving a peak resolution accurate to 0.3 Hz. Typically 16 scans were obtained. Line broadening was set at 0.1 ppm.

^{13}C Spectra

^{13}C spectra were acquired using a power-gated decoupling pulse sequence employing a 90° pulse of 13.6 μs and a 2 sec relaxation delay. Spectra were collected over a 12 kHz window with 32K data points giving a peak resolution that was accurate to 0.7 Hz. Typically 1024 scans were obtained. Line broadening was set at 1.0 ppm.

Bruker Avance 400

^{1}H Spectra

^{1}H spectra were acquired using a standard single 30° pulse of 8.5 μs and relaxation delay of 2 sec. Spectra were collected over a 8224 ppm window with 32K data points resulting in a peak resolution accurate to 0.25 Hz. Typically 16 or 32 scans were obtained.

^{13}C Spectra

^{13}C spectra were acquired using a power-gated decoupling pulse sequence employing a 30° pulse of 13.0 μs and a 2 sec relaxation delay. Typically spectra were collected over a 31847 Hz window with 64K data points giving a peak resolution accurate to 0.49 Hz. The number of scans used ranged from 1024 to 20,000 depending on the amount of available material.

gCOSY Spectra

Gradient accelerated ^{1}H-^{1}H Correlated NMR Spectroscopy (gCOSY) spectra were acquired using the Bruker *cosygp* pulse programme typically over a 3592 Hz window for both F_1 and F_2 in a phase insensitive fashion. A 1024 (t_2) × 256 (t_1) data matrix was used with 1 to 4 FIDs acquired per t_1 increment. The data in F_1 was zero-filled to 512 points. Fourier transformation w.r.t t_1 and t_2 and sine-bell squared apodisation gave a transformed data matrix of 1024 (F_2) × 512 (F_1) real points.

HMQC Spectra

^{1}H detected Heteronuclear Multiple Quantum Coherence (HMQC) spectra were acquired using a Bruker *inv4gstp* pulse programme in a phase sensitive manner. The polarisation transfer delay was set for an assumed $^{1}J_{C\text{-}H}$ of 145 Hz. The size of the F_2 and F_1 spectral windows varied with the compound of interest, typically a F_2 window of 3592 Hz and a F_1 window of 15095 Hz were used with ^{13}C GARP-1 decoupling. A 1024 (t_2) × 128 (t_1) data matrix was used with 32 scans per increment. Spectra were recorded in simultaneous acquisition mode. Zero filling of data in F_1 and F_2, fourier transformations and cosine-bell squared apodisation gave a transformed data matrix of 2048 (F_2) × 1024 (F_1) real points.

HMBC Spectra

^{1}H detected Heteronuclear Multiple Bond Correlation (HMBC) spectra were acquired using a Bruker *inv4gslplrnd* pulse programme in a phase insensitive manner typically over a F_2 spectral window of 3592 Hz and a F_1 window of 18113 Hz with ^{13}C GARP-1 decoupling. A 1024 (t_2) × 256 (t_1) data matrix was acquired using simultaneous acquisition with 32 scans per increment. After F_1 zero filling, fourier transformation and cosine-bell squared apodisation a transformed data matrix of 1024 (F_2) × 512 (F_1) real points was obtained. The delay for evolution of long range coupling was 70 ms.

Data Presentation and Structure Labelling

^{1}H NMR data were presented in parts per million (ppm) downfield shift from tetramethylsilane standard. For each signal, chemical shift (δ), relative integral, multiplicity, coupling constants (*J*), and assignment information was given unless otherwise stated. Chemical shift and assignments were provided for ^{13}C NMR spectral data. Synthetic compounds were labelled for NMR assignment with unique numbers for each carbon. In order to achieve this it was necessary in certain cases (*e.g.* 4-non-1-enyltropolone (**59**)) to use a

numbering system that differed from the numbering system for IUPAC nomenclature. Where possible NMR numbering was kept consistent with numbering for IUPAC nomenclature. Representative structures labelled for NMR assignment are provided in the structural index at the end of this thesis.

7.2 Synthesis

7.2.1 Silylations

Two methods were used for the preparation of trimethylsilyl derivatives. The compound for derivatisation (0.01 g) was dissolved in pyridine (100 μl) and bis(trimethylsilyl)acetamide (BSA) (100 μl) and warmed to 60°C for 1 hr. Pyridine and BSA were blown down with N_2 at 60°C prior to GC/MS injection. Alternatively a solution of the compound for analysis was prepared in CH_2Cl_2 to a concentration of 1 mg/ml or less. Bis(trimethylsilyl)trifluoro-acetamide (BSTFA) with 1% chlorotrimethylsilane (TMCS) (10-30 μl) was added and the mixture warmed to 70°C for 1 hr. The product was injected directly onto the GC/MS column.

7.2.2 Synthesis of *p-n*-Nonylanisole (7)

Preparation of anisole

Dimethyl sulphate (111 ml, 1.17 mol) was added dropwise to phenol (100 g, 1.06 mol) dissolved in 20% aqueous NaOH (186 ml). The reaction mixture was heated under reflux for 1.5 hrs, cooled and the two phases separated. The organic layer was diluted with ether, washed with 10% NaOH, water, dried over $MgSO_4$ and filtered. Ether was removed by evaporation under reduced pressure to give **anisole** as a colourless liquid (94.5 g, 83%). ^{1}H NMR (200 MHz, $CDCl_3$) δ: 3.79 (3H, s, OCH_3), 6.93 (d, J = 8.6 Hz, H2 and H6), 6.97 (overlapping with H2 and H6, t, J = 7.4 Hz, H4), (H4, H2 and H6, 3H), 7.32 (2H, dd, J = 8.6, 7.4 Hz, H3 and H5); ^{13}C NMR (50 MHz, $CDCl_3$) δ: 54.9 (OCH_3), 113.8 (C2, C6), 120.6 (C4), 129.4 (C3, C5), 159.5 (C1); MS (EI, GC/MS) *m/z*: 108 ($M^{\cdot+}$, 100%), 93 (14%), 78 (59%), 77 (18%), 65 (53%), 51 (10%). The product was verified as anisole by comparison of ^{1}H and ^{13}C NMR data with published data (339).

Preparation of nonanoic chloride

Neat nonanoic acid (100 g, 0.63 mol) was added dropwise to thionyl chloride (91.3 ml, 1.26 mol) with stirring over a period of 1.5 hrs. The reaction mixture was warmed to 40°C until evolution of HCl and SO_2 ceased. Excess thionyl chloride was removed by evaporation under reduced pressure to give **nonanoic chloride** as a yellow liquid in quantitative yield (111.8 g, 100%). IR (NaCl, neat) cm^{-1}: 1800.8 (ν C=O); ^1H NMR (200 MHz, $CDCl_3$) δ: 0.88 (3H, t, *J* = 6.7 Hz, H9), 1.2-1.4 (10H, m, H4-H8), 1.71 (2H, p, *J* = 7.2 Hz, H3), 2.88 (2H, t, *J* = 7.4 Hz, H2); ^{13}C NMR (50 MHz, $CDCl_3$) δ: 14.1 (C9), 22.6 (C8), 25.1 (C3), 28.4 (C4), 29.0, 29.0 (C5, C6), 31.7 (C7), 47.1 (C2), 173.8 (C1). The product was verified as nonanoic chloride by comparison of ^1H and ^{13}C NMR data with published data (339).

Preparation of *p*-nonanoylanisole (8)

Anisole (89 g, 0.824 mol) and nonanoyl chloride (157.6 g, 0.893 mol) in dry CS_2 was added dropwise to $AlCl_3$ (109.9 g, 0.824 mol) in dry CS_2 over a period of 3 hrs. The reaction mixture was heated under reflux for 4.5 hours and left to stand overnight. CS_2 was distilled, aqueous HCl (250 ml, 2 mol l^{-1}) was cautiously added, followed by water and ether. The ether extract was washed with 10% NaOH, water, dried over $MgSO_4$, filtered and ether removed under reduced pressure to give *p*-nonanoylanisole as a light brown waxy solid (177.1 g, 86.6%). A portion of the crude product (10 g) was successively recrystallised from aqueous ethanol to give ***p*-nonanoylanisole (8)** as white crystals (4.1 g, 41%), m.p. = 37-38 °C. IR (NaCl, neat) cm^{-1}: 1668 (ν C=O); ^1H NMR (200 MHz, $CDCl_3$) δ: 0.88 (3H, t, *J* = 6.7 Hz, H16), 1.15-1.45 (10H, m, H11-H15), 1.71 (2H, p, *J* = 7.2 Hz, H10), 2.90 (2H, t, *J* = 7.4 Hz, H9), 3.87 (3H, s, H7), 6.93 (2H, d, *J* = 9 Hz, H2 and H6), 7.95 (2H, d, *J* = 9 Hz, H3 and H5); ^{13}C NMR (50 MHz, $CDCl_3$) δ: 14.0 (C16), 22.6 (C15), 24.6 (C10), 29.1, 29.4, 29.4, (C11, C12, C13), 31.8 (C14), 38.2 (C8), 55.3 (C7), 113.6 (C2, C6), 130.1 (C4), 130.2 (C3, C5), 163.2 (C1), 199.2 (C8); MS (EI, GC/MS) *m/z*: 248 ($M^{\cdot+}$, 2%), 163 (10%), 150 (82%), 135 (100%), 107 (8%), 92 (8%). Elemental Analysis: found C, 77.5, H, 9.8; $C_{16}H_{24}O_2$ requires C, 77.4, H, 9.7.

Preparation of *p-n*-nonylanisole (7)

Catalytic Hydrogenation

Recrystallised *p*-nonanoylanisole (**8**) (1 g, 0.004 mol) dissolved in glacial acetic acid was shaken with 10% Pd/C (100 mg) under 2 atmospheres of H_2 for 40 hrs. Water and ether were added. The ether extract was washed with 10% NaOH, water and dried over $MgSO_4$ and filtered. Ether was removed under reduced pressure to give *p-n*-nonylanisole (**7**) as a colourless liquid, (0.41 g, 44%). Characterisation data for *p-n*-nonylanisole (**7**) are provided below.

Wolff-Kishner reduction

p-Nonanoylanisole (**8**) (5 g , 0.020 mol), hydrazine hydrate (3 ml, 0.06 mol), KOH (3.4 g, 0.06 mol) and triethylene glycol (50 ml) were heated under reflux at 150°C for 2 hrs. Water and excess hydrazine hydrate were removed *via* a take-off condenser until the temperature of the reaction mixture reached 200°C. The mixture was heated under reflux for a further 4 hours. The reaction mixture was allowed to cool and poured into 200 ml of ice water. The product was successively extracted into ether and the combined extracts washed with 5 mol l^{-1} HCl and then water, dried over $MgSO_4$ and filtered. Evaporation under reduced pressure yielded a yellow liquid (3.2 g, 68%). GC/MS analysis indicated the crude product was approximately a 1:2 mixture of *p-n*-nonylanisole (**7**) and *p-n*-nonylphenol (**9**). *p-n*-Nonylphenol (**9**) was identified from identical GC/MS results as *p-n*-nonylphenol (**9**) produced by BBr_3 demethylation of *p-n*-nonylanisole (**7**) (see page 216). IR (NaCl, neat) cm^{-1}: 3100-3500 (ν O-H); GC/MS: 2 peaks, (Rt (min), GC peak area, MS *m/z*, compound); 41.5, 37%, 234 ($M^{\cdot+}$, 11%), 134 (2%), 122 (12%), 121 (100%), 91 (4%), 77 (3%), *p-n*-nonylanisole (**7**); 43.0, 63%, 220 ($M^{\cdot+}$, 15%), 120 (2%), 107 (100%), 94 (1%), 77 (5%), *p-n*-nonylphenol (**9**).

The *p-n*-nonylphenol (**9**) in the mixture was methylated by reaction of the crude product (2.3 g) with dimethyl sulphate in 20% aqueous NaOH as described for phenol above. Workup gave *p-n*-nonylanisole (**7**) as an orange liquid (1.3 g, 55%). The calculated overall yield of *p-n*-nonylanisole (**7**) was 37%. Characterisation data for *p-n*-nonylanisole (**7**) are provided below.

Clemmensen reduction

Zn dust (3.2 g, 0.0484 mol) was treated with a solution of 5% $HgCl_2$ in 2% aqueous HCl (10 mls) for 1.5 hrs. The solution as decanted off and *p*-nonanoylanisole (**8**) (2 g, 0.0081 mol), water (5 ml) and concentrated HCl (5 ml) were added. The reaction mixture was heated under reflux for 20 hrs, cooled and ether and water were added. The solution was decanted from the amalgam and the ether layer was separated, washed with 10% aqueous NaOH solution, water, dried over $MgSO_4$ and filtered. Evaporation under reduced pressure gave a yellow liquid (1.6 g, 84%). The crude product contained *p-n*-nonylanisole (**7**) (*ca.* 95%) and possibly *p*-nonenylanisole (**10**) (*ca.* 5%) as indicated by GC/MS. Characterisation data of *p-n*-nonylanisole (**7**) are provided below. *p*-Nonenylanisole (**10**) was tentatively identified from its mass spectrum. MS (EI, GC/MS) *m/z*: 232 ($M^{\cdot+}$, 19%), 147 (100%), 134 (23%), 121 (22%), 115 (11%), 91 (14 %).

Clemmensen reduction with Martin modification

Zn dust (7.92 g, 0.121 mol) was treated with a solution of 6% $HgCl_2$ in 1.5% aqueous HCl (11 mls) for 1 hrs. The solution was decanted and water (5 ml) and *p*-nonanoylanisole (**8**) (5 g, 0.0202 mol) in toluene (7 ml) were added. Concentrated HCl (15 ml) was added cautiously and the reaction mixture heated under reflux for 19 hrs. The mixture was cooled, water (50 ml) was added and the solution decanted from the amalgam. Ether (50 ml) was added and the organic layer was worked up as described above to give a yellow liquid (4.48 g, 95%). Column chromatography on 5% deactivated Al_2O_3 eluting with hexane gave ***p-n*-nonylanisole** (**7**) as a colourless liquid (4.0 g, 85%). ^1H NMR (200 MHz, $CDCl_3$) δ: 0.88 (3H, t, J = 6.7 Hz, H16), 1.1-1.4 (12H, m, H10-H15), 1.56 (2H, p, J = 7.2 Hz, H9), 2.54 (2H, t, J = 7.6 Hz, H8), 3.79 (3H, s, H7), 6.82 (2H, d, J = 8.6 Hz, H2 and H6), 7.09 (2H, d, J = 8.6 Hz, H3 and H5); ^{13}C NMR (50 MHz, $CDCl_3$) δ: 14.1 (C16), 22.7 (C15), 29.3, 29.4, 29.6, 29.6 (C10, C11, C12, C13), 31.8, 31.9 (C9, C14), 35.1 (C8), 55.1 (C7), 113.6 (C2, C6), 129.2 (C3, C5), 135.0 (C4), 157.6 (C1); MS (EI, GC/MS) *m/z*: 234 ($M^{\cdot+}$, 11%), 134 (2%), 122 (12%), 121 (100%), 91 (4%), 77 (3%); Elemental Analysis: found C, 82.3, H, 10.9; $C_{16}H_{26}O$ requires C, 82.0, H, 11.2.

7.2.3 Synthesis of 1-Methyl-4-nonylcyclohexan-1-ol (5)

Preparation of 1-methoxy-4-nonylcyclohexa-1,4-diene (11)

To ammonia (*ca.* 40 ml), freshly distilled and cooled by immersion in a dry ice/acetone bath, was added *p-n*-nonylanisole (**7**) (1 g, 0.0043 mol) in dry tetrahydrofuran (3 ml). Dry 2-methyl-2-propanol (5 ml) was added. Li shot (0.75 g, 0.107 mol) was ground to flat plates, washed free of paraffin with THF and added over a period of 1 hr. The cooling bath was removed and the reaction solution was refluxed for 2 hrs. The blue colour of the reaction solution was discharged by cautious addition of MeOH (4 ml). The ammonia was allowed to evaporate overnight and the residue was partitioned between water and ether. The ether extract was washed with water, dried over $MgSO_4$, filtered and the ether removed under reduced pressure to yield a yellow liquid (0.96 g, 96%). The product was a mixture of components (see below). Column chromatography on 5% deactivated neutral Al_2O_3 eluting with a hexane:CH_2Cl_2 gradient was unsuccessful in separating the main product, 1-methoxy-4-nonylcyclohexa-1,4-diene (**11**) from the by-products and starting material. A crude yield of 1-methoxy-4-nonylcyclohexa-1,4-diene (**11**) was calculated at *ca.* 73% based on the estimated composition of the product mixture from GC/MS evidence.

The spectroscopic evidence for the product mixture was as follows. IR (NaCl, neat) cm^{-1}: 1697 and 1666 (ν C=C); GC/MS: 4 main and 2 minor peaks, (Rt (min), GC peak area, MS *m/z*, compound); 36.7, 10%, 208 ($M^{\cdot+}$, 11%), 123 (3%), 109 (14%), 96 (100%), 81 (93%), 67 (26%), a nonylcyclohex-1-ene possibly 1-nonylcyclohex-1-ene (**15**) or 3-nonylcyclohex-1-ene (**16**); 42.2, 4%, 238 ($M^{\cdot+}$, 11%), 209 (30%), 153 (40%), 111 (57%), 97 (24%), 84 (100%), a methoxynonylcyclohexene, possibly 1-methoxy-4-nonylcyclohex-1-ene (**17**); 42.4, 29%, 236 ($M^{\cdot+}$, 10%), 137 (1%), 123 (100%), 109 (8%), 91 (8%), 79 (4%), 1-methoxy-4-nonylcyclohexa-1,4-diene (**11**); 42.7, 6%, as above, *p-n*-nonylanisole (**7**); 43.3, 47%, 236 ($M^{\cdot+}$, 12%), 137 (3%), 123 (59%), 109 (100%), 91 (11%), 79 (5%), 1-methoxy-4-nonylcyclohexa-1,4-diene (**11**) possibly rearranged to the 1,5-diene (**18**) on GC injection; 43.8, 2%, as above, *p-n*-nonylphenol (**9**).

Major signals in ^{1}H and ^{13}C NMR spectra of the mixture were assignable to **1-methoxy-4-nonylcyclohexa-1,4-diene (11)**. ^{1}H NMR (200 MHz, $CDCl_3$)[1] δ: 0.88 (t, J = 6.6 Hz, H16), 1.1-1.45 (m, H10-H15), 1.58 (m, H9), 1.97 (t, H8), 2.73 (unresolved d, H3 and H6), 3.55 (s, H7), 4.63 (unresolved t, H2), 5.36 (unresolved t, H5); ^{13}C NMR (50 MHz, $CDCl_3$) δ: 14.1 (C16), 22.7 (C15), 27.7, 29.1, 29.3, 29.4, 29.4, 29.6, 29.7 (C3, C6, C9-C13), 32.0 (C14), 36.8 (C8), 53.7 (C7), 90.5 (C2), 117.0 (C5), 135.6 (C4), 153.1 (C1).

Preparation of a 2:1 mixture of 4-nonylcyclohex-2-enone (12) and 4-nonylcyclohex-3-enone (13)

The crude 1-methoxy-4-nonylcyclohexa-1,4-diene (**11**) product was rearranged to a mixture of 4-nonylcyclohexenones by treatment with acid under a variety of conditions. A typical reaction involved treating the crude Birch reduction product (0.84 g) dissolved in 95% ethanol with an equal volume of 12% aqueous HCl. The mixture was warmed to 60-65°C under N_2 for 1 hour. Water was added and the product was extracted into ether. The ether extract was washed with water, dried over $MgSO_4$, filtered and concentrated by solvent evaporation to give a pale yellow liquid (0.68 g). Column chromatography of the crude product (0.65 g) on 5% deactivated neutral Al_2O_3 eluting with hexane gave a mixture of mainly the nonylcyclohexene (**15** or **16**) and *p-n*-nonylanisole (**7**). Elution with 1:1 hexane:CH_2Cl_2 gave approximately a 2:1 mixture of **4-nonylcyclohex-2-enone (12)** and **4-nonylcyclohex-3-enone (13)** as a colourless liquid (0.268 g, 47% (based on the percentage of (**11**) in the starting material). Calculated overall yield from *p-n*-nonylanisole (**7**) was 34%. IR (NaCl, neat) cm^{-1}: 1719 (ν C=O, (**13**)), 1684 (ν C=O, (**12**)); GC/MS: 2 peaks (Rt (min), GC peak area, MS *m/z*, compound); 42.3, 31%, 222 ($M^{\cdot+}$, 36%), 126 (15%), 111 (65%), 95 (56%), 82 (46%), 68 (100%), 4-nonylcyclohex-3-enone (**13**); 43.3, 64%, 222 ($M^{\cdot+}$, 21%), 123 (10%), 109 (74%), 96 (100%), 81 (56%), 68 (45%), 4-nonylcyclohex-2-enone (**12**).

Major signals in the ^{1}H and ^{13}C NMR spectra of the product mixture were assignable to **4-nonylcyclohex-2-enone (12)**. ^{1}H NMR (200 MHz, $CDCl_3$)[2] δ: 0.9 (t, H15), 1.2-1.5 (m, H7-H14), 1.6-1.8 (m, H5), 2.3-2.5 (m, H4 and H6), 5.98 (dd, J = 10.2, 2.4 Hz, H2), 6.87 (ddd, J = 10.2, 2.7, 1.3 Hz, H3); ^{13}C NMR (50 MHz, $CDCl_3$) δ: 13.9 (C15), 22.5 (C14), 26.8 (C8), 28.5,

[1] Relative integrals for 1-methoxy-4-nonylcyclohexa-1,4-diene (**11**) are not shown as the product was a mixture of compounds.

[2] Relative integrals for 4-nonylcyclohex-2-enone (**12**) are not shown as the product was a mixture of two isomers.

29.1, 29.4, 29.5 (C9-C12), 31.7 (C13), 34.4, 35.9 (C5, C7), 36.7 (C6), 53.3 (C4), 128.6 (C2), 155.3 (C3), 199.9 (C1).

A variety of different reaction conditions was employed in an attempt to exclusively form 4-nonylcyclohex-2-enone (**12**). Variations included reaction temperatures of 20°C and 60-65°C, reaction times of 10 min, 30 min, 1 hr, 2.5 hrs and 16 hrs. Various acid and alkali catalysts were employed including 5% aqueous HCl, 10% aqueous HCl, 36% HCl, 10% aqueous H_2SO_4, 10% aqueous oxalic acid and 5% and 10% aqueous NaOH solution. Solvents included ethanol, methanol and acetone.

Preparation of a 6:1 mixture of 4-nonylcyclohex-3-enone (13) and 4-nonylcyclohex-2-enone (12)

The crude 1-methoxy-4-nonylcyclohexa-1,4-diene (**11**) (1 g) product dissolved in 99% ethanol (20 ml) was treated with 12% aqueous HCl under a N_2 atmosphere. The mixture was stirred at room temperature for 1 hour. The product was worked up as described for the rearrangement above to give a pale yellow liquid (0.876 g). Column chromatography of the crude product (0.847 g) on 5% deactivated neutral Al_2O_3 eluting with 1:1 hexane:CH_2Cl_2 gave a 6:1 mixture of **4-nonylcyclohex-3-enone** (**13**) (*ca.* 86%) and **4-nonylcyclohex-2-enone** (**12**) (*ca.* 14%) (based on GC/MS peak areas) as a colourless liquid (0.31 g, 44% (based on the percentage of (**11**) in the starting material). Calculated overall yield from *p-n*-nonylanisole (**7**) was 33%.

Major signals in the ^1H and ^{13}C NMR spectra of the product mixture were assignable to **4-nonylcyclohex-3-enone** (**13**). ^1H NMR (200 MHz, $CDCl_3$)[3] δ: 0.88 (t, J = 6.7 Hz, C15), 1.2-1.45 (m, H8-H15), 2.05 (t, H7), 2.30-2.50 (m, H5 and H6), 2.85 (unresolved heptet, J = 1.7 Hz, H2), 5.43 (unresolved tt, J = 3.7, 1.2 Hz, H3); ^{13}C NMR (50 MHz, $CDCl_3$) δ: 14.0 (C15), 22.5 (C14), 27.5, 28.4, 29.2, 29.4, 29.4 (C8-C12), 31.8 (C13), 37.0 (C7), 38.5 (C5), 39.5 (C6), 40.6 (C2), 117.4 (C3), 138.8 (C4), 211.1 (C1).

[3] Relative integrals for 4-nonylcyclohex-3-enone (**13**) are not shown as the product was a mixture of two isomers.

Preparation of 4-nonylcyclohexanone (20)

To a mixture of 4-nonylcyclohex-2-enone (**12**) and 4-nonylcyclohex-3-enone (**13**) (0.040 g, 0.180 mmol) dissolved in absolute ethanol was added 4 mg Pd/C (5% Pd). The reaction mixture was stirred under 1 atmosphere of H_2 for 16 hrs. The catalyst was filtered and the ethanol removed to give a yellow liquid (0.036 g). Column chromatography on 5% deactivated Al_2O_3 eluting with 3:1 hexane:CH_2Cl_2 gave **4-nonylcyclohexanone** (**20**) as a colourless liquid (0.027 g, 67%). IR (NaCl, neat) cm^{-1}: 1719 (ν C=O); ^1H NMR (400 MHz, $CDCl_3$) δ: 0.85 (3H, t, *J* = 6.9 Hz, H15), 1.20-1.35 (m, H7-H14), 1.30-1.40 (overlapping with H7-H14, dt, *J* = 12.2, 5.1 Hz, H3 and H5), (H7-H14, H3 and H5, 18 H), 1.66 (1H, m, H4), 2.02 (2H, m, H3' and H5'), 2.25-2.38 (4H, m, H2 and H6); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 14.1 (C15), 22.6 (C14), 27.3 (C8), 29.3 (C12), 29.6, 29.6 (C10, C11), 29.7 (C9), 31.9 (C13), 32.7 (C3, C5), 35.5 (C7), 35.9 (C4), 40.8 (C2, C6), 212.5 (C1); MS (EI, GC/MS) *m/z*: 224 ($M^{\cdot+}$, 15%), 195 (15%), 139 (32%), 126 (53%), 97 (100%), 55 (59%). Elemental analysis did not give satisfactory microanalytical data, found C, 79.5, H, 12.6; $C_{15}H_{28}O$ requires C, 80.3, H, 12.6.

Preparation of 1-methyl-4-nonylcyclohexan-1-ol (5)

Iodomethane (3.8 g, 0.027 mol) in dry ether (20 ml) was added to Mg turnings (0.65 g, 0.027 g atoms) under a N_2 atmosphere. The mixture was heated under reflux for 1.5 hrs during which time all the magnesium was consumed. 4-Nonylcyclohexanone (**20**) (2.1 g, 0.0094 mol) in ether (20 ml) was added dropwise and the reaction mixture was heated under reflux for 20 hrs. The mixture was cooled and 2.4% aqueous NH_4Cl (40 ml) was added. The pH of the reaction solution was adjusted to 6.5 with 10% aqueous HCl. Ether was added and the two phases separated. The ether extract was washed with water, dried over $MgSO_4$ and filtered. Ether was removed by evaporation under reduced pressure to give a mixture of *cis* (**21**) and *trans* (**22**) isomers of 1-methyl-4-nonylcyclohexan-1-ol as a pale yellow liquid (2.09 g, 93%). Column chromatography of part of this product (1.4 g) on silica gel eluting with 1:3 hexane:CH_2Cl_2 gave ***cis*-1-methyl-4-nonylcyclohexan-1-ol**[4] (**21**) as a colourless liquid which crystallised to give white needles (419 mg, 28%), m.p. = 37-38°C. IR (neat) cm^{-1}: 3100-3500 (ν O-H); ^1H NMR (400 MHz, $CDCl_3$) δ: 0.86 (3H, t, *J* = 6.7 Hz, H16), 1.10-1.30 (m, H4, H3 and H5, H8-H15), 1.18 (overlapping with H3-H5, H8-H15, s, H7), 1.34 (overlapping with H3-

H5, H8-H15, dt, H2 and H6), (H4, H3 and H5, H8-H15, H7, H2 and H6, 24H), 1.54 (2H, broad d (unresolved), H3' and H5'), 1.61 (2H, md (unresolved), H2' and H6'); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 14.1 (C16), 22.7 (C15), 27.0 (C9), 28.5 (C3, C5), 29.3 (C13), 29.6, 29.7 (C11, C12), 29.9 (C10), 31.2 (C7), 31.9 (C14), 36.8, 36.9 (C8, C4), 38.6 (C2, C6), 69.3 (C1); MS (EI, GC/MS) 1 peak, Rt = 34.3 min, *m/z*: 240 ($M^{\cdot+}$, 2%), 225 (11%), 124 (5%), 95 (9%), 81 (9%), 71 (100%); Elemental Analysis: found C, 80.1, H, 13.6; $C_{16}H_{32}O$ requires C, 79.9, H, 13.4.

Elution with CH_2Cl_2 gave ***trans*-1-methyl-4-nonylcyclohexan-1-ol**[4] **(22)** as a colourless liquid (543 mg, 36%), m.p. = 25-26°C. IR (neat) cm^{-1}: 3100-3500 (ν O-H); ^1H NMR (400 MHz, acetone-d_6) δ: 0.86 (3H, t, *J* = 6.7 Hz, H16), 1.02 (2H, m, H3 and H5), 1.13 (3H, s, H7), 1.15-1.24 (17 H, m, H4 and H8-H15), 1.41 (2H, dt, *J* = 12.5, 3.8 Hz, H2 and H6), 1.58 (2H, unresolved td, H2' and H6'), 1.67 (2H, m, H3' and H5'); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 14.0 (C16), 22.6 (C15), 25.9 (C7), 27.2 (C9), 29.3 (C13), 29.6, 29.6 (C11, C12), 29.9 (C10), 30.3 (C3, C5), 31.9 (C14), 36.1 (C8), 36.7 (C4), 39.8 (C2, C6), 71.0 (C1); MS (EI, GC/MS) 1 peak, Rt = 34.7 min, *m/z*: 240 ($M^{\cdot+}$, 2%), 225 (8%), 124 (5%), 95 (9%), 81 (8%), 71 (100%), Elemental Analysis: found C, 80.0, H, 13.3; $C_{16}H_{32}O$ requires C, 79.9, H, 13.4.

Autoxidation of 4-nonylcyclohex-3-enone (13) and 4-nonylcyclohex-2-enone (12)

The 6:1 mixture of 4-nonylcyclohex-3-enone (**13**) and 4-nonylcyclohex-2-enone (**12**) when left standing at room temperature in the presence of air for 3 days underwent partial autoxidation. Column chromatography on the resulting mixture (280 mg) on 5% deactivated Al_2O_3 eluting with hexane gave an 8:1 mixture of 4-nonylcyclohex-3-enone (**13**) and 4-nonylcyclohex-2-enone (**12**) respectively. Elution with 1:3 hexane:ether gave a 6:1 mixture of two autoxidation products as a colourless liquid (35 mg). Based on the following spectroscopic evidence the major autoxidation product (*ca.* 86%) was tentatively characterised as **4-hydroxy-4-nonylcyclohex-2-enone (23)**. IR (neat) cm^{-1}: 3100-3500 (ν O-H), 1668 (ν C =O); ^1H NMR (200 MHz, $CDCl_3$) δ: 0.88 (3H, t, *J* = 6.7 Hz, H15), 1.2-1.45 (14H, m, H9-H14), 1.50-1.70 (overlapping with H7, m, H8), 1.78 (m, H7), (H8, H7, 4H), 2.05 (1H, m, H5), 2.4 (2H, m, H6), 2.65 (1H, m, H5'), 6.05 (1H, d, *J* = 10.4 Hz, H2), 6.85 (1H, dd, *J* = 10.4, 0.9 Hz, H3); ^{13}C NMR (50 MHz, $CDCl_3$) δ: 14.1 (C15), 22.6 (C14), 23.4 (C8), 29.3, 29.4, 29.5,

[4] *Cis* and *trans* refers to the two highest order groups OH and C_9H_{19}.

29.5 (C9-C12), 30.0 (C5), 31.6 (C13), 34.4, 35.8 (C6, C7), 81.6 (C4), 130.5 (C2), 151.6 (C3), 199.7 (C1); MS (EI, GC/MS) *m/z*: 238 ($M^{\cdot+}$, 1%), 196 (2%), 181 (3%), 111 (100%), 97 (8%), 83 (35%). The mass spectrum of the minor autoxidation product (*ca.* 14%) was as follows. MS (EI, GC/MS) *m/z*: 254 ($M^{\cdot+}$, 3%), 142 (15%), 127 (81%), 99 (100%), 84 (18%), 71 (48%).

Preparation of 4-nonylcyclohexanone (20) from *p-n*-nonylanisole (7)

Birch reduction and acid rearrangement of *p-n*-nonylanisole (**7**) (10 g, 0.043 mol), as described above, gave a mixture of 4-nonylcyclohex-2-enone (**12**) (*ca.* 36%), 4-nonylcyclohex-3-enone (**13**) (*ca.* 51%) and a 4-nonylcyclohexene as a pale yellow liquid (9.37 g). This crude product was dissolved in absolute ethanol (150 ml) and 10% Pd/C (1 g) was added. The reaction mixture was agitated at room temperature under 2 atmospheres of H_2 for 2 hrs. The catalyst was filtered and the ethanol removed to give a colourless liquid (10.3 g). Column chromatography of the crude product (8.8 g) on 5% deactivated neutral Al_2O_3 (150 g) eluting with 3:1 hexane:CH_2Cl_2 gave 4-nonylcyclohexanone (**20**) (1.90 g, 20%). Elution with hexane gave a mixture of four compounds (5.85 g). GC/MS analysis indicated these compounds were possibly 4-nonylcyclohexane (**26**) (*ca.* 11%), 1,1-diethoxy-4-nonylcyclohexane (**28**) (*ca.* 36%) and *cis* and *trans* diastereomers of 1-ethoxy-4-nonylcyclohexane (**27**) (*ca.* 54%). GC/MS: 4 peaks (Rt (min), GC peak area, MS *m/z*, compound); 28.7, 11%, 210 ($M^{\cdot+}$, 7%), 126 (2%), 97 (7%), 82 (100%), 67 (18%), 55 (68%), possibly 4-nonylcyclohexane (**26**); 34.4, 38%, 254 ($M^{\cdot+}$, 0.9%), 208 (11%), 179 (8%), 96 (21%), 85 (100%), 57 (30%), 1-ethoxy-4-nonylcyclohexane (**27**) isomers; 35.2, 16%, 254 ($M^{\cdot+}$, 0.2%), 208 (14%), 179 (6%), 96 (24%), 85 (100%), 57 (31%), 1-ethoxy-4-nonylcyclohexane (**27**) isomer; 36.2, 36%, 252 ($M^{\cdot+}$, 23%), 223 (53%), 167 (66%), 125 (59%), 98 (100%), 70 (60%), possibly 1,1-diethoxy-4-nonylcyclohexane (**28**). Further isolation and characterisation work helped to identify 1-ethoxy-4-nonylcyclohexane (**27**) (see below).

To the above mixture (5.24 g) dissolved in acetone (45 ml) was added 5% aqueous HCl. After stirring at 20°C for 10 minutes acetone was removed by evaporation under reduced pressure and the product extracted into ether. The ether layer was washed with water, dried over $MgSO_4$, filtered and the solvent removed to give a colourless liquid (4.11 g). Column chromatography on 5% deactivated Al_2O_3 eluting with hexane gave a mixture of three compounds (2.76 g), which were tentatively identified as 4-nonylcyclohexane (**26**) (*ca.* 12%) and the *cis* and *trans* isomers of 1-ethoxy-4-nonylcyclohexane (**27**) (*ca.* 88%). Elution with

3:1 hexane:CH_2Cl_2 gave 4-nonylcyclohexanone (**20**) (1.03 g, 11%). Characterisation data for 4-nonylcyclohexanone (**20**) are given above. The yield of 4-nonylcyclohexanone (**20**) on the basis of the *p-n*-nonylanisole (**7**) starting material was calculated at 31%.

Isolation and identification of *trans*-1-ethoxy-4-nonylcyclohexane isomer (27)

The catalytic hydrogenation in the above preparation of 4-nonylcyclohexanone (**20**) gave a crude product (10.3 g) containing the mixture of compounds described above. A sample (1 g) of this mixture was column chromatographed on 5% deactivated Al_2O_3 (70 g). Elution with hexane gave a colourless liquid (581 mg, fraction 1) which GC/MS analysis suggested was a mixture of three products, possibly 4-nonylcyclohexane (**26**), 1,1-diethoxy-4-nonylcyclohexane (**28**) and one diastereomer of 1-ethoxy-4-nonylcyclohexane (**27**). Elution with 3:1 hexane:CH_2Cl_2 gave a colourless liquid (214 mg, fraction 2) which GC/MS analysis suggested was a mixture of 1,1-diethoxy-4-nonylcyclohexane (**28**) and the other diastereomer of 1-ethoxy-4-nonylcyclohexane (**27**). Elution with 1:1 hexane:CH_2Cl_2 gave 4-nonylcyclohexanone (**20**) (209 mg, fraction 3). Fraction 2 was treated with dilute aqueous HCl in acetone and worked up as described above to give a clear liquid (90 mg). Column chromatography on 5% deactivated Al_2O_3 eluting with hexane gave a single compound as a colourless liquid (20 mg) which the NMR evidence suggested was the *trans* isomer of **1-ethoxy-4-nonylcyclohexane** (**27**). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.85 (t, J = 6.7 Hz, H17), 0.80-0.92 (overlapping with H17, m, H3 and H5), (H17, H3 and H5, 5H), 1.18 (t, J = 7.0 Hz, H8), 1.10-1.30 (overlapping with H8, m, H2 and H6, H4, H9-H16), (H8, H2 and H6, H4, H9-H16, 22H), 1.75 (2H, broad d, J = 13.2 Hz, H3' and H5'), 2.00 (2H, broad d, J = 10.9 Hz, H2' and H6'), 3.14 (1H, tt, J = 11.0, 4.2 Hz, H1), 3.50 (2H, q, J = 7.0 Hz, H7); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 14.1 (C17), 15.7 (C8), 22.7 (C16), 27.2 (C9), 29.3 (C13), 29.6, 29.7 (C11, C12), 30.0 (C10), 31.4 (C3, C5), 31.9 (C15), 32.4 (C2, C6), 36.8 (C9), 37.1 (C4), 63.1 (C7), 78.3 (C1); MS (EI, GC/MS) *m/z*: 254 ($M^{\cdot+}$, 0.3%), 208 (19%), 179 (9%), 96 (27%), 85 (100%), 57 (28%).

7.2.4 Synthesis of 2-Isopropyl-4-nonylphenol (4)

$AlCl_3$ catalysed Friedel-Crafts isopropylation of *p-n*-nonylanisole (7)

A mixture of *p-n*-nonylanisole (**7**) (10 g, 0.0427 mol) and 2-chloropropane (3.36 g, 0.0427 mol) in dry CH_3NO_2 was added dropwise to $AlCl_3$ (5.69 g, 0.0427 mol) in dry CH_3NO_2 in a reaction flask flushed with N_2. The reaction mixture was heated under reflux for 2.5 hrs. Nitromethane was distilled under reduced pressure (water aspirator) and 5% aqueous HCl (100 ml) and ether (150 ml) were added. The ether phase was separated, washed with 5% HCl, water, dried over $MgSO_4$, filtered and concentrated to a black oil (9.36 g, 79%). The crude product was a mixture of mainly mono- and di-isopropyl nonyl products, di-nonyl products and starting material. 2-Isopropyl-4-nonylanisole (**29**) made up *ca.* 43% of the crude product as indicated by GC/MS analysis. Column chromatography on 5% deactivated neutral Al_2O_3 eluting with a hexane:CH_2Cl_2 gradient was unsuccessful in separating 2-isopropyl-4-nonylanisole (**29**) from the by-products and starting material. The mass spectrum of 2-isopropyl-4-nonylanisole (**29**) was as follows. MS (EI, GC/MS) *m/z*: 276 ($M^{\cdot+}$, 21%), 261 (9%), 163 (100%), 149 (9%), 133 (7%), 121 (6%).

Attempts were made to improve the yield of 2-isopropyl-4-nonylanisole (**29**) by varying the reaction stoichiometry (0.5 mol equivalent of $AlCl_3$), reaction time (1 hr, 3 hrs), temperature (60°C) and solvent (CS_2). The conditions described in the above synthesis were the most successful. In a series of reactions in CH_3NO_2, isopropanol was used in place of 2-chloropropane. The Friedel-Crafts alkylation was carried out under a variety of conditions, including reagent stoichiometry ratios (nonylanisole:isopropanol:$AlCl_3$) of 1:1:1, 1:1:2, 2:1:1 and 2:1:2 and reaction temperatures of 20-60°C. The progress of the reactions was monitored by GC/MS analysis.

$FeCl_3$ catalysed Friedel-Crafts isopropylation of *p-n*-nonylanisole (7)

A mixture of *p-n*-nonylanisole (**7**) (1 g, 4.27 mmol) and 2-chloropropane (3.69 g, 4.69 mmol) in dry CH_3NO_2 was added dropwise to $FeCl_3$ (0.762 g, 4.69 mmol) in dry CH_3NO_2 in a reaction flask flushed with N_2. The reaction mixture was heated under reflux for 2.5 hrs and worked up as described above to give a black oil, (0.797 g). Analysis by GC/MS revealed the product was a mixture of starting material (MW = 234 Da), mono- and di-isopropyl

nonylanisoles (MW = 276 and 318 Da) and di-nonylated anisoles (MW = 360 and 402 Da). 2-Isopropyl-4-nonylanisole (**29**) constituted *ca.* 22% of the crude product as indicated by GC/MS analysis.

Demethylation of the isopropyl nonylanisole mixture

To a solution of the $AlCl_3$ catalysed Friedel-Crafts isopropylation crude product (9.36 g) in dry CH_2Cl_2, cooled to <0°C (with ice/salt bath), was added dropwise BBr_3 (0.79 g, 0.00315 mol) in dry CH_2Cl_2. After stirring for 1 hr the reaction mixture was allowed to return to room temperature and stirred for an additional 16 hrs under a N_2 atmosphere. CH_2Cl_2 and CH_3Br were distilled and water was added. The mixture was heated under reflux for 1 hr, cooled and ether was added. The two phases were separated and the ether extract was washed with water and aqueous NaCl solution, dried over $MgSO_4$ and filtered. Ether was removed by evaporation under reduced pressure to yield a black oil (8.77 g, 99%). The product (8.77 g) was loaded onto a column packed with 470 g of 5% deactivated neutral Al_2O_3. Elution with 3:1 hexane:ether gave **2-isopropyl-4-nonylphenol** (**4**) (1.36 g) as a black oil in 91% purity (based on GC/MS peak areas). The calculated yield of 2-isopropyl-4-nonylphenol (**4**) from *p-n*-nonylanisole (**7**) was 12%. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.86 (3H, t, J = 7.0 Hz, H18), 1.16-1.36 (18 H, m and s overlapping, H12-H17, H8 and H9), 1.56 (2H, p, $J \approx$ 7.3 Hz, H11), 2.51 (2H, t, J = 7.5 Hz, H10), 3.16 (1H, heptet, J = 6.9 Hz, H7), 4.61 (1H, s, OH), 6.64 (1H, d, J = 8.0 Hz, H6), 6.85 (1H, dd, J = 8.0, 2.1 Hz, H5), 6.97 (1H, d, J = 2.1 Hz, H3); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 14.1 (C18), 22.7, 22.7 (C8, C9, C17), 27.0 (C7), 29.4, 29.4, 29.6, 29.6 (C12-C15), 31.9, 31.9 (C11, C16), 35.4 (C10), 115.1 (C6), 126.3, 126.3 (C3, C5), 134.1 (C2), 135.3 (C4), 150.6 (C1); MS (EI, GC/MS) *m/z*: 262 ($M^{\cdot+}$, 23%), 247 (7%), 149 (100%), 135 (12%), 107 (8%), 91 (4%); High resolution MS (EI, DIP): found 262.2284 Da; $C_{18}H_{30}O$ requires 262.2297 Da. The presence of minor impurities meant that satisfactory micro-analytical data were not obtained, found C, 81.3, H, 11.2; $C_{18}H_{30}O$ requires C, 82.4, H, 11.5. GC/MS analysis suggested the impurities (*ca.* 9%) were two isomers of di-isopropylphenol. MS (EI, GC/MS) *m/z*: 178 ($M^{\cdot+}$, 21%), 164 (12%), 163 (100%), 121 (14%), 91 (8%), 74 (8%).

Attempts to separate the di-isopropylnonylphenols (MW = 304 Da) and di-nonylphenols (MW = 346 and 388 Da) by column chromatography on 5% deactivated neutral Al_2O_3 yielded a di-nonylphenol in 95% purity but only mixtures of the di-isopropylnonyl and isopropyldi-nonyl products. The following spectroscopic evidence suggested the isolated di-nonylphenol was

possibly **2,4-di-nonylphenol (30)**. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.86 (6H, t, *J* = 7.0 Hz, H15 and H15'), 1.16-1.40 (24H, m, H9-H14 and H9'-H14'), 1.56 (4H, tt, *J* = 7.8, 6.7 Hz, H8 and H8'), 2.48 (2H, t, *J* = 7.8 Hz, H7'), 2.54 (2H, t, *J* = 7.8 Hz, H7), 4.57 (1 H, s, OH), 6.65 (1H, d, *J* = 8.0 Hz, H6), 6.85 (1H, dd, *J* = 8.0, 2.1 Hz, H5), 6.9 (1H, d, *J* = 2.1 Hz, H3); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 14.1 (C15, C15'), 22.7 (C14, C14'), 29.4, 29.5, 29.6 (C10-C12, C9'-C12'), 29.9, 30.0 (C9, C7), 31.8, 31.9 (C8, C8', C13, C13'), 35.1 (C7'), 114.9 (C6), 126.6 (C5), 128.2 (C2), 130.1 (C3), 135.1 (C4), 151.2 (C1); MS (EI, GC/MS) *m/z*: 346 ($M^{\bullet+}$, 45%), 233 (14%), 147 (5%), 121 (100%), 107 (9%), 91 (7%).

Preparation of *p-n*-nonylphenol (9)

To a solution of *p-n*-nonylanisole (**7**) (0.6 g, 0.0026 mol) in dry CH_2Cl_2, immersed in a dry ice/acetone bath, was added BBr_3 (0.64 g, 0.0026 mol) in dry CH_2Cl_2. The reaction mixture was returned to room temperature and stirred for 16 hrs under a N_2 atmosphere. CH_2Cl_2 and CH_3Br were distilled and unreacted BBr_3 was hydrolysed by cautious addition of water. Additional water (10 ml) was added and the mixture heated under reflux for 10 minutes to hydrolyse the boron esters. The mixture was cooled, ether and water were added and the two phases separated. The ether extract was washed with water, dried over $MgSO_4$, and filtered. Ether was removed by evaporation under reduced pressure to give ***p-n*-nonylphenol (9)** as a light brown solid (0.52 g, 91.5%). Recrystallisation of a sample from aqueous ethanol gave colourless crystals, m.p. = 41-42°C (literature m.p. = 41-43°C (237). IR (NaCl, neat) cm^{-1}: 3200-3500 (ν O-H); ^{1}H NMR (200 MHz, $CDCl_3$) δ: 0.87 (3H, t, *J* = 6.8 Hz, H15), 1.15-1.32 (12H, m, H9-H14), 1.54 (2H, p, *J* = 7.4 Hz, H8), 2.54 (2H, t, *J* = 7.7 Hz, H7), 4.73 (1H, broad s, OH), 6.73 (H2, d, *J* = 8.3 Hz, H2 and H6), 7.02 (2H, d, *J* = 8.3 Hz, H3 and H5); ^{13}C NMR (50 MHz, $CDCl_3$) δ: 14.1 (C15), 22.7 (C14), 29.2, 29.3, 29.5, 29.6 (C9, C10, C11, C12), 31.7 (C8), 31.9 (C13), 35.0 (C7), 115.0 (C2, C6), 129.4 (C3, C5), 135.2 (C4), 153.2 (C1); MS (EI, GC/MS) *m/z*: 220 ($M^{\bullet+}$, 15%), 120 (2%), 107 (100%), 94 (1%), 77 (5%). The product was verified as *p-n*-nonylphenol (**9**) by comparison of measured and published melting point data (237).

Friedel-Crafts isopropylation of *p-n*-nonylphenol (9)

A mixture of *p-n*-nonylphenol (**9**) (0.1 g, 0.46 mmol) and 2-chloropropane (0.036 g, 0.46 mmol) in dry CH_3NO_2 (2 ml) was added dropwise to $AlCl_3$ (0.120 g, 0.90 mmol) in dry

CH_3NO_2 (1 ml) under N_2. The reaction mixture was heated under reflux for 4 hrs and the course of the reaction was monitored by GC/MS analysis of aliquots taken after 1 and 2.5 hrs. Nitromethane was distilled under reduced pressure, 5% aqueous HCl and ether were added. The ether extract was separated, washed with 5% aqueous HCl, water and dried over $MgSO_4$. Filtration and solvent evaporation yielded a black oil (0.077 g). Analysis by GC/MS indicated the oil was a mixture of nonylphenol and mono- and di-isopropylated nonylphenol. 2-Isopropyl-4-nonylphenol (**4**) made up *ca.* 20% of the mixture (as indicated by GC/MS analysis).

7.2.5 Synthesis of 1-Isopropyl-2-naphthol (6)

Preparation of 1-isopropyl-2-naphthol (6)

Na metal (3.2 g, 0.139 mol) was dissolved in dry methanol and 2-naphthol (**31**) (20 g, 0.139 mol) was added. The mixture was stirred until the naphthol completely dissolved. Methanol was removed by evaporation under reduced pressure and dry toluene was added. The toluene was removed to give sodium β-naphthoxide as a white crystalline solid. To this was added toluene (100 ml) and 2-chloropropane (25.4 ml, 0.139 mol). The heterogeneous mixture was warmed to 60°C and stirred for 11 days under a N_2 atmosphere. At no time during the reaction did the mixture become completely homogeneous. The reaction mixture was cooled and 10% aqueous NaOH (200 ml) and ether (200 ml) were added and after shaking the two phases were separated. The NaOH layer was extracted with a second volume of ether (100 ml) and the two ether extracts combined. After washing with water, drying over $MgSO_4$ and filtering the ether was removed to give a black oil (4.24 g). The NaOH layer was acidified with concentrated HCl to pH *ca.* 5 and extracted with ether. The ether layer was worked up as above to give 2-naphthol (**31**) as a brown solid (16.0 g).

The black oil (4.24 g) was loaded onto a column packed with 5% deactivated neutral Al_2O_3 (120 g). Elution with hexane gave possibly 2-(1-methylethoxy)naphthalene (**32**) as a colourless oil (94%) (0.7 g, 2.7%). 2-(1-Methylethoxy)naphthalene (**32**) was tentatively characterised from GC/MS evidence. MS (EI, GC/MS) *m/z*: 186 ($M^{\cdot+}$, 17%), 144 (100%), 127 (3%), 116 (8%), 115 (22%), 89 (3%). Elution with 3:1 hexane:ether gave a pale yellow oil which crystallised on cooling (2.25 g, 8.7%). Recrystalisation from ethyl acetate/hexane gave

1-isopropyl-2-naphthol (**6**) as white needles, m.p. = 72-73°C (literature m.p. = 72-74°C (264)). IR (NaCl, neat) cm^{-1}: 3100-3500 (ν O-H), ^1H NMR (300 MHz, $CDCl_3$) δ: 1.51 (6H, d, *J* = 7.0 Hz, H10 and H11), 3.90 (1H, p, *J* = 7.0 Hz, H9), 4.98 (1H, s, OH), 6.97 (1H, d, *J* = 8.6 Hz, H3), 7.30 (1H, ddd, *J* = 8.0, 7.0, 1.2 Hz, H6), 7.45 (1H, ddd, *J* = 8.5, 7.0, 1.5 Hz, H7), 7.58 (1H, d, *J* = 8.7 Hz, H4), 7.75 (1H, dd, *J* = 8.0 Hz, H5), 8.11 (1H, d, *J* = 8.5 Hz, H8); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 21.1 (C10, C11), 26.3 (C9), 118.8 (C3), 122.8 (C6), 123.2 (C8), 125.5 (C1), 126.1 (C7), 127.7 (C4), 128.8 (C5), 129.7 (C4a), 132.9 (C8a), 151.0 (C2); MS (EI, GC/MS) *m/z*: 186 ($M^{\bullet+}$, 48%), 171 (100%), 152 (19%), 143 (8%), 128 (17%), 115 (14%); MS (EI, GC/MS, TMSi derivative) *m/z*: 258 ($M^{\bullet+}$, 43%), 243 (100%), 152 (7%), 141 (7%), 115 (6%), 73 (65%). This product was verified as 1-isopropyl-2-naphthol (**6**) by comparison of melting point and ^1H NMR data with published data (264).

Autoxidation of 1-isopropyl-2-naphthol (6)

1-Isopropyl-2-naphthol (**6**) standing at room temperature in the presence of air underwent slow autoxidation. After 18 days, column chromatography of this partially oxidised product (0.5 g) on 5% deactivated neutral Al_2O_3 eluting with 1:1 hexane:ether afforded 1-isopropyl-2-naphthol (**6**) (225 mg). Elution with 1:3 hexane:ether gave a yellow solid (94 mg). Recrystallisation from ethyl acetate/hexane gave **1-hydroperoxy-1-isopropyl-2-(1*H*)-naphthalenone** (**33**) as white needles, m.p. = 134-137°C (literature m.p. = 135-137°C (264)). IR (KBr disc) cm^{-1}: 3200-3450 (ν O-H), 1654 (ν C=O); ^1H NMR (400 MHz, acetone-d_6) δ: 0.80, 0.85 (2 d, *J* = 7 Hz, 6H, H10 and H11), 2.10 (overlapping with solvent, m, *J* = 7 Hz, H9), 6.12 (1H, d, *J* = 9.9 Hz, H3), 7.43 (t, *J* = 7.3 Hz, H6), 7.46 (overlapping with H6 and H7, d, H5), 7.49 (dd, H7), 7.55 (d, *J* = 9.9 Hz, H4), (H4-H7, 4H), 7.68 (1H, d, *J* = 7.5 Hz, H8), 10.69 (s, OH); ^{13}C NMR (100 MHz, acetone-d_6) δ: 16.0, 16.6 (C10, C11), 39.6 (C9), 91.3 (C1), 126.6 (C3), 127.8 (C8), 128.4 (C6), 129.4 (C5), 129.7 (C7), 132.7 (C4a), 142.5 (C8a), 145.1 (C4), 199.6 (C2); MS (EI, GC/MS) *m/z*: 202 ($M^{\bullet+}$-O, 1%), 160 (100%), 131 (38%), 115 (5%), 103 (14%), 77 (13%); MS (EI, GC/MS, TMSi derivative) *m/z*: 274 ($M^{\bullet+}$-O, 3%), 259 (9%), 232 (19%), 216 (100%), 203 (33%), 186 (21%). The presence of the hydroperoxy group was confirmed by the rapid reduction of 1-hydroperoxy-1-isopropyl-2-(1*H*)-naphthalenone (**33**) by iodide ion or permanganate ion. This product was verified as gave 1-hydroperoxy-1-isopropyl-2-(1*H*)-naphthalenone (**33**) by comparison of melting point and ^1H NMR data with published data (264).

7.2.6 Synthesis of 4-Nonylcyclohexan-1,2-epoxide (39)

Halo-de-alkoxylation of 1-ethoxy-4-nonylcyclohexane (27)

A mixture of the *cis* and *trans* isomers of 1-ethoxy-4-nonylcyclohexane (**27**) (*ca.* 88%) and possibly 4-nonylcyclohexane (**26**) (*ca.* 12%) was obtained during the preparation of 4-nonylcyclohexanone (**20**) from *p-n*-nonylanisole (**7**) (see Section 7.2.3). This mixture (0.78 g, *i.e.* 0.69 g, 2.70 mmol of (**27**)) was heated under reflux with 54% HI for 3.5 hrs, cooled and 5% aqueous $Na_2S_2O_3$ and ether were added. The ether layer was washed with 5% aqueous $Na_2S_2O_3$, water, dried over $MgSO_4$ and filtered. Evaporation of the solvent gave a pale yellow liquid (1 g) which contained three iodo-4-nonylcyclohexane isomers in 78% purity (0.78 g, calculated yield was 86%). GC/MS: 5 peaks (Rt (min), GC peak area, MS *m/z*, compound); 28.8, 12%, 210 ($M^{\cdot+}$, 7%), 126 (2%), 97 (7%), 82 (100%), 67 (18%), 55 (68%), possibly 4-nonylcyclohexane (**26**); 29.2, 7%, 208 ($M^{\cdot+}$, 12%), 109 (8%), 96 (76%), 81 (100%), 67 (59%), 54 (29%), possibly 4-nonylcyclohex-1-ene (**42**) (possible artifact of GC/MS analysis); 38.9, 16%, 209 ($M^{\cdot+}$- 127(-I), 22%), 111 (31%), 97 (80%), 83 (100%), 69 (64%), 55 (96%), iodononylcyclohexane isomer; 39.1, 10%, 209 ($M^{\cdot+}$- 127(-I), 25%), 111 (32%), 97 (80%), 83 (100%), 67 (60%), 55 (97%), iodononylcyclohexane isomer; 39.4, 52%, 209 ($M^{\cdot+}$- 127(-I), 26%), 111 (31%), 97 (77%), 83 (100%), 67 (65%), 55 (96%), 1-iodo-4-nonylcyclohexane isomer (**41**).

Signals in the NMR spectra of the mixture assignable to the major **1-iodo-4-nonylcyclohexane** isomer (**41**) (*ca.* 52%) were as follows: ^{1}H NMR (400 MHz, $CDCl_3$)[5] δ: 0.86 (t, J = 6.8 Hz, H15), 0.96 (m, H3 and H5), 1.12 (m, H7), 1.2-1.4 (m, H4, H8-H14), 1.61 (m, H3' and H5'), 1.95 (m, H2 and H6), 2.37 (m, H2' and H6'), 4.09 (m, H1); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 14.1 (C15), 22.7 (C14), 26.8 (C8), 29.3, 29.6, 29.6, 29.9 (C9-C12), 30.9 (C1), 31.9 (C13), 35.5 (C3, C5), 36.2 (C4), 37.0 (C7), 40.5 (C2, C6).

Dehydrohalogenation of the mixture of iodononylcyclohexanes

To KOH (1.33 g, 2.37 mmol) dissolved in 96% ethanol (5 ml) under a nitrogen atmosphere was added dropwise the mixture of iodononylcyclohexanes (1 g, (*ca.* 78% iodononylcyclo-

[5] The mixture of iodononylcyclohexane isomers made complete ^{1}H and ^{13}C assignment impractical. Chemical shifts for the major 1-iodo-4-nonylcyclohexane isomer were determined from the HMQC experiment.

hexanes, 2.32 mmol)) suspended in ethanol (3 ml). The mixture was heated under reflux for 9.5 hrs, during which time KI precipitated. The mixture was cooled, water and ether were added. The ether layer was washed with 5% aqueous HCl, water, dried over $MgSO_4$ and filtered. Ether was removed under reduced pressure to give a yellow liquid, which appeared to be a mixture of two compounds. GC/MS: 2 peaks (Rt (min), GC peak area, MS *m/z*, compound); 28.8, 15%, 210 ($M^{\cdot+}$, 8%), 126 (2%), 83 (100%), 82 (90%), 67 (17%), 55 (58%), possibly 4-nonylcyclohexane (**26**); 29.2, 85%, 208 ($M^{\cdot+}$, 9%), 109 (10%), 96 (75%), 81 (100%), 67 (49%), 54 (35%), 4-nonylcyclohex-1-ene (**42**). Major signals in ^1H and ^{13}C NMR spectra of the mixture were assignable to **4-nonylcyclohex-1-ene** (**42**). ^1H NMR (400 MHz, $CDCl_3$)[6] δ: 0.86 (t, *J* = 6.8 Hz, H15), 1.20-1.40 (m, H7-H14), 1.50 (m, H5), 1.61 (m, H6), 1.67 (m, H5'), 1.71 (m, H4), 2.01 (m, H3), 2.07 (dd, H6'), 5.63, 5.64 (overlapping m, H1 and H2); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 14.1 (C15), 22.7 (C14), 25.3 (C3), 27.5 (C8), 29.0 (C4), 29.4, 29.7, 29.7, 30.0 (C9-C12), 32.0 (C6), 33.5 (C5), 36.8 (C7), 126.7 and 127.0 (C1, C2).

Preparation of 4-nonylcyclohexan-1,2-epoxide (39)

To a mixture of 4-nonylcyclohex-1-ene (**42**) (*ca.* 85%) and possibly 4-nonylcyclohexane (*ca.* 15%) (0.467 g, 1.84 mmol of (**42**)) dissolved in dry CH_2Cl_2 was added dropwise 80-85% *m*-chloroperbenzoic acid (426 mg, 2.48 mmol) in CH_2Cl_2. The mixture was stirred at 30°C for 1 hr, during which time *m*-chlorobenzoic acid precipitated from the solvent. A solution of 5% $NaHSO_3$ was added until the excess oxidant was consumed as indicated by starch-iodine paper. The CH_2Cl_2 layer was washed with 5% aqueous $NaHCO_3$ and aqueous NaCl solution, dried over $MgSO_4$ and filtered. Removal of the solvent by evaporation under reduced pressure gave a pale yellow liquid (0.405 g). This product was combined with that of an earlier reaction to give 0.478 g of crude product and loaded onto a column of packed silica gel. Elution with hexane gave possibly 4-nonylcyclohexane (**26**) as colourless liquid (32 mg). Elution with 1:1 hexane:CH_2Cl_2 gave 4-nonylcyclohexan-1,2-epoxide (**39**) as a pale yellow liquid (176 mg). Based on the amount 4-nonylcyclohex-1-ene (**42**) in the starting material the yield was calculated at 35%. In a subsequent reaction a yield of 48% was obtained.

[6] Relative integrals for 4-nonylcyclohex-1-ene (**42**) are not provided as the product was a mixture of compounds.

The spectroscopic evidence for **4-nonylcyclohexan-1,2-epoxide (39)** as the product was as follows. ^{1}H NMR (400 MHz, $CDCl_3$)[7] Diastereomer 1 δ: 0.86 (t, *J* = 6.8 Hz, H15), 1.06 (m, H5), 1.13 (m, H4, H7), 1.18-1.35 (m, H8-H14), 1.30 (m, H5'), 1.39 (dd, H3), 1.67 (ddd, H6), 2.01 (m, H3'), 2.11 (m, H6'), 3.10 (m, H2), 3.11 (m, H1); Diastereomer 2 δ: 0.85 (m, H5), 0.86 (t, *J* = 6.8 Hz, H15), 1.12 (m, H7), 1.18-1.35 (m, H8-H14), 1.30 (m, H3), 1.42 (m, H4), 1.46 (m, H5'), 1.80 (ddd, H6), 1.95 (m, H6'), 2.14 (m, H3'), 3.10 (m, H1), 3.14 (m, H2); ^{13}C NMR (100 MHz, $CDCl_3$) Diastereomer 1 δ: 14.0 (C15), 22.6 (C14), 24.5 (C5), 26.7 (C8), 25.4 (C6), 29.3, 29.6, 29.9, 29.8 (C9-C12), 30.8 (C3), 31.9 (C13), 32.7 (C4), 36.9 (C7), 51.9 (C2), 52.6 (C1); Diastereomer 2 δ: 14.0 (C15), 22.6 (C14), 23.6 (C6), 26.7 (C8), 27.2 (C5), 29.3, 29.6, 29.9, 29.8 (C9-C12), 29.4 (C4), 31.9 (C13), 31.9 (C3), 36.3 (C7), 51.9 (C1), 53.1 (C2); MS (EI, GC/MS) *m/z*: 224 ($M^{\cdot+}$, 0.3%), 125 (12%), 111 (12%), 97 (100%), 84 (76%), 67 (35%), 55 (50%); High resolution MS (EI, DIP): found 224.2145 Da; $C_{15}H_{28}O$ requires 224.2140 Da. The product did not give unsatisfactory microanalytical data, found C, 79.7, H, 12.3; $C_{15}H_{28}O$ requires C, 80.3, H, 12.6.

7.2.7 Synthesis of 4-Ethyl-1-methyl-1,2,3,4-tetrahydronaphth-1-ol (40) and 5,9-Dimethyl-6,7,8,9-tetrahydrobenzocyclohepten-5-ol (44)

Preparation of 4-ethyl-α-tetralone (45) and 9-methyl-6,7,8,9-tetrahydrobenzocyclohepten-5-one (46)

To γ-caprolactone (5 g, 0.044 mol) in dry benzene (50 ml) was added $AlCl_3$ (23.5 g, 0.176 mol) in small amounts over a period of 30 minutes. The reaction mixture was heated under reflux for 18 hrs under a N_2 atmosphere. Benzene was distilled and the mixture cooled to room temperature. Aqueous HCl (6%) was cautiously added and then ether. The ether layer was separated, washed with water, 20% KOH, water and then dried over $MgSO_4$. After filtration, the ether was evaporated under reduced pressure to give a black oil (7.94 g). This product was combined with product from an earlier small scale preparation (1.68 g) in a Claisen flask with Vigreux column. The product was distilled under reduced pressure using a Dynvac dual vacuum pump system. Two products, 4-ethyl-α-tetralone (**45**) and 9-methyl-6,7,8,9-tetrahydrobenzocycloheptan-5-one (**46**) co-distilled as a colourless liquid (6.26 g,

[7] Relative integrals are not shown as the product was a mixture of diastereomers. Multiplicities were impossible to determine in many cases due to overlapping signals. Chemical shifts were obtained from the HMQC spectrum.

82%) at 94-96°C and 0.090-0.098 torr. Three fractions (F1, F2 and F3) were collected. GC/MS analysis indicating that mixtures of 4-ethyl-α-tetralone (**45**) and 9-methyl-6,7,8,9-tetrahydrobenzocycloheptan-5-one (**46**) were obtained in the following ratios: F1, 1.78 g, 43:57; F2, 3.42 g, 45:55; F3, 1.06 g, 50:50. The yields of 4-ethyl-α-tetralone (**45**) and 9-methyl-6,7,8,9-tetrahydrobenzocycloheptan-5-one (**46**) were calculated at 37% and 45% respectively.

The spectroscopic evidence for **4-ethyl-α-tetralone (45)** was as follows. IR (NaCl, neat) cm^{-1}: 2935 (ν C-H), 1685 (ν C=O); ^{1}H (400 MHz, $CDCl_3$)[8] δ: 1.00 (t, *J* = 7.4 Hz, H10), 1.72 (m, H9), 2.05 (m, H3), 2.22 (m, H3'), 2.56 (overlapping with H6 (**46**), m, H2), 2.74 (overlapping with H6' (**46**), m, H2'), 2.81 (overlapping with H2', m, H4), 7.27 (d, H5), 7.28 (overlapping with H5, H1 (**46**) and H3 (**46**), dd, H7), 7.48 (overlapping with H2 (**46**), dd, *J* = 7.8 Hz, H6), 8.00 (d, *J* = 7.7 Hz, H8); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 12.0 (C10), 26.2 (C3), 27.3 (C9), 34.8 (C2), 39.4 (C4), 126.4 (C7), 127.2 (C8), 128.2 (C5), 131.8 (C8a), 133.2 (C6), 148.1 (C4a), 198.3 (C1); MS (EI, GC/MS): Rt = 28.7 min, *m/z*: 174 (41%), 145 (100%), 131 (18%), 117 (47%), 115 (42%), 91 (20%); High resolution MS (EI, GC/MS): Rt = 17.2 min, found 174.1034 Da; $C_{12}H_{14}O$ requires 174.1045 Da.

The spectroscopic evidence for **9-methyl-6,7,8,9-tetrahydrobenzocyclohepten-5-one (46)** was as follows. IR (NaCl, neat) cm^{-1}: 2936 (ν C-H), 1681 (ν C=O); ^{1}H (400 MHz, $CDCl_3$)[9] δ: 1.36 (3H, d, *J*= 6.6 Hz, H10), 1.51 (overlapping with H7, m, H8), 1.58 (m, H7), (H8, H7, 2H), 1.86 (m, H7'), 1.96 (overlapping with H7', hextet (ddd), $J \approx 6$ Hz, H8'), (H7', H8', 2H), 2.58 (1H, ddd, *J* = 18.0, 12.4, 3.0 Hz, H6), 2.71 (1H, ddd, *J* = 18.0, 5.9, 2.6 Hz, H6'), 3.08 (1H, heptet (qdd), *J* = 11, 5.9, 5.9 Hz, H9), 7.25 (overlapping with H3, d, *J* = 7.6 Hz, H1), 7.26 (dd, *J* = 7.6, 7.6 Hz, H3), (H1, H3, 2H), 7.44 (1H, dd, *J* = 7.6, 7.6 Hz, H2), 7.52 (1H, d, *J* = 7.5 Hz, H4); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 19.2 (C10), 20.3 (C7), 34.1 (C8), 34.2 (C9), 41.1 (C6), 125.2 (C1), 126.4 (C3), 127.7 (C4), 131.9 (C2), 139.4 (C4a), 143.1 (C9a), 208.3 (C5); MS (EI, GC/MS): Rt = 28.3 min, *m/z*: 174 (60%), 145 (30%), 131 (100%), 103 (34%), 91 (30%), 77 (42%); High resolution MS (EI, GC/MS): Rt = 17.0 min, found 174.1040 Da; $C_{12}H_{14}O$

[8] For certain ^{1}H signals, multiplicities, *J* values and relative integrals were impossible to determine due to overlapping of signals. In these cases chemical shifts were determined from the HMQC spectrum.

[9] Spectra of pure (**46**) isolated unreacted from Grignard reaction product.

requires 174.1045 Da. The ^{1}H and ^{13}C NMR data was identical to that previously reported for this compound (301).

Preparation of 4-ethyl-1-methyl-1,2,3,4-tetrahydronaphth-1-ol (40) and 5,9-dimethyl-6,7,8,9-tetrahydrobenzocyclohepten-5-ol (44)

To magnesium turnings (0.21 g, 8.6 g atoms) was added slowly MeI (1.22 g, 8.6 mmol) in dry ether under N_2. The mixture was stirred for 15 minutes at room temperature then heated under reflux for 1 hr. The mixture of 4-ethyl-α-tetralone (**45**) and 9-methyl-6,7,8,9-tetrahydro-benzocycloheptan-5-one (**46**) (0.50 g, 2.87 mmol) in dry ether was added dropwise and the reaction mixture was heated under reflux for 16 hrs. The mixture was cooled and NH_4Cl (0.74 g, 13.8 mmol) in water (10 ml) was added cautiously. The mixture was acidified to pH 6.7 with 10% aqueous HCl. Ether was added and the ether layer washed with H_2O (×3), dried over $MgSO_4$ and the solvent removed to give a pale yellow oil (0.485 g). The crude product was loaded onto a column of 35 g silica gel and eluted with a hexane:CH_2Cl_2 gradient. Eluting with 1:1 hexane:CH_2Cl_2 gave 9-methyl-6,7,8,9-tetrahydrobenzocyclohepten-5-one (**46**) as a colourless liquid (0.096 g, 35% of the original amount of (**46**) in starting mixture). Structural characterisation data for (**46**) are given above.

Eluting with 1:3 hexane:CH_2Cl_2 gave a 1:3.7 mixture of 4-ethyl-1-methyl-1,2,3,4-tetrahydronaphth-1-ol (**40**) and 5,9-dimethyl-6,7,8,9-tetrahydrobenzocyclohepten-5-ol (**44**) as a colourless oil (0.180 g, 33%). GC/MS: 3 peaks (Rt (min), GC peak areas, MS *m/z*, compound); 28.2, 21%, 190 ($M^{\cdot+}$, 4%), 175 (100%), 143 (56%), 128 (21%), 115 (12%), 91 (17%), 4-ethyl-1-methyl-1,2,3,4-tetrahydronaphth-1-ol (**40**); 28.8, 68%, 190 ($M^{\cdot+}$, 49%), 175 (100%), 157 (39%), 147 (45%), 129 (64%), 91 (55%), 5,9-dimethyl-6,7,8,9-tetrahydrobenzo-cyclohepten-5-ol isomer (**44**); 29.2, 11%, 190 ($M^{\cdot+}$, 2%), 172 (92%), 157 (100%), 143 (56%), 129 (60%), 91 (38%), 5,9-dimethyl-6,7,8,9-tetrahydrobenzocyclohepten-5-ol isomer (**44**). Signals in the ^{1}H and ^{13}C NMR spectra of the mixture which were assignable to the major **5,9-dimethyl-6,7,8,9-tetrahydrobenzocyclohepten-5-ol** (**44**) diastereomer were as follows. ^{1}H (400 MHz, $CDCl_3$)[10] δ: 1.36 (m, H8), 1.37 (overlapping with H8, d, J = 6.9 Hz, H11), 1.58 (s, H10), 1.64 (m, H7), 1.80 (overlapping with H8, m, H7'), 1.81 (m, H8'), 1.90 (m, H6), 2.04

[10] For certain ^{1}H signals, multiplicities and J values were impossible to determine due to overlapping of signals, in these cases chemical shifts were determined from the HMQC spectrum. Relative integrals are not shown as the product was a mixture of compounds.

(ddd, J = 14.0, 8.5, 2 Hz, H6'), 3.11 (m, H9), 7.23 (d, H1), 7.24 (overlapping with H1, m, H2 and H3), 7.79 (dd, J = 7.5, 1.9 Hz, H4); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 20.6 (C11), 23.1 (C7), 29.6 (C10), 34.9 (C9), 35.9 (C8), 42.3 (C6), 76.4 (C5), 125.2 (C1), 125.8 (C4), 126.1 (C3), 127.0 (C2), 141.4 (C9a), 146.9 (C4a).

Elution with CH_2Cl_2 gave a 5.7:1 mixture of 4-ethyl-1-methyl-1,2,3,4,-tetrahydronaphth-1-ol (**40**) and 5,9-dimethyl-6,7,8,9-tetrahydrobenzocyclohepten-5-ol (**44**) as a colourless oil (0.190 g, 36%). GC/MS: 2 peaks (Rt (min), GC peak areas, *m/z*, compound); 28.2, 85%, 190 ($M^{\bullet+}$, 4%), 175 (100%), 143 (56%), 128 (21%), 115 (12%), 91 (17%), 4-ethyl-1-methyl-1,2,3,4-tetrahydronaphth-1-ol (**40**, both diastereomers); 28.8, 15%, 190 ($M^{\bullet+}$, 49%), 175 (100%), 157 (39%), 147 (45%), 129 (64%), 91 (55%), 5,9-dimethyl-6,7,8,9-tetrahydrobenzocyclohepten-5-ol isomer (**44**). Signals in ^{1}H and ^{13}C NMR spectra which were assignable to the major **4-ethyl-1-methyl-1,2,3,4-tetrahydronaphth-1-ol** (**40**) diastereomer (*ca.* 60% of mixture) were as follows. ^{1}H (400 MHz, $CDCl_3$)[10] δ: 0.98 (t, J = 7.5 Hz, H11), 1.51 (s, H9), 1.70 (m, H10), 1.86 (overlapping with H2, m, H3), 1.86 (m, H2), 1.91 (overlapping with H2', m, H3), 1.99 (m, H2'), 2.64 (hextet (dtd), J = 9.3, 4.6, 4.6 Hz, H4), 7.16 (d, H5), 7.19 (m, H6), 7.22 (m, H7), 7.57 (d, H8); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 12.2 (C11), 23.9 (C3), 29.3 (C10), 31.1 (C9), 36.0 (C2), 39.4 (C4), 71.0 (C1), 126.1, 126.2 (C7, C8), 127.0 (C6), 128.4 (C5), 140.4 (C4a), 142.8 (C8a).

Signals in ^{1}H and ^{13}C NMR spectra which were assignable to the minor **4-ethyl-1-methyl-1,2,3,4-tetrahydronaphth-1-ol** (**40**) diastereomer (*ca.* 25% of mixture) were as follows. ^{1}H (400 MHz, $CDCl_3$) δ: 0.95 (t, J = 7.5 Hz, H11), 1.55 (s, H9), 1.66 (m, H3), 1.70 (m, H10), 2.03 (overlapping with H2', m, H3), 1.82 (m, H2), 2.03 (m, H2'), 2.73 (hextet (dtd), J = 9.4, 4.7, 4.7 Hz, H4), 7.14-7.25 and 7.55-7.60 (overlapping with H5-H9 major isomer, m, H5-H8); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 11.5 (C11), 23.9 (C3), 28.6 (C10), 30.5 (C9), 36.7 (C2), 39.3 (C4), 70.3 (C1), 126.0, 126.2 (C7, C8), 127.1 (C6), 128.2 (C6), 140.1 (C4a), 142.8 (C8a).

A subsequent chromatographic separation on silica gel eluting with a hexane:CH_2Cl_2 gradient gave small amounts of 4-ethyl-1-methyl-1,2,3,4-tetrahydronaphth-1-ol (**40**) and 5,9-dimethyl-6,7,8,9-tetrahydrobenzocyclohepten-5-ol (**44**) in *ca.* 94% and *ca.* 93% purity respectively (as well as mixtures of the two tertiary alcohols); High resolution GC/MS analysis was preformed on these samples. 5,9-Dimethyl-6,7,8,9-tetrahydrobenzocyclohepten-5-ol (**44**) (*ca.* 93%): (EI,

GC/MS), 2 diastereomers (*cis* and *trans*), Rt = 20.6 min, found 190.1354 Da; Rt = 21.2 min, found 190.1354 Da; $C_{13}H_{18}O$ requires 190.1358 Da. 4-Ethyl-1-methyl-1,2,3,4-tetrahydro-naphth-1-ol (**40**) (*ca.* 94%): (EI, GC/MS), 2 diastereomers (*cis* and *trans*) co-elute, Rt = 20.3 min, found 190.1361 Da; $C_{13}H_{18}O$ requires 190.1358 Da.

7.2.8 Synthesis of Alkyltropolones from 3-Carboxy-4-carboxymethyl-tropolone (49)

Preparation of Purpurogallin (50) (2,3,4,6-Tetrahydroxy-(5*H*)-benzocyclohepten-5-one)

Purpurogallin was prepared by the method of Evans and Dehn (306). To pyrogallol (20 g, 0.159 mol) in water (40 ml) was added dropwise an aqueous solution of $NaIO_3$ (16 g, 0.81 mol, 200 ml water) with stirring. Carbon dioxide was evolved and the brown precipitate which formed was collected by Buchner filtration, washed with water and air dried to give crude purpurogallin as a brown solid (12.76 g, 73%). Recrystallisation from anisole gave **purpurogallin** (**50**) as red-brown plates (8.52 g, 49%), m.p. = 265-266°C (literature m.p. = 276°C (306)). IR (KBr disc) cm^{-1}: 3000-3600 (ν O-H), 1624 (ν C=O); ^1H NMR (400 MHz, DMSO-d_6) δ: 6.73 (1H, dd, J = 11.3, 9.5 Hz, H8), 6.89 (1H, s, H1), 7.06 (1H, dd, J = 9.5, 0.66 Hz, H7), 7.34 (1H, d, J = 11.3 Hz, H9), 9.26 (2H, s, OH), 10.48 (1H, s, OH), 15.20 (1H, s, OHα to C4); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 110.2 (C1), 114.8 (C4a), 116.4 (C7), 123.5 (C8), 133.0 (C9a), 134.2 (C9), 134.6 (C3), 151.5 (C2), 151.7 (C4), 154.6 (C6), 182.2 (C5); MS (EI, DIP) *m/z*: 220 ($M^{\bullet+}$, 100%), 192 (65%), 146 (15%), 118 (17%), 89 (15%).

Preparation of 3-carboxy-4-carboxymethyltropolone (49)

3-Carboxy-4-carboxymethyltropolone (**49**) was prepared by oxidation of purpurogallin (**50**) using the method of Haworth and Hobson (305). In a typical reaction, recrystallised purpurogallin (8.52 g, 0.0387 mol) was dissolved in 23% aqueous KOH (554 g) and warmed to 90-95°C. H_2O_2 (21.5 ml, 100 vol.) was added dropwise and the reaction mixture stirred for 30 minutes at 90-95°C. The reaction solution was cooled and $Na_2S_2O_5$ (4.2 g, 0.022 mol) in water was added. The solution was acidified to pH *ca.* 1.5 with 40% H_2SO_4. Precipitated K_2SO_4 was collected by Buchner filtered and the filter cake washed with three volumes of ether. The filtrate was continuously extracted with ether for *ca.* 48 hrs. 3-Carboxy-4-carboxy-

methyltropolone (**49**) (3.313 g, 38%) crystallised from the ether extract and was separated. The ether extract was dried with $MgSO_4$, filtered and the ether removed by evaporation under reduced pressure to yield a black oil (2.263 g). Brown crystals crystallised from the oil and were washed with ether (1.343 g, 15%). The crude 3-carboxy-4-carboxymethyltropolone (**49**) was recrystallised from acetic acid. The yield of **3-carboxy-4-carboxymethyltropolone (49)** varied from 50-55% with various preparations. Oxidation of crude purpurogallin consistently gave lower yields in the 20-30% range. M.p. = 177-178°C (literature m.p. = 183-184°C decomp. (305)). IR (KBr disc) cm^{-1}: 3000-3600 (ν O-H), 3260, broad, (ν O-H, tropolone OH), 2706, 2596, 2512, weak, (ν O-H, COOH), 1740 (ν C=O, CH_2COOH), 1686 (ν C=O, COOH), 1611 (ν C=O, tropolone); ^1H NMR (400 MHz, acetone-d_6) δ: 3.79 (2H, s, H9), 7.17 (1H, d, *J* = 11.0 Hz, H5), 7.29 (1H, d, *J* = 9.9 Hz, H7), 7.42 (1H, dd, *J* = 11.0, 9.9 Hz, H6); ^{13}C NMR (100 MHz, acetone-d_6) δ: 43.2 (C9), 118.9 (C7), 133.1 (C5), 136.7 (C6), 137.7 (C3), 143.1 (C4), 168.1 (C8), 168.4 (C1), 170.7 (C10), 173.5 (C2), MS (DIP) *m/z*: 224 ($M^{\cdot+}$, 14%), 180 (85%), 162 (95%), 134 (100%), 105 (87%), 78 (65%).

Preparation of 4-methyltropolone (51)

4-Methyltropolone (**51**) was prepared by the method of Haworth and Hobson (305) of heating 3-carboxy-4-carboxymethyltropolone (**49**) (0.10 g, 0.446 mmol) to its melting point. Purification by sublimation gave **4-methyltropolone (51)** as white needles (0.035 g, 58%), m.p. = 75-76°C (literature m.p. = 76-77°C (305)). IR (KBr disc) cm^{-1}: 3203 (ν O-H, tropolone), 1606 (ν C=O); ^1H NMR (400 MHz, acetone-d_6) δ: 2.44 (3H, s, H8), 6.98 (1H, dd, *J* = 10.0, 0.9 Hz, H5), 7.13 (1H, d, *J* = 10.8 Hz, H7), 7.22 (1H, s, H3), 7.32 (1H, dd, *J* = 10.8, 10.0 Hz, H6); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 27.2 (C8), 122.0 (C7), 125.8 (C3), 129.9 (C5), 136.8 (C6), 150.0 (C4), 170.7, 170.8 (C1, C2); MS (GC/MS) *m/z*: 136 ($M^{\cdot+}$, 100%), 108 (38%), 107 (67%), 90 (11%), 84 (10%), 79 (27%); MS (GC/MS, TMSi derivative) *m/z*: 208 ($M^{\cdot+}$, 0.5%), 193 (100%), 177 (2%), 163 (5%), 89 (12%), 75 (6%).

Preparation of the anhydride (52) of 3-carboxy-4-carboxymethyltropolone (49)

To 3-carboxy-4-carboxymethyltropolone (**49**) (0.25 g, 1.12 mmol) dissolved in dry 1,4-dioxane was added dropwise dicyclohexylcarbodiimide (DCC) (0.25 g, 1.23 mmol) in dry 1,4-dioxane under a N_2 atmosphere. The reaction mixture was stirred at room temperature for 1-2 hrs. The mixture was filtered to remove precipitated DCC urea and the dioxane evaporated

under reduced pressure to give a red solid (0.247 g). The product was washed with dry ether and the ether removed by decanting and evaporation to give red crystals, (0.202 g, 88%). A sample was recrystalised from anisole to give the **anhydride (52) of 3-carboxy-4-carboxymethyltropolone** as red crystals, m.p. = decomposes from 204°C (literature m.p. = 205-208°C decomp. (309). IR (KBr disc) cm^{-1}: 3400-3600 (ν O-H), 3232, broad (ν O-H, tropolone), 1700 (ν C=O, anhydride), 1629 (ν C=O, tropolone); ^{1}H NMR (400 MHz, THF-d_8) δ: 5.45 (1H, s, H9), 6.46 (s, H7), 6.48 (overlapping with H7, dd, *J* = 14.8, 9.3 Hz, H6), (H6, H7, 2H), 6.64 (1H, dd, *J* = 9.0, 3.5 Hz, H5), 9.41 (1H, broad s, OH (enol)), 10.8 (1H, broad s, OH (tropolone)); ^{13}C NMR (100 MHz, THF-d_8) δ: 97.4 (C9), 100.6 (C3), 116.0 (C7), 130.2 (C6), 130.9 (C5), 149.9 (C4), 152.9 (C2), 155.0 (C10), 171.0 (C1), C8 not observed; MS (EI, DIP) *m/z*: 206 ($M^{\cdot+}$, 56%), 178 (100%), 150 (62%), 121 (25%), 104 (25%), 76 (30%). The product was verified as the tropolone anhydride (**52**) by comparison of measured and published melting point data and IR spectra (309).

Preparation of esters of 3-carboxy-4-carboxymethyltropolone (49)

In a typical reaction the anhydride (**52**) of 3-carboxy-4-carboxymethyltropolone (**49**) (0.192 g, 0.932 mmol) and octanol (1.2 g, 9.32 mmol) were heated together at 100°C for 17 hrs. Octanol was removed by azeotropic distillation with water. The crude product (272 mg) was dissolved in ether and extracted with 5% aqueous $NaHCO_3$. The ether layer was washed with water, dried over $MgSO_4$, filtered and concentrated to give a 2:1 mixture of 3-octylcarboxy-4-methyltropolone (**55**) and 4-octylcarboxymethyltropolone (**56**) as a yellow oil (0.146 g, 54%). The aqueous $NaHCO_3$ layer was acidified with concentrated HCl to pH *ca.* 3 and extracted with ether. The ether extract was worked up as above to give 3-carboxy-4-octylcarboxy-methyltropolone (**53**) a pale yellow solid (0.033 g, 11%).

Reaction of the anhydride (**52**) of 3-carboxy-4-carboxymethyltropolone (**49**) (200 g, 0.971 mmol) and octanol (1.24 g, 9.71 mmol) at 60°C for 3 hrs gave in the ether partition 3-octylcarboxy-4-methyltropolone (**55**) as yellow crystals (84 mg, 28%) and in the $NaHCO_3$ partition a 2:1 mixture of 3-carboxy-4-octylcarboxymethyltropolone (**53**) and possibly 3-octylcarboxy-4-carboxymethyltropolone (**54**) (176 g, 51%).

Attempts to separate the ester tropolone mixtures, namely the mixture of (**53**) and possibly (**54**) and the mixture of (**55**) and (**56**) by column chromatography were unsuccessful in yielding pure compounds. Hence the possible product 3-octylcarboxy-4-carboxymethyl-tropolone (**54**) was not isolated and could only be tentatively identified from signals in the ^{1}H and ^{13}C NMR of the mixture of (**53**) and possibly (**54**). Therefore by the two reaction described above it was possible to obtain 3-carboxy-4-octylcarboxymethyltropolone (**53**) and 3-octylcarboxy-4-methyltropolone (**55**). The spectroscopic evidence for these products was as follows.

3-carboxy-4-octylcarboxymethyltropolone (**53**): IR (KBr disc) cm^{-1}: 3300-3600 (ν O-H, COOH), 3195 (ν O-H, tropolone), 1729 (ν C=O, ester), 1606 (ν C=O, tropolone); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.86 (3H, t, *J* = 7.0 Hz, H18), 1.20-1.32 (10H, m, H13-H14), 1.61 (2H, p, *J* = 6.9 Hz, H12), 3.88 (2H, s, H9), 4.11 (2H, t, *J* = 6.8 Hz, H11), 7.11 (1H, d, *J* = 10.2 Hz, H5), 7.34 (1H, d, *J* = 9.8 Hz, H7), 7.40 (1H, dd, *J* = 10.2, 9.8 Hz, H6); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 14.0 (C18), 22.6 (C17), 25.7 (C13), 28.3 (C12), 29.1 (C14, C15), 31.7 (C16), 43.9 (C9), 66.0 (C11), 119.3 (C7), 132.6 (C5), 135.0 (C3), 137.3 (C6), 144.0 (C4), 167.9 (C1), 168.9 (C8), 169.8 (C10), 172.8 (C2); MS (EI, DIP) *m/z*: 336 ($M^{\cdot+}$, 6%), 290 (9%), 189 (35%), 178 (100%), 160 (39%), 150 (54%), 136 (50%). Microanalytical data were not obtained.

3-octylcarboxy-4-methyltropolone (**55**): IR (KBr disc) cm^{-1}: 3224 (ν O-H, tropolone), 1732 (ν C=O, ester), 1616 (ν C=O, tropolone); ^{1}H NMR (400 MHz, acetone-d_6) δ: 0.86 (3H, t, *J* = 7.0 Hz, H16), 1.20-1.33 (m, H12-H15), 1.42 (m, H11), (H12-H15, H11, 10H), 1.75 (2H, tt, *J* = 7.1, 6.8 Hz, H10), 2.40 (3H, s, H17), 4.32 (2H, t, *J* = 6.8 Hz, H9), 7.08 (1H, d, *J* = 10.7 Hz, H5), 7.22 (1H, d, *J* = 10.1 Hz, H7), 7.35 (1H, dd, *J* = 10.7, 10.1 Hz, H6); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 14.0 (C16), 22.6 (C15), 24.7 (C17), 25.9 (C11), 28.5 (C10), 29.1 (C12, C13), 31.7 (C14), 66.0 (C9), 118.6 (C7), 131.4 (C5), 134.6 (C3), 136.4 (C6), 146.5 (C4), 167.7 (C8), 168.2 (C1), 171.4 (C2); MS (EI, DIP) *m/z*: 292 ($M^{\cdot+}$, 15%), 179 (80%), 163 (78%), 134 (100%), 106 (24%), 77 (31%); MS (EI, DIP, acetylated) *m/z*: 334 ($M^{\cdot+}$, 3%), 292 (28%), 179 (100%), 163 (72%), 134 (74%), 106 (16%). Microanalytical data were not obtained.

Signals in the ^{1}H and ^{13}C NMR spectra of the mixture of (**53**) and possibly (**54**) that could be assigned to the possible product, **3-octylcarboxy-4-carboxymethyltropolone** (**54**) were as

follows. ^{1}H NMR (400 MHz, $CDCl_3$)[11] δ: 1.75 (p, H10), 3.70 (s, H17), 4.40 (t, J = 6.8 Hz, H9), 7.05 (d, J = 10.7 Hz, H5); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 14.0 (C16), 22.6 (C15), 25.8 (C11), 28.4 (C10), 29.1 (C12, C13), 31.7 (C14), 43.0 (C17), 66.5 (C9), 119.9 (C7), 131.7 (C5), 134.8 (C3), 137.0 (C6), 141.3 (C4), 167.4 (C8), 168.7 (C1), 171.6 (C2), 173.4 (C18).

Preparation of 4-octylcarboxymethyltropolone (56)

3-Carboxy-4-octylcarboxymethyltropolone (**53**) (50 mg, 0.149 mmol) was decarboxylated by heating under reflux in pyridine for 3.5 hrs. Pyridine was removed by evaporation under reduced pressure and ether and 10% $NaHCO_3$ solution were added. Insolubles were removed by filtration and the ether layer was washed with aqueous $NaHCO_3$ and then water. The filtrate was dried over $MgSO_4$ and solvent removed to give **4-octylcarboxymethyltropolone (56)** as a pale yellow solid (26 mg, 60%). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.86 (3H, t, J = 7.0 Hz, H17), 1.20-1.35 (10H, m, H12-H16), 1.59 (2H, p, J = 6.8 Hz, H11), 3.58 (2H, s, H8), 4.08 (2H, t, J = 6.8 Hz, H10), 6.97 (1H, d, J = 9.5 Hz, H5), 7.24 (overlapping with H6, d, J = 10.6 Hz, H7), 7.30 (overlapping with H3, t, H6), 7.30 (s, H3), (H7, H6, H3, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 14.0 (C17), 22.6 (C16), 25.8 (C12), 28.4 (C11), 29.1 (C13, C14), 31.7 (C15), 46.0 (C8), 65.7 (C10), 123.8 (C7), 125.2 (C3), 129.9 (C5), 137.1 (C6), 144.9 (C4), 170.0 (C9), 170.2 (C2), 171.8 (C1); MS (EI, DIP) *m/z*: 292 ($M^{\cdot+}$, 16%), 179 (40%), 163 (44%), 136 (100%), 134 (45%), 107 (35%). Microanalytical data were not obtained.

Preparation of 4-(2-hydroxynonyl)-3-carboxylic acid δ-lactone tropolone (57)

To the anhydride (**52**) of 3-carboxy-4-carboxymethyltropolone (**49**) (0.424 g, 2.06 mmol) dissolved in pyridine (5 ml) was added a 3 mol excess of octanal (0.799 g, 6.24 mmol) in pyridine (5 ml). The reaction mixture was warmed to 60-65°C for 3 hrs. Pyridine was removed by evaporation under reduced pressure and excess octanal was distilled under reduced pressure. The crude product was dissolved in CH_2Cl_2, washed with 3.6% HCl, water, dried over $MgSO_4$, filtered and the CH_2Cl_2 removed by evaporation under reduced pressure to give a brown solid (0.493 g, 83%). The crude product was recrystallised from CH_2Cl_2:hexane to give **4-(2-hydroxynonyl)-3-carboxylic acid δ-lactone tropolone (57)** as pale brown crystals (0.328 g, 55%), m.p. = 115-116°C. IR (KBr disc) cm^{-1}: 3227, broad, (ν O-H,

[11] Only certain ^{1}H NMR signals could be assigned due to overlap with signals associated with 3-carboxy-4-octylcarboxymethyltropolone (**53**).

tropolone), 1723 (ν C=O, δ-lactone), 1617 (ν C=O, tropolone); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.85 (3H, t, *J* = 7.0 Hz, H17), 1.18-1.35 (overlapping with H12, m, H13-H16), 1.40 (m, H12), 1.52 (m, H12'), (H13-H16, H12, H12', 10 H), 1.66 (1H, m, H11), 1.80 (1H, m, H11), 2.84 (1H, dd, *J* = 16.7, 2.3 Hz, H9), 2.99 (1H, dd, *J* = 16.7, 11.1 Hz, H9'), 4.38 (1H, m, H10), 6.79 (1H, d, *J* = 10.1 Hz, H5), 7.12 (1H, d, *J* = 10.5 Hz, H7), 7.29 (1H, dd, *J* = 10.5, 10.1 Hz, H6); ^{13}C NMR (100 MHz, CD_3OD) δ: 14.4 (C17), 23.7 (C16), 25.9 (C12), 30.3, 30.1 (C13, C14), 31.9 (C15), 35.2 (C11), 40.4 (C9), 78.6 (C10), 118.8 (C7), 124.6 (C3), 128.8 (C5), 140.9 (C6), 153.0 (C4), 167.2 (C8), 171.1 (C1), 176.2 (C2); MS (EI, DIP) *m/z*: 290 ($M^{\bullet+}$, 33%), 262 (32%), 191 (35%), 163 (86%), 148 (57%), 134 (100%); Elemental Analysis: found C, 70.1, H, 7.6; $C_{17}H_{22}O_4$ requires C, 70.3, H, 7.6.

Saponification of 4-(2-hydroxynonyl)-3-carboxylic acid δ-lactone tropolone (57)

4-(2-Hydroxynonyl)-3-carboxylic acid δ-lactone tropolone (**57**) (0.05 g, 0.173 mmol) was dissolved in 2% aqueous NaOH (*ca.* 2 ml) and heated to reflux for 18 hrs. The reaction solution was extracted with ether and the two phases separated. The aqueous phase was acidified to pH 1.5 with concentrated HCl and extracted with ether. The ether layer was separated, washed with water, dried over $MgSO_4$, filtered and the solvent removed to give a crude product as a yellow oil (0.01 g, 19%), which was tentatively identified as mainly 3-carboxy-4-(2-hydroxynonyl)tropolone (**58**). The spectroscopic evidence suggested the crude product contained a small amount of the lactone tropolone (**57**) (*ca.* 20% of product). The spectroscopic evidence indicating that the main product was **3-carboxy-4-(2-hydroxynonyl)-tropolone** (**58**) was as follows. IR (NaCl, neat oil) cm^{-1}: 3400-3600 (ν O-H, COOH), 3213, broad, (ν O-H, tropolone), 2601 (ν O-H, COOH), 1718 (ν C=O, COOH), 1617 (ν C=O, tropolone); ^{1}H NMR (400 MHz, acetone-d_6)[12] δ: 0.9 (t, *J* = 6.9 Hz, H17), 1.30-1.40 (m, H12-H16), 1.55 (m, H11), 2.74 (dd, *J* = 13.4, 9.0 Hz, H9), 2.92 (dd, *J* = 13.4, 3.8 Hz, H9'), 3.95 (m, H10), 7.22 (d, *J* = 11.1 Hz, H5), 7.25 (d, *J* = 10.0 Hz, H7), 7.37 (dd, *J* = 11.1, 10.0 Hz, H6); ^{13}C NMR (100 MHz, acetone-d_6) δ: 13.8 (C17), 22.8 (C16), 25.9 (C12), 29-30 (C13, C14, overlapping with acetone-d_6), 32.1 (C15), 38.4 (C11), 46.5 (C9), 71.8 (C10), 117.4 (C7), 132.8 (C5), 135.7 (C6), 137.5 (C3), 148.2 (C4), 167.0 (C1), 168.4 (C8), 174.2 (C2).

[12] Relative integrals are not provided as the product was a mixture of compounds.

Attempts to optimise this reaction and produce pure 3-carboxy-4-(2-hydroxynonyl)tropolone (**58**) were not undertaken for two reasons. Firstly, it was thought that (**58**) would be susceptible to spontaneous lactonisation as suggested by the presence of some lactone tropolone (**57**) in the product. Secondly, subsequent saponification and decarboxylations of (**57**) (see below) revealed that the saponification was a redundant step in developing a pathway to 4-non-1-enyltropolone (**59**).

Saponification and decarboxylation of 4-(2-hydroxynonyl)-3-carboxylic acid δ-lactone tropolone (57)

4-(2-Hydroxynonyl)-3-carboxylic acid δ-lactone tropolone (**57**) (0.1 g, 0.345 mmol) was heated to reflux with 3% aqueous NaOH (1.1 ml) for 2 hrs. Water (10 ml) was added, the mixture stirred for 5 minutes and then extracted with ether (10 ml). The aqueous layer was acidified to pH 1.5 with 10% HCl. The precipitated product was extracted with ether and the two phases separated. Additional product subsequently precipitated and was extracted with a second volume of ether. The two ether extracts were combined and worked up in the usual manner to give a brown oil (0.05 g). The crude product was identified as approximately a 1:1 mixture of 3-carboxy-4-(2-hydroxynonyl)tropolone (**58**) and 4-(2-hydroxynonyl)-3-carboxylic acid δ-lactone tropolone (**57**) from the ^{1}H NMR spectrum. This product was dissolved in quinoline, Cu powder (5 mg) was added and the reaction mixture was heated to 170°C for 2 hrs. Quinoline was distilled under reduced pressure. Ether and 10% aqueous HCl were added and the mixture was shaken for 5 minutes. The ether layer was separated, filtered and washed with 10% aqueous HCl and water. After drying over $MgSO_4$ and filtration, the solvent was removed to give crude 4-non-1-enyltropolone (**59**) as a brown oil (0.018 g, 19%). Characterisation data for 4-non-1-enyltropolone (**59**) are provided below. This combined saponification and decarboxylation reaction was not optimised to produced pure 4-non-1-enyltropolone (**59**) as the saponification step was proved unnecessary by the subsequent decarboxylation reaction (see below).

Decarboxylation of 4-(2-hydroxynonyl)-3-carboxylic acid δ-lactone tropolone (57)

4-(2-Hydroxynonyl)-3-carboxylic acid δ-lactone tropolone (**57**) (0.05 g, 0.172 mmol) dissolved in dry quinoline and Cu powder (5 mg) were heated to 160°C with stirring for 1 hr. The reaction mixture was cooled, ether (15 ml) and 10% aqueous HCl (10 ml) were added and

the mixture stirred for 5 minutes. The ether phase was separated, washed with 10% aqueous HCl and water, dried over $MgSO_4$ and filtered. Evaporation of the solvent gave a black oil (36 mg). Column chromatography on silica gel eluting with CH_2Cl_2 gave 4-non-1-enyltropolone (**59**) as a orange oil (11 mg, 26%). Characterisation data for 4-non-1-enyltropolone (**59**) are provided below.

Preparation of 4-non-1-enyltropolone (59)

The tropolone anhydride (**52**) (0.488 g), prepared from 3-carboxy-4-carboxymethyltropolone (**49**) (0.50 g, 2.23 mmol) with DCC (0.51 g, 2.45 mmol) as previously described, was dissolved in pyridine (5 ml) and octanal (0.309 g, 2.41 mmol) in pyridine (5 ml) was added. The reaction mixture was heated under reflux for 4 hrs. Pyridine was removed by evaporation under reduced pressure and the product was taken up in CH_2Cl_2. The CH_2Cl_2 extract was washed with 10% aqueous HCl and water and dried over $MgSO_4$. Filtration and solvent evaporation gave a black oil (0.428 g, 78%). Column chromatography on silica gel eluting with CH_2Cl_2 and then 19:1 CH_2Cl_2:ether gave **4-non-1-enyltropolone (59)** as an orange oil (269 mg, 49%). IR (NaCl, neat) cm^{-1}: 3205, broad, (ν O-H, tropolone), 2926, 2855 (ν C-H), 1601 (ν C=O, tropolone); ^1H NMR (400 MHz, $CDCl_3$) δ: 0.86 (3H, t, *J* = 7.0 Hz, H16), 1.21-1.36 (8H, m, H12-H15), 1.46 (2H, p, *J* = 7.2 Hz, H11), 2.24 (2H, dt, *J* = 7.2, 6.7 Hz, H10), 6.34 (1H, d, *J* = 15.7 Hz, H8), 6.43 (1H, dt, *J* = 15.7, 6.7 Hz, H9), 7.00 (1H, d, *J* = 10.3 Hz, H5), 7.17 (1H, d, *J* = 10.9 Hz, H7), 7.31 (1H, dd, *J* = 10.9, 10.3 Hz, H6), 7.44 (1H, d, *J* = 1.4 Hz, H3); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 14.1 (C16), 22.6 (C15), 29.0 (C11), 29.1, 29.2 (C12, C13), 31.8 (C14), 33.3 (C10), 119.7 (C3), 123.8 (C7), 126.8 (C5), 132.0 (C8), 137.3 (C6), 138.2 (C9), 147.2 (C4), 168.9 (C2), 172.8 (C1); MS (EI, GC/MS) *m/z*: 246 ($M^{\cdot+}$, 63%), 148 (50%), 133 (73%), 120 (100%), 115 (27%), 77 (30%); MS (EI, GC/MS, TMSi derivative) *m/z*: 318 ($M^{\cdot+}$, 0.2%), 303 (100%), 217 (14%), 205 (4%), 179 (2%), 115 (3%); High resolution MS (EI, DIP): found 246.1615 Da; $C_{16}H_{22}O_2$ requires 246.1620 Da.

Preparation of 4-nonyltropolone (60)

4-Non-1-enyltropolone (**59**) (0.102 g, 0.415 mmol) dissolved in ethyl acetate and 10% Pd/C (0.01 g) were stirred at room temperature under 1 atmosphere of H_2 for 2 hrs. The reaction mixture was filtered and the ethyl acetate removed by evaporation under reduced pressure to give **4-nonyltropolone (60)** as an orange oil (0.084 g, 82%). IR (NaCl, neat) cm^{-1}: 3205,

broad, (ν O-H, tropolone), 2925, 2855 (ν C-H), 1612 (ν C=O, tropolone); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.86 (3H, t, *J* = 7.0 Hz, H16), 1.20-1.36 (12H, m, H10-H15), 1.62 (2H, p, *J* = 7.6 Hz, H9), 2.59 (2H, t, *J* = 7.6 Hz, H8), 6.88 (1H, ddd, *J* = 9.8, 1.5, 1.0 Hz, H5), 7.18 (1H, dd, *J* = 10.8, 1.0 Hz, H7), 7.24 (overlapping with H3, dd, *J* = 10.8, 9.8 Hz, H6), 7.25 (d, *J* = 1.5 Hz, H3), (H6, H3, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 14.0 (C16), 22.6 (C15), 29.1 (C10), 29.2 (C11), 29.4, 29.4 (C12, C13), 31.3 (C9), 31.8 (C14), 41.4 (C8), 122.1 (C7), 125.2 (C3), 129.4 (C5), 136.9 (C6), 154.6 (C4), 170.9 (C2), 171.0 (C1); MS (EI, GC/MS) *m/z*: 248 ($M^{\cdot+}$, 19%), 149 (50%), 136 (100%), 123 (14%), 108 (32%), 107 (41%); MS (EI, GC/MS, TMSi derivative) *m/z*: 320 ($M^{\cdot+}$, 0.2%), 305 (100%), 205 (7%), 192 (9%), 177 (5%), 73 (7%); High resolution MS (EI, DIP): found 248.17761 Da; $C_{16}H_{24}O_2$ requires 248.17763 Da.

Preparation of 4-hept-1-enyltropolone (61)

The tropolone anhydride (**52**) (0.447 g), prepared from 3-carboxy-4-carboxymethyltropolone (**49**) (0.50 g, 2.23 mmol) and DCC (0.505 g, 2.45 mmol), was dissolved in pyridine (5 ml) and hexanal (0.239 g, 2.39 mmol) in pyridine (5 ml) was added. The reaction mixture was heated under reflux for 2.5 hrs. Pyridine was removed by evaporation under reduced pressure and the product extracted into ether. The ether extract was decanted from insolubles and washed with 4% aqueous HCl and water. After drying with $MgSO_4$ and filtration, solvent evaporation gave an orange oil (0.318 g, 65%). This product was combined with the product of an earlier synthesis to give 0.432 g of crude 4-hept-1-enyltropolone. Column chromatography on silica gel eluting with CH_2Cl_2 afforded **4-hept-1-enyltropolone (61)** as an orange oil (0.284 g, calculated overall yield was 43%). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.90 (3H, t, *J* = 6.8 Hz, H14), 1.26-1.38 (4H, m, H12, H13), 1.48 (2H, p, *J* = 7.2 Hz, H11), 2.24 (2H, dt, *J* = 7.2, 6.6 Hz, H10), 6.34 (1H, d, *J* = 15.7 Hz, H8), 6.43 (1H, dt, *J* = 15.7, 6.6 Hz, H9), 7.00 (1H, d, *J* = 10.5 Hz, H5), 7.17 (1H, d, *J* = 11.0 Hz, H7), 7.30 (1H, dd, *J* = 11.0, 10.5 Hz, H6), 7.44 (1H, d, *J* = 1.3 Hz, H3); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 14.0 (C14), 22.5 (C13), 28.6 (C11), 31.4 (C12), 33.2 (C10), 119.7 (C3), 123.8 (C7), 126.8 (C5), 132.0 (C8), 137.3 (C6), 138.2 (C9), 147.2 (C4), 169.0 (C2), 172.8 (C1); MS (EI, GC/MS) *m/z*: 218 ($M^{\cdot+}$, 82%), 148 (39%), 133 (88%), 120 (100%), 115 (30%), 77 (33%); MS (EI, GC/MS, TMSi derivative) *m/z*: 290 ($M^{\cdot+}$, 0.2%), 275 (100%), 217 (16%), 205 (4%), 186 (2%), 115 (4%); High resolution MS (EI, DIP): found 218.1306 Da; $C_{14}H_{18}O_2$ requires 218.1307 Da.

Preparation of 4-pent-1-enyltropolone (62)

3-Carboxy-4-carboxymethyltropolone (**49**) (0.50 g, 2.23 mmol) was dehydrated with DCC (0.530 g, 2.57 mmol) to form the tropolone anhydride (**52**) (0.423 g) as previously described. The tropolone anhydride (**52**) was dissolved in pyridine (5 ml) and butanal (0.163 g, 2.26 mmol) in pyridine (5 ml) was added. The reaction mixture was heated under reflux and stirred for 2 hrs. Pyridine was removed by evaporation under reduced pressure and the residue was dissolved in ether. The mixture was agitated in an ultrasonic bath and insolubles were removed by filtration. The ether extract was washed with 3.6% aqueous HCl and water, dried over $MgSO_4$, filtered and the solvent removed to give an orange oil (0.293 g, 69%). This product was combined with the product of an earlier synthesis to give 0.381 g of crude 4-pent-1-enyltropolone. Column chromatography on silica gel eluting with CH_2Cl_2 gave **4-pent-1-enyltropolone (62)** as an orange oil (0.281 g, calculated overall yield was 51%). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.94 (3H, t, *J* = 7.3 Hz, H12), 1.50 (2H, hextet, *J* = 7.3 Hz, H11), 2.22 (2H, dt, *J* = 7.3, 6.6 Hz, H10), 6.34 (1H, d, *J* = 15.8 Hz, H8), 6.42 (1H, dt, *J* = 15.8, 6.6 Hz, H9), 6.99 (1H, d, *J* = 10.0 Hz, H5), 7.16 (1H, d, *J* = 11.0 Hz, H7), 7.29 (1H, dd, *J* = 11.0, 10.0 Hz, H6), 7.43 (1H, s, H3); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 13.6 (C12), 22.1 (C11), 35.2 (C10), 119.7 (C3), 123.8 (C7), 126.8 (C5), 132.2 (C8), 137.3 (C6), 137.9 (C9), 147.1 (C4), 169.0 (C2), 172.8 (C1); MS (EI, GC/MS) *m/z*: 190 ($M^{\cdot+}$, 60%), 133 (100%), 120 (27%), 115 (14%), 105 (19%), 77 (24%); MS (EI, GC/MS, TMSi derivative) *m/z*: 262 ($M^{\cdot+}$, 0.3%), 247 (100%), 217 (22%), 203 (4%), 186 (3%), 115 (5%); High resolution MS (EI, DIP): found 190.0991 Da; $C_{12}H_{14}O_2$ requires 190.0994 Da.

Preparation of 4-heptyltropolone (63)

4-Hept-1-enyltropolone (**61**) (0.124 g, 0.569 mmol) dissolved in ethyl acetate and 10% Pd/C (0.01 g) were stirred at room temperature under 1 atmosphere of H_2 for 2 hrs. The reaction mixture was filtered and the ethyl acetate removed by evaporation under reduced pressure to give **4-heptyltropolone (63)** as an orange oil (0.101 g, 81%). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.88 (3H, t, *J* = 7.0 Hz, H14), 1.21-1.38 (8H, m, H10-H13), 1.63 (2H, p, H9), 2.61 (2H, t, *J* = 7.6 Hz, H8), 6.92 (1H, d, *J* = 9.8 Hz, H5), 7.21 (1H, dd, *J* = 10.9, 1.0 Hz, H7), 7.28 (overlapping with H3, dd, *J* = 10.9, 9.8 Hz, H6), 7.29 (s, H3), (H6, H3, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 14.0 (C14), 22.6 (C13), 29.0, 29.0 (C10, C11), 31.3 (C9), 31.6 (C12), 41.3 (C8), 122.2 (C7), 125.3 (C3), 129.5 (C5), 137.0 (C6), 154.7 (C4), 170.9 (C1, C2); MS (EI,

GC/MS) *m/z*: 220 ($M^{\cdot+}$, 25%), 149 (22%), 136 (100%), 123 (15%), 108 (42%), 107 (32%); MS (EI, GC/MS, TMSi derivative) *m/z*: 292 ($M^{\cdot+}$, 0.1%), 277 (100%), 205 (5%), 192 (8%), 177 (5%), 73 (5%); High resolution MS (EI, DIP): found 220.1460 Da; $C_{14}H_{20}O_2$ requires 220.1463 Da.

Preparation of 4-pentyltropolone (64)

4-Pent-1-enyltropolone (**62**) (0.127 g, 0.668 mmol) dissolved in ethyl acetate and 10% Pd/C (0.01 g) were stirred at room temperature under 1 atmosphere of H_2 for 2 hrs. The reaction mixture was filtered and the ethyl acetate removed by evaporation under reduced pressure to give **4-pentyltropolone (64)** as an orange oil (0.106 g, 83%). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.91 (3H, t, *J* = 6.9 Hz, H12), 1.35 (4H, m, H10-H11), 1.66 (2H, p, *J* = 7.5 Hz, H9), 2.63 (2H, t, *J* = 7.6 Hz, H8), 6.93 (1H, d, *J* = 9.8 Hz, H5), 7.23 (1H, d, *J* = 10.7 Hz, H7), 7.29 (overlapping with H3, dd, *J* = 10.7, 9.8 Hz, H6), 7.30 (s, H3), (H6, H3, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 13.9 (C12), 22.4 (C11), 31.0 (C9), 31.2 (C10), 41.3 (C8), 122.2 (C7), 125.3 (C3), 129.5 (C5), 137.0 (C6), 154.7 (C4), 170.9 (C1, C2); MS (EI, GC/MS) *m/z*: 192 ($M^{\cdot+}$, 39%), 136 (100%), 121 (9%), 108 (51%), 107 (42%), 77 (29%); MS (EI, GC/MS, TMSi derivative) *m/z*: 264 ($M^{\cdot+}$, 0.1%), 249 (100%), 192 (12%), 177 (7%), 162 (4%), 73 (5%); High resolution MS (EI, DIP): found 192.1152 Da; $C_{12}H_{16}O_2$ requires 192.1150 Da.

7.2.9 Synthesis of 4-Styryltropolones

Preparation of 3-carboxy-4-styryltropolone (65)

The tropolone anhydride (**52**) (0.453 g), prepared from 3-carboxy-4-carboxymethyltropolone (**49**) (0.30 g, 1.34 mmol) as previously described, was dissolved in dry pyridine and benzaldehyde (0.142 g, 1.33 mmol) in dry pyridine was added. The reaction mixture was warmed to 50-55°C and stirred for 3.5 hrs. Pyridine was removed by evaporation under reduced pressure and ether was added. After agitation in an ultrasonic bath to promote dissolution, insolubles were removed by filtration. The ether layer was washed with 4% aqueous HCl and water, then dried over $MgSO_4$ and the solvent removed to give a yellow residue (0.169 g). Unreacted benzaldehyde was removed by washing the product with toluene. Residual toluene was removed by evaporation under reduced pressure to give yellow crystals (0.110 g, 31%). Recrystalisation from ethyl acetate gave **3-carboxy-4-styryltropolone (65)**,

m.p. = 164-165°C (literature m.p. = 163-166°C (340)). IR (KBr disc) cm^{-1}: 3400-3600 (ν O-H, COOH), 3220, broad, (ν O-H, tropolone), 2558, (ν O-H, COOH), 1743 (ν C=O, COOH), 1624 (ν C=O, tropolone), 1576 (ν C=C); ^{1}H NMR (400 MHz, acetone-d_6) δ: 7.28 (1H, d, J = 10.1 Hz, H7), 7.34 (1H, d, J = 16.0 Hz, H9), 7.40 (1H, d, J = 7.1 Hz, 1H, H4'), 7.45 (overlapping with H10, t, $J \approx$ 7.1 Hz, H3' and H5'), 7.48 (overlapping with H6, d, J = 16.0 Hz, H10), 7.49 (t , $J \approx$ 10.3 Hz, H6), (H3' and H5', H10, H6, 4H), 7.61 (overlapping with H5, d, J = 7.2 Hz, H2' and H6'), 7.62 (d, J = 11.1 Hz, H5), (H2' and H6', H5, 3H); ^{13}C NMR (100 MHz, acetone-d_6) δ: 118.3 (C7), 126.8 (C5), 127.6 (C9), 127.7 (C2', C6'), 129.3 (C3', C5'), 129.6 (C4'), 134.9 (C3), 136.4 (C6), 136.7 (C1'), 137.6 (C10), 143.8 (C4), 168.0 (C1, C8), 172.5 (C2); MS (EI, DIP) *m/z*: 268 ($M^{\bullet+}$, 100%), 239 (29%), 223 (28%), 194 (22%), 178 (19%), 165 (95%). The product was verified as 3-carboxy-4-styryltropolone (**65**) by comparison of measured and published melting points (340).

Preparation of 4-styryltropolone (66)

To the anhydride (**52**) of 3-carboxy-4-carboxymethyltropolone (**49**) (0.453 g, 2.1 mmol) dissolved in pyridine was added benzaldehyde (0.224 g, 2.1 mmol) in pyridine. The reaction mixture was heated under reflux and stirred for 2.5 hrs. The mixture was cooled, pyridine was removed by evaporation under reduced pressure and ether (20 ml) was added. The mixture was agitated in an ultrasonic bath for 5 minutes and the ether layer decanted from the insolubles. A second volume of ether was added and the mixture was again agitated and filtered. The ether extracts were combined, washed with 4% aqueous HCl, water, dried over $MgSO_4$ and filtered. The solvent was removed to give **4-styryltropolone (66)** as a yellow solid (0.335 g, 59%). An analytical sample was recrystallised from hexane to give yellow crystals, m.p. = 90-91°C (literature m.p. = 90-91°C (317)). IR (KBr disc) cm^{-1}: 3203, broad, (ν O-H, tropolone), 1628 (ν C=O, tropolone), 1593 (ν C=C); ^{1}H NMR (400 MHz, acetone-d_6) δ: 7.20 (1H, d, J = 11.0 Hz, H7), 7.32 (d, J = 16.5 Hz, H8), 7.35 (overlapping with H8 and H4', dd, J = 10.1, 1.3 Hz, H5), 7.37 (d, J = 7.3 Hz, H4'), (H5, H8, H4', 3H), 7.43 (2H, dd, J = 7.3 Hz, H3' and H5'), 7.50 (overlapping with H9, dd, J = 11.0, 10.1 Hz, H6), 7.52 (d, J = 16.2 Hz, H9), (H6, H9, 2H), 7.66 (1H, d, J = 1.3 Hz, H3), 7.70 (2H, d, J = 7.3 Hz, H2' and H6'); ^{13}C NMR (100 MHz, acetone-d_6) δ: 118.7 (C3), 125.0 (C7), 127.5 (C5), 127.7 (C2', C6'), 129.2 (C3', C4', C5'), 130.8 (C8), 134.5 (C9), 136.9 (C1'), 137.8 (C6), 146.6 (C4), 168.6 (C2), 173.9 (C1); MS (EI, GC/MS, TMSi derivative) 1 peak *m/z*: 296 ($M^{\bullet+}$, 0.5%), 281 (100%), 207

(5%), 178 (4%), 140 (4%), 133 (5%); MS (APCI) +ve ion, *m/z*: 225.2 $(M+H)^+$; MS^2 225.2@40% CE, *m/z*: 207.2 (85%), 197.1 (15%), 179.3 (100%), 169.2 (12%), 100.1 (20%); MS (APCI) -ve ion, *m/z*: 223.3 $(M-H)^-$; MS^2 223.3@40% CE, *m/z*: 223.1 (78%), 195.3 (7%), 167.3 (100%); High resolution MS (EI, DIP): found 224.0838 Da; $C_{15}H_{12}O_2$ requires 224.0837 Da.

Attempted preparation of 4-(3',4'-dihydroxy)styryltropolone

To the tropolone anhydride (**52**), prepared from 3-carboxy-4-carboxymethyltropolone (**49**) (0.200 g, 0.89 mmol), dissolved in dry pyridine was added 3,4-dihydroxybenzaldehyde (0.123 g, 0.89 mmol) in dry pyridine. The mixture was heated to reflux for 2.5 hrs under N_2. Pyridine was removed by evaporation under reduced pressure. The residue was extracted successively with ether and the extracts filtered to remove insolubles. The ether extracts were combined, washed with 4% HCl, water and dried over $MgSO_4$. Evaporation of the ether gave unreacted 3,4-dihydroxybenzaldehyde as yellow crystals (0.12 g) and no condensation product.

The attempted reaction of tropolone anhydride (**52**) (0.101 g, 0.45 mmol) with 2,4-dihydroxybenzaldehyde (0.101 g, 0.49 mmol), under the same conditions also gave no condensation product.

Preparation of 3-carboxy-4-(3',4',5'-trimethoxy)styryltropolone (67)

The tropolone anhydride (**52**) was prepared from 3-carboxy-4-carboxymethyltropolone (**49**) (0.500 g, 2.23 mmol) as described earlier. 3,4,5-Trimethoxybenzaldehyde (1.5 g, 7.63 mmol) was added and the mixture was melted at 100°C and stirred for 1 hr. CO_2 was evolved. Upon cooling, the residue was taken up in toluene. The insolubles were collected by Buchner filtration, washed with toluene and the solvent removed by drying in a vacuum oven to give an orange solid (0.454 g, 57%). Recrystallisation from ethyl acetate/hexane gave **3-carboxy-4-(3',4',5'-trimethoxy)styryltropolone (67)** as orange crystals, m.p. = 186-187°C. IR (KBr disc) cm^{-1}: 3400-3600 (ν O-H, COOH), 3227, broad, (ν O-H, tropolone), 1728 (ν C=O, COOH), 1618 (ν C=O, tropolone), 1584 (ν C=C); ^1H NMR (400 MHz, acetone-d_6) δ: 3.78 (3H, s, H8'), 3.88 (6H, s, H7', H9'), 6.93 (2H, s, H2' and H6'), 7.23 (overlapping with H7, d, J = 15.8 Hz, H9), 7.27 (d, $J \approx$ 10 Hz, H7), (H9, H7, 2H), 7.40 (1H, d, J = 15.8 Hz, H10), 7.47 (1H, dd, J = 11.0, 10 Hz, H6), 7.58 (1H, d, J = 11.0 Hz, H5); ^{13}C NMR (100 MHz,

acetone-d_6) δ: 56.1 (C7', C9'), 60.2 (C8'), 105.7 (C2', C6'), 118.0 (C7), 126.8 (C5), 126.9 (C9), 132.2 (C1'), 134.7 (C3), 136.3 (C6), 137.8 (C10), 140.4 (C4'), 144.1 (C4), 154.2 (C3', C5'), 167.8, 167.9 (C1, C8), 172.5 (C2); MS (APCI) +ve ion, *m/z*: 359.0 ($(M+H)^+$, 3%), 315.1 ($(M+H)^+$-CO_2, 100%); MS^2 359.0@40% CE, *m/z*: 359.1 (48%), 344.1 (42%), 341.0 (100%), 331.1 (66%), 327.1 (76%), 299.1 (28%); MS (APCI) -ve ion, *m/z*: 357.2 ($(M-H)^-$, 6%), 313.2 ($(M-H)^-$-CO_2, 100%); MS^2 357.2@40% CE, *m/z*: 357.2 (61%), 342.1 (100%), 341.1 (16%), 313.1 (60%), 283.2 (28%).

Preparation of 4-(3',4',5'-trimethoxy)styryltropolone (68)

3-Carboxy-4-(3',4',5'-trimethoxy)styryltropolone (**67**) (0.350 g, 0.98 mmol) was stirred at 160°C in dry quinoline with Cu powder (35 mg) for 1.5 hrs. The reaction mixture was cooled and 10% aqueous HCl and $CHCl_3$ were added. The organic and aqueous layers were separated and the aqueous layer was washed with $CHCl_3$. The $CHCl_3$ extracts were combined, washed with 10% HCl, water, dried over $MgSO_4$ and the solvent removed to give a brown solid. The product was successively washed with ether and the solvent evaporated to give a yellow solid (0.180 g, 59%). Recrystallisation from ethyl acetate/hexane gave **4-(3',4',5'-trimethoxy)-styryltropolone (68)** as yellow crystals, m.p. = 173-174°C. IR (KBr disc) cm^{-1}: 3193 (ν O-H, tropolone), 1627 (ν C=O, tropolone), 1585 (ν C=C); ^1H NMR (400 MHz, acetone-d_6) δ: 3.80 (3H, s, H8'), 3.90 (6H, s, H7' and H9'), 7.06 (2H, s, H2' and H6'), 7.16 (1H, d, *J* = 11 Hz, H7), 7.28 (overlapping with H5, d, *J* = 16.4 Hz, H8), 7.33 (d, *J* = 10 Hz, H5), (H5, H8, 2H), 7.45 (overlapping with H6, d, *J* = 16.4 Hz, H9), 7.48 (dd, *J* = 11, 10 Hz, H6), (H6, H9, 2H), 7.62 (1H, d, *J* = 1.4 Hz, H3); ^{13}C NMR (100 MHz, acetone-d_6) δ: 56.1 (C7', C9'), 60.2 (C8'), 105.6 (C2', C6'), 118.9 (C3), 124.4 (C7), 127.1 (C5), 130.0 (C8), 132.5 (C1'), 134.8 (C9), 137.6 (C6), 140.1 (C4'), 146.9 (C4), 154.2 (C3', C5'), 168.8 (C2), 173.6 (C1); MS (APCI) +ve ion, *m/z*: 315.2 $(M+H)^+$; MS^2 315.2@40% CE, *m/z*: 300 (35%), 297.1 (100%), 284.0 (59%), 238.1 (35%), 218.0 (44%); MS (APCI) -ve ion, *m/z*: 313.2 $(M-H)^-$; MS^2 313.2@40% CE, *m/z*: 313.1 (99%), 298.0 (89%), 283.2 (12%), 257.2 (55%), 242.2 (100%). Elemental Analysis: found C, 68.5, H, 5.7; $C_{18}H_{18}O_5$ requires C, 68.8, H, 5.8.

Preparation of 4-(3',5'-dimethoxy)styryltropolone (69)

The tropolone anhydride (**52**) was prepared from 3-carboxy-4-carboxymethyltropolone (**49**) (1.0 g, 4.46 mmol), as described previously and dissolved in dry pyridine. 3,5-Dimethoxy-

benzaldehyde (0.63 g, 3.8 mmol) was added and the mixture was heated under reflux for 4.5 hrs. The reaction mixture was cooled to room temperature and left to stand overnight. Pyridine was removed by distillation under reduced pressure. The residue was extracted successively with ether with agitation in a ultrasonic bath. The ether extracts were decanted from the insolubles. The insolubles were treated with 10% aqueous HCl and extracted with ether. The ether extracts were combined. Yellow crystals, which deposited from the ether extracts were collected, washed with ether and dried to give 4-(3',5'-dimethoxy)styryl-tropolone (**69**) (0.065 g, 6%). The ether extract was washed with 10% aqueous HCl, water, dried over $MgSO_4$ and filtered. Ether was evaporated under reduced pressure to give a mixture of 4-(3',5'-dimethoxy)styryltropolone and 3,5-dimethoxybenzaldehyde (0.693 g). The 3,5-dimethoxybenzaldehyde was removed by successive extraction with small volumes of ether to give a yellow solid (0.387 g, 36%). The product was recrystallised from ethyl acetate/hexane to give **4-(3',5'-dimethoxy)styryltropolone** (**69**) as yellow crystals, m.p. = 103°C. IR (KBr disc) cm^{-1}: 3191 (ν O-H tropolone), 1604 (ν C=O, tropolone), 1552 (ν C=C); ^1H NMR (400 MHz, $CDCl_3$) δ: 3.83 (6H, s, H7' and H8'), 6.45 (1H, t, *J* = 2.0 Hz, H4'), 6.66 (2H, d, *J* = 2.0 Hz, H2' and H6'), 7.01 (1H, d, *J* = 16.1 Hz, H8), 7.15 (overlapping with H9, d, *J* = 9.9 Hz, H5), 7.17 (overlapping with H7, d, *J* = 16.1 Hz, H9), 7.21 (d, *J* = 11.1 Hz, H7), (H5, H7, H9, 3H), 7.38 (1H, dd, *J* = 11.1, 9.9 Hz, H6), 7.59 (1H, d, *J* = 1.3 Hz, H3); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 55.4 (C7', C8'), 101.2 (C4'), 105.2 (C2', C6'), 118.7 (C3), 125.0 (C7), 127.4 (C5), 130.7 (C8), 134.5 (C9), 137.4 (C6), 137.8 (C1'), 146.2 (C4), 161.0 (C3', C5'), 168.1 (C2), 173.6 (C1); MS (APCI) +ve ion, *m/z*: 285.1 $(M+H)^+$; MS^2 285.1@40% CE, *m/z*: 267.1 (100%), 257.1 (22%), 239.2 (76%), 201.2 (26%), 188.1 (57%), 281.0 (28%); MS (APCI) -ve ion, *m/z*: 283.2 $(M-H)^-$; MS^2 283.2@40% CE, *m/z*: 283.1 (54%), 268.2 (3%), 255.2 (7%), 227.2 (100%); Elemental Analysis: found C, 72.0, H, 5.8; $C_{17}H_{16}O_4$ requires C, 71.8, H, 5.7.

Preparation of 4-formyltropolone (71)

4-Formyltropolone (**71**) was prepared by the method of Tarbell *et al.* (317). To 4-styryltropolone (**66**) (0.200 g, 0.89 mmol) dissolved in 3:1 dioxane:water was added a catalytic amount of OsO_4 (a few mg). The reaction mixture was stirred for 15 minutes during which time the mixture turned brown in colour. Solid $NaIO_4$ (0.419 g, 1.96 mmol) was added to the reaction mixture in small portions over a period of 45 minutes. The reaction mixture was stirred for 3 hrs at 25°C. Dioxane was added and the precipitated $NaIO_3$ removed by filtration. The filter cake was washed with ether and combined with the filtrate. Ether, dioxane

and water were removed by evaporation under reduced pressure using a water aspirator and benzaldehyde was removed using an oil pump to give a yellow solid (0.195 g). The crude product was placed in a sublimation tube and heated under vacuum with an oil bath. **4-Formyltropolone (71)** sublimed as a yellow solid (0.099 g, 74%) at 0.25 torr over a temperature range of 110-130°C. A sample was recrystallised from benzene, m.p. = 157-158°C (literature m.p. = 158-160°C (317)). IR (KBr disc) cm^{-1}: 3198 (ν O-H, tropolone), 1684 (ν C=O, CHO), 1615 (ν C=O, tropolone); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.48 (overlapping with H7, dd, *J* = 9.0, 1.0 Hz, H5), 7.49 (d, *J* = 11.5 Hz, H7), (H5, H7, 2H), 7.64 (1H, dd, *J* = 11.5, 9.0 Hz, H6), 7.74 (1H, d, *J* = 1.0 Hz, H3), 9.87 (1H, s, H8); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 115.0 (C3), 132.6 (C5), 132.9 (C7), 137.4 (C6), 141.1 (C4), 166.4 (C2), 176.9 (C1), 192.0 (C8); MS (EI, GC/MS) *m/z*: 150 ($M^{\bullet+}$, 100%), 121 (75%), 93 (18%), 84 (7%), 65 (18%), 44 (22%); MS (EI, GC/MS, TMSi derivative) *m/z*: 222 ($M^{\bullet+}$, 0.2%), 207 (100%), 179 (21%), 149 (8%), 135 (6%), 73 (3%).

Attempted preparation of 3-carboxy-4-(ethenyltropolone)tropolone

The tropolone anhydride (**52**) was prepared from 3-carboxy-4-carboxymethyltropolone (**49**) (0.194 g, 0.78 mmol) with DCC (0.177 g, 0.86 mmol) as previously described. The tropolone anhydride was dissolved in dry pyridine, warmed to 40°C and 4-formyltropolone (**71**) (0.090 g, 0.6 mmol) in pyridine was added dropwise. The reaction mixture was stirred for 3.5 hrs at 50°C under a N_2 atmosphere. Pyridine was removed by evaporation under reduced pressure and the product was treated with 10% aqueous HCl and then extracted with 3 volumes of CH_2Cl_2. The extracts were filtered from insolubles, combined, washed with water and dried over $MgSO_4$. The solvent was removed by evaporation to give a yellow residue (0.32 g). The crude product was analysed by GC/MS and NMR. No evidence of 3-carboxy-4-(ethenyltropolone)tropolone was found. Repeating the reaction at reflux temperature for 5.5 hrs gave a brown solid as a crude product in approximately 13% yield. This product was insoluble in ether and MeOH, but soluble in DMSO. The product displayed signals in both the ^{1}H and ^{13}C NMR spectra that were conceivably consistent with a condensation product of tropolone anhydride (**52**) and 4-formyltropolone (**71**). However, the evidence was less than convincing and the yield too poor to warrant further investigation.

7.2.10 Syntheses Towards β-Thujaplicin (47) from 1-Methyl-3,4,6-trihydroxy-(5*H*)-benzocyclohepten-5-one (72)

Preparation of 1-methyl-3,4,6-trihydroxy-(5*H*)-benzocyclohepten-5-one (72) (4'-methyl-1',2'-dihydroxy-3,4-benzotropolone)

1-Methyl-3,4,6-trihydroxy-(5*H*)-benzocyclohepten-5-one (**72**) was prepared by the method of Collier (321). To a solution of $NaIO_4$ (1.72 g, 8.1 mmol) in water (20 ml) cooled in a ice/water bath was added dropwise 4-methylcatechol (1 g, 8.1 mmol) in water (10 ml). After stirring for 5 minutes, pyrogallol (1.02 g, 8.1 mmol) in water (10 ml) was added dropwise. The cooling bath was removed and the mixture was stirred at room temperature for 15 minutes. Solid $Na_2S_2O_4$ (1.40 g, 8.1 mmol) was added and after stirring for 5 minutes the brown precipitate was collected by Buchner filtration. The product was washed with cold water and air dried to give a brown solid (1.41 g, 80%). The crude product was recrystallised from ethanol to yield **1-methyl-3,4,6-trihydroxy-(5*H*)-benzocyclohepten-5-one (72)** as red-brown crystals, m.p. = indeterminate, decomposes from 155°C (literature m.p. = 208°C (321), decomposes from 175°C (341)). IR (KBr disc) cm^{-1}: 3200-3600 (ν O-H), 1612 (ν C=O); ^1H NMR (400 MHz, acetone-d_6) δ: 2.61 (3H, s, H10), 6.91 (1H, dd, *J* = 11.8, 9.3 Hz, H8), 7.27 (1H, d, *J* = 9.3 Hz, H7), 7.45 (1H, s, H2), 7.73 (1H, d, *J* = 11.8 Hz, H9), 8.23 (1H, s, OH), 8.77 (1H, s, OH), 14.78 (1H, s, OHα to C4); ^{13}C NMR (100 MHz, acetone-d_6) δ: 21.9 (C10), 118.2 (C7), 121.6 (C4a), 123.5 (C8), 124.9 (C2), 130.4 (C9a), 130.6 (C1), 130.9 (C9), 146.1 (C3), 148.9 (C4), 155.4 (C6), 184.7 (C5); MS (EI, DIP) *m/z*: 218 ($M^{\cdot+}$, 100%), 190 (78%), 161 (6%), 144 (14%), 129 (5%), 115 (48%); MS (EI, DIP, TMSi derivative) *m/z*: 419 ($M^{\cdot+}$-CH_3, 62%, 3 $SiMe_3$ groups), 362 (10%), 347 (48%), 331 (41%), 290 (17%), 275 (56%), 73 (100%).

Preparation of 1-methyl-3,4-dioxo-6-hydroxy-(5*H*)-benzocyclohepten-5-one (73) (4-methylbenzotropolone-1,2-quinone)

1-Methyl-3,4-dioxo-6-hydroxy-(5*H*)-benzocyclohepten-5-one (**73**) was prepared by a modification of the method of Collier (321). To *o*-chloranil (1.13 g, 4.60 mmol) in ether (30 ml) cooled to 3°C was added dropwise 1-methyl-3,4,6-trihydroxy-(5*H*)-benzocyclohepten-5-one (**72**) (1.0 g, 4.58 mmol) in 300 ml ether. The reaction mixture was cooled to *ca.* -23°C by

immersion in a CCl_4/dry ice bath for 1.5 hrs. The crystallised product was collected by Buchner filtration, washed with ether and dried to give **1-methyl-3,4-dioxo-6-hydroxy-(5*H*)-benzocyclohepten-5-one** (**73**) as red/brown crystals (0.813 g, 82%), indeterminate m.p. (literature m.p. = indeterminate, decomp. from 150°C (321)). IR (KBr disc) cm^{-1}: 3442, broad, (ν O-H), 1630 (ν C=O, quinone), 1600 (ν C=O, tropolone); ^1H NMR (400 MHz, $CDCl_3$) δ: 2.45 (3H, s, H10), 6.64 (1H, s, H2), 6.88 (1H, d, *J* = 11.9 Hz, H7), 7.08 (1H, d, *J* = 9.0 Hz, H9), 7.27 (1H, dd, *J* =11.9, 9.0 Hz, H8); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 22.9 (C10), 113.6 (C4a), 127.9 (C9), 129.4 (C2), 133.1 (C7), 135.1 (C9a), 137.0 (C8), 154.7 (C1), 177.7 and 178.3 (C5, C6), 183.1 and 183.6 (C3, C4); MS (EI, GC/MS, TMSi derivative) 1 peak, *m/z*: 434 ($M^{\bullet+}$, 1%), 420 (35%), 419 (100%), 331 (3%), 273 (2%), 73 (40%), triTMSi derivative of 1-methyl-3,4-dioxo-6-hydroxy-(5*H*)-benzocyclohepten-5-one.

m-Chloroperbenzoic acid oxidation of 1-methyl-3,4-dioxo-6-hydroxy-(5*H*)-benzocyclohepten-5-one (73)

To 1-methyl-3,4-dioxo-6-hydroxy-(5*H*)-benzocyclohepten-5-one (**73**) (0.612 g, 2.83 mmol) dissolved in dry CH_2Cl_2 was added dropwise *m*-chloroperbenzoic acid (0.537 g, 3.11 mmol) in dry CH_2Cl_2 at room temperature. The mixture was stirred at room temperature for four days during which time a brown precipitate formed. The precipitate was collected by filtration and washed with CH_2Cl_2 to give a brown solid (200 mg, 28%). Workup of the CH_2Cl_2 filtrate gave *m*-chlorobenzoic acid. Characterisation of the brown product by IR, ESI and APCI MS and ^1H and ^{13}C NMR spectroscopy gave the following results. IR (KBr disc) cm^{-1}: 3000-3600, (ν O-H), 3244, broad, (ν O-H, tropolone), 1760 and 1734 (ν C=O); MS (ESI) +ve ion, *m/z*: 251.1 $(M+H)^+$; MS (ESI) -ve ion, *m/z*: 248.9 $(M-H)^-$; MS^2 248.9@40% Collision Energy (CE), *m/z*: 231.0 (6%), 205.0 (100%), 187.1 (68%), 177.0 (35%); MS (APCI) +ve ion, *m/z*: 233.0 $(M+H)^+$; MS (APCI) -ve ion, *m/z*: 231.1 $(M-H)^-$. ^1H NMR (400 MHz, DMSO-d_6) δ: 2.16 (3H, s), 6.15 (1H, s), 7.18 (1H, t), 7.20 (1H, d), 7.25 (1H, d); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 19.6, 93.9, 118.8, 123.3, 124.3, 127.4, 129.0, 133.2, 151.0, 163.6, 168.4, 169.3. Possible products from the oxidation reaction were the tropolone diacid (**74**) and the tropolone anhydride (**75**). In general the spectroscopic evidence points toward the tropolone diacid (**74**) as the product although it is difficult to assign ^{13}C NMR signals at δ 93.9 and 151.0 ppm to this structure. The unambiguous identification of this product was not achieved when further research in this area was abandoned (see Section 4.4).

7.2.11 Alkylations of Tropolone (48) and β-Thujaplicin (47) with Diethyl Acetals

Preparation of 7,7-dichlorobicyclo(3.2.0)hept-2-en-6-one (76)

7,7-Dichlorobicyclo(3.2.0)hept-2-en-6-one (**76**) was prepared by the method of Minns (332). Dicyclopentadiene was cracked to cyclopentadiene by heating under a column (200 mm by 20 mm diameter) filled with glass rings. Cyclopentadiene distilled at 40°C and was collected in a receiver maintained at -78°C. To dichloroacetyl chloride (50 g, 0.34 mol), cyclopentadiene (68 g, 1.03 mol) and hexane (350 ml) heated to reflux under N_2 and rapidly stirred, was added triethylamine (35.4 g, 0.35 mol) in hexane (150 ml) over a period of 4 hrs. The reaction mixture was heated under reflux for a further 2 hrs and allowed to stand overnight. Water (150 ml) was added and the mixture stirred until the triethylamine hydrochloride dissolved. The hexane layer was separated and the aqueous layer was extracted with hexane (100 ml). The hexane extracts were combined and dried by passage through absorbent cotton. Hexane and excess cyclopentadiene were removed by evaporation under reduced pressure to give an orange liquid, which was fractionally distilled under reduced pressure through a 350 mm Vigreux column. The first fraction (7.83 g), collected between 32-42°C at 2.5-1.4 torr, was mainly dicyclopentadiene as indicated by its ^{1}H NMR spectrum. **7,7-Dichlorobicyclo-(3.2.0)hept-2-en-6-one (76)** (47.34 g, 79%) distilled between 64-66°C at 0.63-0.54 torr as a colourless liquid (literature b.p. = 66-68°C (2 mm Hg) (332)). IR (KBr, neat liquid) cm^{-1}: 1802 (ν C=O); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.55 (1H, qdd, J = 17.5, 8.8, 2.1 Hz, H4), 2.81 (1H, md (unresolved), J = 17.5 Hz, H4'), 4.05 (1H, ddd, J = 9.3, 4.7, 2.3 Hz, H5), 4.24 (1H, dd, J = 7.7, 7.7 Hz, H1), 5.71 (1H, td, J = 7.8, 2.3 Hz, H3), 6.03 (1H, m, H2); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 35.1 (C4), 58.6 (C1), 59.6 (C5), 88.1 (C7), 128.4 (C3), 136.8 (C2), 197.7 (C6); MS (EI, GC/MS) *m/z*: 178 ($M^{\cdot+}$, 2%), 141 (4%), 115 (29%), 113 (89%), 77 (100%), 66 (61%).

Preparation of tropolone (48)

Tropolone (**48**) was prepared by solvolysis of 7,7-dichlorobicyclo(3.2.0)hept-2-en-6-one (**76**) as described by Minns (332). NaOH pellets (47 g, 1.175 mol) were dissolved in glacial acetic acid (240 ml) by warming to *ca.* 70°C with stirring. 7,7-Dichlorobicyclo(3.2.0)hept-2-en-6-

one (**76**) (46.7 g, 0.264 mol) was added and the reaction mixture was heated under reflux under a N_2 atmosphere for 8 hrs. The mixture was cooled and allowed to stand overnight. Concentrated HCl was added until the pH of the mixture was *ca.* 1. Benzene (450 ml) was added and the mixture was filtered. The NaCl filter cake was washed with three 50 ml volumes of benzene. The two phases were separated and the aqueous phase continuously extracted with benzene for 16 hrs. Benzene was removed by distillation. Acetic acid was removed by distillation under reduced pressure. The resultant liquid was distilled under reduced pressure through a 350 mm Vigreux column. When the last of the acetic acid distilled the condenser was replaced with a round bottom flask immersed in ice water. Tropolone (**48**) distilled at 71-75°C at 0.2-0.13 torr and crystallised as a pale yellow solid (27.24 g, 85%).

The crude product (27.24 g) was dissolved in CH_2Cl_2 (62.5 ml) and diluted with pentane (250 ml). Activated carbon (1.2 g) was added and the mixture heated under reflux for 5 minutes. The mixture was filtered hot and the filtrate cooled to -20°C until crystallisation was complete. Tropolone (**48**) recrystallised as white needles (19.63 g) and was collected by filtration. The filtrate was evaporated under reduced pressure to dryness and the residue dissolved in pentane (300 ml) on heating to reflux. The solution was filtered while warm and cooled to -20°C. Tropolone (**48**) crystallised as white needles (3.46 g) and was collected by filtration. The total yield of **tropolone** (**48**) was 21.1 g (72%), m.p. = 50-51°C (literature m.p. = 50-51°C (332)). ^{1}H NMR (400 MHz, acetone-d_6) δ: 7.13 (1H, t, J = 9.7 Hz, H5), 7.31 (2H, d, J = 10.8 Hz, H3 and H7), 7.51 (2H, dd, J_1 = 10.8, 9.8 Hz, H4 and H6); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 123.9 (C3, C7), 128.2 (C5), 137.7 (C4, C6), 171.8 (C1, C2); MS (EI, GC/MS, TMSi derivative) *m/z*: 194 ($M^{\cdot+}$, 0.7%), 180 (29%), 179 (100%), 163 (4%), 149 (31%), 82 (14%).

Preparation of octanal diethyl acetal (77)

Octanal diethyl acetal (**77**) was prepared by the method of Zhu *et al.* (333). To freshly distilled octanal (1 g, 7.8 mmol) in ethanol (99.7-100%) was added neat triethyl orthoformate (1.56 ml, 9.36 mmol) under a N_2 atmosphere. Solid NH_4NO_3 (31 mg, 0.39 mmol) was added and reaction mixture was stirred for 3 hrs at 40°C and then 12 hrs at room temperature. Solid Na_2CO_3 (45 mg, 0.43 mmol) was added and the mixture stirred for 20 minutes. The mixture was filtered, EtOH was removed by evaporation under reduced pressure and the product was

taken up in CH_2Cl_2. Filteration from insolubles and solvent evaporation gave **octanal diethyl acetal (77)** as a pale yellow liquid (1.20 g, 76%). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.87 (3H, t, *J* = 7.0 Hz, H8), 1.20 (overlapping with H3-H7, t, *J* = 7.0 Hz, H10), 1.23-1.40 (m, H3-H7), (H10, H3-H7, 16 H), 1.60 (2H, m, H2), 3.49 (2H, qd, H9), 3.63 (2H, qd, *J* = 9.1, 7.1 Hz, H9'), 4.48 (1H, t, *J* = 5.8 Hz, H1); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 14.0 (C8), 15.3 (C10), 22.6 (C7), 24.7 (C3), 29.2, 29.4 (C4, C5), 31.7 (C6), 33.5 (C2), 60.7 (C9), 102.9 (C1); MS (EI, GC/MS) *m/z*: 202 ($M^{\bullet+}$, 0.04%), 157 (15%), 103 (100%), 75 (24%), 69 (23%), 57 (13%).

Preparation of *trans*-cinnamaldehyde diethyl acetal (78)

trans-Cinnamaldehyde diethyl acetal (**78**) was prepared by the method of Klein and Bergmann (334). To *trans*-cinnamaldehyde (7.07 g, 54 mmol) dissolved in freshly distilled absolute ethanol (50 ml) was added triethyl orthoformate (10.8 ml, 65 mmol) and solid NH_4NO_3 (0.216 g, 2.7 mmol). The reaction mixture was warmed to 40-45°C for 3.5 hrs, cooled to room temperature and stirred for a further 16 hrs. Solid Na_2CO_3 (0.315 g, 2.97 mmol) was added and the mixture stirred for 20 minutes. The mixture was filtered and ethanol was removed by evaporation under reduced pressure. Ether was added, insolubles were removed by filtration and the filtrate was evaporated under reduced pressure to give a yellow liquid (9.31 g, 84.4%). The crude product was distilled under reduced pressure. ***trans*-Cinnamaldehyde diethyl acetal (78)** distilled as a colourless liquid at 75-77°C and 0.28 torr (6.56 g, 59%), (literature b.p. = 155°C (23 mm Hg) (334)). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.24 (6H, t, *J* = 7.1 Hz, H11), 3.54, 3.56 (2H, 2 × q, *J* = 7.0 Hz, H10), 3.68, 3.70 (2H, 2 × q, *J* = 7.1 Hz, H10'), 5.05 (1H, d, *J* = 5.4 Hz, H9), 6.20 (1H, dd, *J* = 16.1, 5.4 Hz, H8), 6.68 (1H, d, *J* = 16.1 Hz, H7), 7.25 (1H, d, *J* = 6.6 Hz, H4), 7.30 (2H, dd, *J* = 7.5, 6.6 Hz, H3 and H5), 7.39 (2H, d, *J* = 7.5 Hz, H2 and H6); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 15.2 (H11), 60.9 (H10), 101.4 (C9), 126.5 (C8), 126.6 (C2, C6), 127.9 (C4), 128.4 (C1, C5), 132.8 (C7), 136.0 (C1); MS (EI, GC/MS) *m/z*: 206 ($M^{\bullet+}$, 10%), 161 (100%), 133 (56%), 115 (29%), 105 (32%), 77 (26%).

An earlier fraction (1.1 g) distilling at 75°C (0.28 torr) contained 87% cinnamaldehyde diethyl acetal (**78**) with cinnamaldehyde as an impurity (as determined by GC/MS analysis). Overall yield of cinnamaldehyde diethyl acetal (**78**) from the reaction was calculated at 68% from these two fractions.

Attempted preparation of *trans*-2-octenal diethyl acetal (80)

To *trans*-2-octenal (technical grade, 94%) (5.0 g, 39.6 mmol) dissolved in freshly distilled absolute ethanol (40 ml), was added triethyl orthoformate (7.3 ml, 43.9 mmol) and solid NH_4NO_3 (0.158 g, 1.98 mmol). The reaction mixture was warmed to 40-45°C for 4 hrs, cooled to room temperature and stirred for a further 16 hrs. Solid Na_2CO_3 (0.233 g, 2.2 mmol) was added and the mixture stirred for 20 minutes. The mixture was filtered and ethanol was removed by evaporation under reduced pressure. Ether was added, insolubles were removed by filtration and the filtrate was evaporated under reduced pressure to give a yellow liquid (7.0 g, 88%). The crude product appeared to contain mainly *trans*-2-octenal diethyl acetal (**80**) with possibly 1-ethoxy-1,3-octadiene (**81**) as a by-product based on the GC/MS evidence. MS (EI, GC/MS) 4 peaks (Rt (min), GC peak areas, MS *m/z*, compound); 18.0, 4.5%, 154 ($M^{\cdot+}$, 21%), 125 (2%), 111 (46%), 83 (100%), 70 (11%), 55 (24%), possibly 1-ethoxy-1,3-octadiene isomer (**81**); 18.5, 4.5%, 154 ($M^{\cdot+}$, 22%), 125 (2%), 111 (46%), 83 (100%), 70 (11%), 55 (23%), possibly 1-ethoxy-1,3-octadiene isomer (**81**); 21.1, 9%, 202 ($M^{\cdot+}$, not observed), 155 (75%), 129 (34%), 109 (23%), 103 (29%), 85 (100%), 57 (68%), possibly *cis*-2-octenal diethyl acetal; 21.7, 81%, 202 ($M^{\cdot+}$, not observed), 155 (100%), 129 (27%), 109 (22%), 103 (21%), 85 (85%), 57 (78%), possibly *trans*-2-octenal diethyl acetal (**80**).

An attempt to purify the possible product, *trans*-2-octenal diethyl acetal (**80**) by distillation under reduced pressure was unsuccessful. GC/MS analysis of distillation fractions indicated that additional by-products, possibly 1-ethoxy-1,3-octadiene (**81**) were formed upon distillation. The products of the transacetalation were only tentatively identified from the GC/MS results as the NMR spectra of the distillation mixtures were impossible to interpret. The use of octenal diethyl acetal (**80**) was abandoned in favour of commercially available *trans*-2-hexenal diethyl acetal (**79**).

Preparation of 1-ethoxy-2-hexene (82)

Na metal (0.46 g, 0.02 g atoms) in dry toluene was heated to reflux and stirred until all the Na had melted. The mixture was cooled with efficient stirring to produced finely divided Na suspended in toluene. 2-Hexen-1-ol (2 g, 0.02 mol) in toluene was added slowly and when the initial reaction had subsided the mixture was heated under reflux and stirred for 3 hrs. Toluene was removed by evaporation under reduced pressure and dry THF was added. Ethyl bromide

(4.36 g, 0.04 mol) in dry THF was added and the reaction mixture was heated under reflux for 16 hrs. The mixture was cooled and ether and water were added. The two phases were separated, and the ether extract was washed with water, dried over $MgSO_4$ and the ether distilled to give a pale yellow liquid (1.163 g). The crude product was loaded onto a column of silica gel and eluted with 10:1 pentane:ether to give a colourless liquid (0.681 g, 27%). The spectroscopic evidence suggested this product was **1-ethoxy-2-hexene (82)**. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.87 (3H, t, J = 7.4 Hz, H6), 1.18 (3H, t, J = 7.0 Hz, H8), 1.38 (2H, hextet (tq), $J \approx$ 7.4, 7.4 Hz, H5), 1.99 (2H, q, J = 7.1 Hz, H4), 3.44 (2H, q, J = 7.0 Hz, H7), 3.88 (2H, d, J = 6.1 Hz, H1), 5.53 (1H, td, J = 15.3, 6.1 Hz, H2), 5.67 (1H, td, J = 15.3, 6.5 Hz, H3); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 13.7 (C6), 15.2 (C8), 22.2 (C5), 34.4 (C4), 65.3 (C7), 71.4 (C1), 126.5 (C2), 134.4 (C3); MS (EI, GC/MS) *m/z*: 128 ($M^{\cdot+}$, 5%), 99 (14%), 85 (65%), 71 (11%), 57 (100%), 55 (23%).

Attempted condensation of octanal diethyl acetal (77) with tropolone (48)

Tropolone (**48**) and octanal diethyl acetal (**77**) were heated together at 160-170°C for 17 hrs under a N_2 atmosphere. An aliquot of the crude product was taken and analysed by GC/MS. GC/MS analysis indicated the crude product contained the unreacted starting materials and two by-products, which were possibly isomers of 1-ethoxy-1-octene formed by thermal expulsion of ethanol. GC/MS (EI, GC/MS, TMSi derivative): 4 peaks, (Rt (min), GC peak area, MS *m/z*, compound); 19.2, 28%, 156 ($M^{\cdot+}$, 13%), 127 (3%), 113 (7%), 85 (83%), 57 (100%), 41 (15%), possibly an isomer of 1-ethoxy-1-octene; 20.6, 28%, 156 ($M^{\cdot+}$, 13%), 127 (3%), 113 (7%), 85 (83%), 57 (100%), 41 (15%), possibly an isomer of 1-ethoxy-1-octene; 25.7, 18%, 202 ($M^{\cdot+}$, 0.04%), 157 (15%), 103 (100%), 75 (24%), 69 (23%), 57 (13%), octanal diethyl acetal (**77**); 30.0, 25%, 194 ($M^{\cdot+}$, 0.2%), 179 (100%), 149 (18%), 91 (1%), 77 (5%), 73 (4%), tropolone trimethyl silyl ether.

Attempted neat reaction of *trans*-cinnamaldehyde diethyl acetal (78) with tropolone (48)

trans-Cinnamaldehyde diethyl acetal (**78**) (0.675 g, 3.28 mmol) was heated with tropolone (**48**) (0.200 g, 1.64 mmol) at 150-160°C under an argon atmosphere for 2 hrs, after which time the reaction mixture became a black tar and stirring was prevented. Ether was added to the tar and the mixture stirred and agitated in an ultrasonic bath to break up the insolubles. Insoluble material was collected as a brown solid on filtration. The filtrate was evaporated under

reduced pressure to give an orange oil (0.643 g). GC/MS analysis showed this product consisted mainly of cinnamaldehyde, no alkylated tropolone was detected. The ^{1}H NMR spectrum of the brown ether insoluble solid indicated it was polymeric in nature. The same result was obtained operating at 150°C and 180°C under a N_2 atmosphere. Heating cinnamaldehyde diethyl acetal and tropolone at 110°C under an argon atmosphere for 28 hrs resulted in no reaction.

Reaction of *trans*-cinnamaldehyde diethyl acetal (78) with β-thujaplicin (47)

trans-Cinnamaldehyde diethyl acetal (**78**) (0.251 g, 1.22 mmol) was heated with β-thujaplicin (**47**) (0.10 g, 0.61 mmol) at 150-160°C under an argon atmosphere for 6 hrs during which time the reaction mixture became a black tar. Excess cinnamaldehyde diethyl acetal was removed by distillation under reduced pressure using an oil pump. The black tar was solubilised in ethyl acetate and loaded onto a column packed with silica gel. Elution with 15:2 hexane:EtOAc gave a mixture of two products as a viscous yellow oil (0.52 mg, 24%). The spectroscopic evidence suggested these two products were the *cis* and *trans* diastereomers of 3-(1-phenyl-3-ethoxy)prop-2-enyl-6-isopropyltropolone (**84**, **85**). Microanalytical data were not obtained. MS (EI, GC/MS) *m/z*: 324 ($M^{\bullet+}$, 77%), 295 (22%), 279 (66%), 265 (67%), 223 (100%), 189 (39%); MS (EI, GC/MS, TMSi derivative): 2 overlapping peaks, Rt = 48.7 min and 49.0 min, *m/z*: 396 ($M^{\bullet+}$, 11%), 381 (15%), 337 (100%), 323 (13%), 293 (12%), 207 (21%), *cis*- and *trans*-3-(1-phenyl-3-ethoxy)prop-2-enyl-6-isopropyltropolone trimethyl silane.

Signals in the NMR spectra of the product that were assignable to ***trans*-3-(1-phenyl-3-ethoxy)prop-2-enyl-6-isopropyltropolone (85)** (*ca.* 72% of the product) were as follows: ^{1}H NMR (400 MHz, $CDCl_3$)[13] δ: 1.24 (t, *J* = 6.9 Hz, H14 and H15), 1.25 (t, *J* = 7.0 Hz, H12), 2.84 (p, *J* = 7.0 Hz, H13), 3.76 (dq, *J* = 7.0, 2.6 Hz, H11), 5.18 (dd, *J* = 12.5, 8.6 Hz, H9), 5.37 (d, *J* = 8.6 Hz, H8), 6.29 (d, *J* = 12.5 Hz, H10), 6.89 (dd, *J* = 10.5, 1.6 Hz, H5), 7.18 (t, *J* = 6.8 Hz, H4'), 7.21-7.29 (m, H2' and H6', H3' and H5' (overlapping)), 7.30 (d, *J* = 1.6 Hz, H7), 7.37 (d, *J* = 10.5 Hz, H4); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 14.7 (C12), 23.4 (C14, C15), 38.4 (C13), 45.3 (C8), 65.1 (C11), 104.9 (C9), 120.8 (C7), 126.3 (C5), 126.4 (C4'), 128.1 (C3', C5'), 128.3 (C2', C6'), 138.5 (C4), 141.7 (C3), 143.1 (C1'), 148.8 (C10), 158.3 (C6), 168.2 (C1), 170.7 (C2).

[13] Relative integrals are not provided as the product is a mixture of two diastereomers.

Signals in the NMR spectra of the product that were assignable to ***cis*-3-(1-phenyl-3-ethoxy)prop-2-enyl-6-isopropyltropolone (84)** (*ca.* 28% of the product) were as follows: ^{1}H NMR (400 MHz, $CDCl_3$)[13] δ: 1.17 (t, *J* = 7.0 Hz, H12), 1.24 (t, *J* = 6.9 Hz, H14 and H15), 2.84 (p, *J* = 7.0 Hz, H13), 3.79 (q, *J* = 7.1 Hz, H11), 4.86 (dd, *J* = 9.3, 6.2 Hz, H9), 5.79 (d, *J* = 9.3 Hz, H8), 6.13 (d, *J* = 6.2 Hz, H10), 6.89 (d, H5), 7.18-7.30 (m, H7, H4', H2' and H6', H3' and H5' (overlapping)), 7.47 (d, *J* = 10.5 Hz, H4); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 15.2 (C12), 23.4 (C14, C15), 38.4 (C13), 42.6 (C8), 67.9 (C11), 106.8 (C9), 121.0 (C7), 126.0, 126.1 (C5, C4'), 127.7 (C2', C6'), 128.2 (C3', C5'), 138.4 (C4), 141.3 (C3), 143.6 (C1'), 146.0 (C10), 158.1 (C6), 168.6 (C1), 170.6 (C2).

Reaction of *trans*-2-hexenal diethyl acetal (79) with β-thujaplicin (47)

trans-2-Hexenal diethyl acetal (**79**) (0.210 g, 2.44 mmol) was heated with β-thujaplicin (**47**) (0.200 g, 1.22 mmol) at 160°C for 8 hrs under a N_2 atmosphere. Excess hexenal diethyl acetal was removed by distillation at 100°C under reduced pressure. The crude product was dissolved in $CHCl_3$ and loaded onto a column packed with silica gel. Elution with 15:1 and 15:2 hexane:EtOAc gave approximately 3:10 (0.178 g) and 5:4 (0.045 g) mixtures respectively of two products as yellow oils. The spectroscopic evidence suggested these two products were *cis*- and *trans*-3-(1-propyl-3-ethoxy)prop-2-enyl-6-isopropyltropolone (**86, 87**). The yield of the proposed product 3-(1-propyl-3-ethoxy)prop-2-enyl-6-isopropyltropolone (**86, 87**) was calculated at 59%. Microanalytical data were not obtained. MS (EI, GC/MS, TMSi derivatives): 2 peaks (Rt (min), GC peak areas, MS *m/z*, compound); 42.0, 23%, 362 ($M^{\cdot+}$, 50%), 347 (80%), 333 (100%), 303 (38%), 275 (54%), 73 (68%), *cis*-3-(1-propyl-3-ethoxy)prop-2-enyl-6-isopropyltropolone trimethyl silane; 43.1, 77%, 362 ($M^{\cdot+}$, 49%), 347 (68%), 333 (100%), 303 (28%), 275 (52%), 73 (67%), *trans*-3-(1-propyl-3-ethoxy)prop-2-enyl-6-isopropyltropolone trimethyl silane.

Signals in the NMR spectra of the product that were assignable to ***trans*-3-(1-propyl-3-ethoxy)prop-2-enyl-6-isopropyltropolone (87)** were as follows: ^{1}H NMR (400 MHz, $CDCl_3$)[14] δ: 0.88 (t, *J* = 7.3 Hz, H15), 1.21 (t, *J* = 6.9 Hz, H12), 1.24 (d, *J* = 6.9 Hz, H17, H18), 1.24-1.42 (overlapping with H17, H18, m, H14), 1.61 (overlapping with $H13_{cis}$, m, H13), 2.85 (overlapping with $H16_{cis}$, p, *J* = 6.9 Hz, H16), 3.70 (overlapping with $H11_{cis}$, q, *J* =

[14] Relative integrals are not provided as the product was a mixture of two diastereomers.

6.9 Hz, H11), 3.96 (q, J = 8-9 Hz, H8), 4.92 (dd, J = 12.5, 9.0 Hz, H9), 6.34 (d, J = 12.5 Hz, H10), 6.90 (dd, J = 10.8, 1.6 Hz, H5), 7.29 (d, J = 1.6 Hz, H7), 7.34 (d, J = 10.8 Hz, H4); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 14.0 (C15), 14.7 (C12), 20.7 (C14), 23.4 (C17, C18), 37.7 (C13), 38.4 (C16), 39.8 (C8), 65.0 (C11), 106.2 (C9), 121.7 (C7), 126.8 (C5), 137.1 (C4), 142.1 (C3), 147.5 (C10), 158.1 (C6), 169.3 (C1), 169.8 (C2).

Signals in the NMR spectra of the product that were assignable to ***cis*-3-(1-propyl-3-ethoxy)prop-2-enyl-6-isopropyltropolone (86)** were as follows: ^{1}H NMR (400 MHz, $CDCl_3$)[14] δ: 0.89 (t, J = 7.3 Hz, H15), 1.15 (t, J = 7.0 Hz, H12), 1.23 (d, J = 7.0 Hz, H17, H18), 1.24-1.42 (overlapping with H17, H18, m, H14), 1.61 (overlapping with H13$_{trans}$, m, H13), 2.84 (overlapping with H16$_{trans}$, p, J = 7.0 Hz, H16), 3.72 (overlapping with H11$_{trans}$, q, J = 7.0 Hz, H11), 4.36 (q, $J \approx 8$ Hz, H8), 4.62 (dd, J = 8.9, 6.2 Hz, H9), 5.97 (d, J = 6.2 Hz, H10), 6.87 (dd, J = 10.8, 1.6 Hz, H5), 7.27 (d, J = 1.6 Hz, H7), 7.40 (d, J = 10.8 Hz, H4); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 14.0 (C15), 15.1 (C12), 20.8 (C14), 23.4 (C17, C18), 37.5 (C13), 37.9 (C8), 38.4 (C16), 67.6 (C11), 108.5 (C9), 121.9 (C7), 126.6 (C5), 137.7 (C4), 141.8 (C3), 145.7 (C10), 157.9 (C6), 169.6 (C1, C2).

Reaction of *trans*-2-hexenal diethyl acetal (79) with tropolone (48)

trans-2-Hexenal diethyl acetal (**79**) (155 mg, 0.90 mmol) was heated with tropolone (**48**) (100 mg, 0.82 mmol) at 150-160°C for 5 hrs under a N_2 atmosphere. Unreacted 2-hexenal diethyl acetal was removed by vacuum distillation to yield a black oil, which was dissolved in CH_2Cl_2 and loaded onto a column packed with silica gel. Elution with 15:2 hexane:EtOAc gave an orange oil (28 mg, 14%). This product appeared to be a mixture of compounds the composition of which changed with time. Major signals in the ^{1}H NMR spectrum suggested 3-(1-ethoxy)hex-2-enyltropolone (**89**) was a possible major component of the initial product. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.93 (t, J = 7.0 Hz, H13), 1.18 (t, J = 6.9 Hz, H15), 1.36 (m, H12), 2.03 (m, H11), 3.64 (m, overlapping with H14, H8), 3.68 (m, H14), 3.96 (p, H14'), 5.41 (m, H10), 6.76-7.30 (overlapping m, H4-H7, H9). MS (DIP, EI) *m/z*: 248 ($M^{\cdot+}$, 29%), 219 (24%), 173 (100%), 159 (63%), 147 (36%), 131 (32%). Signals assignable to 3-(1-propyl-3-ethoxy)prop-2-enyltropolone (**90**, **91**) and 3-(1-formylmethyl)butyltropolone (**92**) (as detailed below) become more apparent in the ^{1}H and ^{13}C NMR spectra with time as the original product possibly (**89**) underwent rearrangement and hydrolysis. Ultimately these changes lead

to 3-(1-formylmethyl)butyltropolone (**92**) (see characterisation data below) as the sole product.

Note: Although the evidence of the proposed initial product (**89**) was limited, the results of this experiment were included to illustrate that the neat reaction of tropolone (**48**) with 2-hexenal diethyl acetal (**79**) gave a product mixture which underwent rearrangement. This result differed from the reaction of 2-hexenal diethyl acetal (**79**) with tropolone (**48**) in pyridine (see below).

Preparation of 3-(1-formylmethyl)butyltropolone (92)

trans-2-Hexenal diethyl acetal (**79**) (1.41 g, 8.2 mmol) was heated with tropolone (**48**) (0.50 g, 4.1 mmol) at 150-160°C for 5 hrs under a N_2 atmosphere. Excess hexenal diethyl acetal was removed by vacuum distillation to yield a black oil. The crude product was dissolved in acetone (16 ml), 5% aqueous HCl (5 ml) was added and the mixture was stirred for 15 minutes at room temperature. Acetone was removed by evaporation under reduced pressure and ether was added. The ether extract was washed with water, dried over $MgSO_4$ and the ether removed to give a black oil (0.891 g). The crude product was loaded onto a column of silica gel and eluted with a hexane:EtOAc gradient. Elution with 3:1 hexane:EtOAc gave **3-(1-formylmethyl)butyltropolone** (**92**) as an orange oil (0.302 g, 33%). IR (KBr, neat) cm^{-1}: 3183 (ν O-H), 1723 (ν C=O, CHO), 1615 (ν C=O, tropolone); ^1H NMR (400 MHz, $CDCl_3$) δ: 0.87 (3H, t, *J* = 7.3 Hz, H11), 1.22 (2H, m, H10), 1.70 (2H, m, H9), 2.78 (2H, m, H12), 3.94 (1H, p, *J* = 7.0 Hz, H8), 7.01 (1H, ddd, *J* = 10.1, 10.1, 1.5 Hz, H5), 7.29 (overlapping with H7, dd, *J* = 10.1, 10.1 Hz, H6), 7.33 (dd, *J* = 10.1, 1.5 Hz, H7), (H6, H7, 2H), 7.42 (1H, d, *J* = 10.1 Hz, H4), 9.78 (1H, s, H13); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 14.0 (C11), 20.6 (C10), 36.4 (C9), 37.1 (C8), 49.2 (C12), 121.1 (C7), 127.4 (C5), 136.4 (C6), 138.0 (C4), 142.2 (C3), 168.4 (C1), 173.8 (C2), 201.5 (C13); MS (EI, GC/MS) *m/z*: 220 ($M^{\cdot+}$, 3%), 191 (33%), 177 (17%), 163 (31%), 149 (100%), 136 (51%), 107 (31%); Elemental Analysis: found C, 70.7, H, 7.3; $C_{13}H_{16}O_3$ requires C, 70.9, H, 7.3.

Attempted reaction of 1-ethoxy-2-hexene (82) with tropolone (48)

1-Ethoxy-2-hexene (**82**) and tropolone (**48**) were heated under reflux (*ca.* 150°C) for 7 hrs under a N_2 atmosphere. On cooling tropolone crystallised. Analysis of the product by ^1H and

^{13}C NMR spectroscopy indicated the presence of no condensation product only unreacted 1-ethoxy-2-hexene (**82**) and tropolone (**48**).

Reaction of *trans*-cinnamaldehyde diethyl acetal (78) with tropolone (48) in pyridine

trans-Cinnamaldehyde diethyl acetal (**78**) (0.675 g, 3.28 mmol) and tropolone (**48**) (0.200 g, 1.64 mmol) were heated under reflux in anhydrous pyridine for 64 hrs. Pyridine was removed by evaporation under reduced pressure and ether was added. The ether extract was washed with 10% aqueous HCl and 10% aqueous NaCl, dried over $MgSO_4$ and filtered. The solvent was removed by evaporation under reduced pressure and excess cinnamaldehyde diethyl acetal (**78**) and cinnamaldehyde were removed by vacuum distillation (oil pump). The crude product was loaded onto a column packed with silica gel and eluted with a hexane:EtOAc gradient. Elution with 15:2 hexane:EtOAc gave a orange oil (129 mg) which consisted of mainly *trans*-3-(1-phenyl-3-ethoxy)prop-2-enyltropolone (**94**) (*ca.* 90%) based on spectroscopic evidence. Elution with 15:3 hexane:EtOAc gave a mixture of two products as an orange oil (110 mg). The spectroscopic evidence suggested these two products were *cis*- and *trans*-3-(1-phenyl-3-ethoxy)prop-2-enyltropolone (**93**, **94**). The calculated yield of 3-(1-phenyl-3-ethoxy)prop-2-enyltropolone was 49%. Microanalytical data were not obtained. MS (EI, GC/MS): 2 peaks (Rt (min), GC peak areas, MS *m/z*, compound); 53.8, 50%, 282 ($M^{\cdot+}$, 38%), 237 (21%), 223 (24%), 207 (32%), 181 (100%), 152 (30%), 3-(1-phenyl-3-ethoxy)prop-2-enyltropolone diastereomer; 54.4, 50%, 282 ($M^{\cdot+}$, 41%), 237 (24%), 207 (17%), 181 (100%), 153 (32%), 91 (21%), 3-(1-phenyl-3-ethoxy)prop-2-enyltropolone diastereomer.

Signals in the NMR spectra of the product that were assignable to ***trans*-3-(1-phenyl-3-ethoxy)prop-2-enyltropolone (94)** were as follows: ^1H NMR (400 MHz, $CDCl_3$)[15] δ: 1.26 (t, J = 7.0 Hz, H12), 3.78 (dq, J = 7.0, 2.6 Hz, H11), 5.19 (dd, J = 12.5, 8.6 Hz, H9), 5.40 (d, J = 8.6 Hz, H8), 6.29 (d, J = 12.5 Hz, H10), 6.99 (t, H5), 7.10-7.35 (m, H6, H7, H2'-H6' (overlapping)), 7.49 (d, J = 10.5 Hz, H4); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 14.8 (C12), 45.8 (C8), 65.2 (C11), 105.0 (C9), 120.6 (C7), 126.5 (C4'), 127.3 (C5), 128.2 (C3', C5'), 128.4 (C2', C6'), 136.0 (C6), 138.8 (C4), 143.0 (C3), 144.2 (C1'), 148.9 (C10), 168.3 (C1), 172.2 (C2).

[15] Relative integrals are not provided as the product is a mixture of two diastereomers.

Signals in the NMR spectra of the product that were assignable to ***cis*-3-(1-phenyl-3-ethoxy)prop-2-enyltropolone (93)** were as follows: ^{1}H NMR (400 MHz, $CDCl_3$)[15] δ: 1.17 (t, J = 7.0 Hz, H12), 3.78 (q, J = 7.0 Hz, H11), 4.87 (dd, J = 9.3, 6.2 Hz, H9), 5.82 (d, J = 9.3 Hz, H8), 6.13 (d, J = 6.2 Hz, H10), 6.99 (t, H5), 7.10-7.35 (m, H6, H7, H2'-H6' (overlapping)), 7.58 (d, J = 10.5 Hz, H4); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 15.2 (C12), 43.1 (C8), 68.0 (C11), 106.8 (C9), 120.7 (C7), 126.1 (C4'), 127.2 (C5), 127.8 (C2', C6'), 128.3 (C3', C5'), 135.9 (C6), 138.7 (C4), 143.5, 143.8 (C3, C1'), 146.1 (C10), 168.6 (C1), 172.2 (C2).

Reaction of *trans*-hexenal diethyl acetal (79) with tropolone (48) in pyridine

trans-Hexenal diethyl acetal (**79**) (0.311 g, 1.84 mmol) and tropolone (**48**) (0.200 g, 1.64 mmol) were heated under reflux in anhydrous pyridine for 64 hrs. Pyridine was removed by rotary evaporation and unreacted hexenal diethyl acetal by distillation under reduced pressure to give the crude product plus unreacted tropolone (**48**). This was loaded onto a silica gel column and eluted with a hexane:EtOAc gradient. Elution with 15:2 hexane:EtOAc gave two products as a orange oil (73 mg, 18%). The spectroscopic evidence suggested the two products were *cis*- and *trans*-3-(1-propyl-3-ethoxy)prop-2-enyltropolone (**90**, **91**). Micro-analytical data were not obtained. MS (EI, GC/MS): 2 peaks (Rt (min), GC peak areas, MS *m/z*, compound); 44.1, 59%, 248 ($M^{\cdot+}$, 27%), 219 (22%), 173 (100%), 159 (53%), 147 (32%), 131 (32%), *trans*-3-(1-propyl-3-ethoxy)prop-2-enyltropolone (**91**); 46.8, 37%, 248 ($M^{\cdot+}$, 26%), 219 (25%), 173 (100%), 159 (45%), 133 (30%), 91 (54%), *cis*-3-(1-propyl-3-ethoxy)prop-2-enyltropolone (**90**).

Signals in the NMR spectra of the product that were assignable to ***trans*-3-(1-propyl-3-ethoxy)prop-2-enyltropolone (91)** were as follows: ^{1}H NMR (400 MHz, $CDCl_3$)[16] δ: 0.88 (t, J = 7.3 Hz, H15), 1.23 (t, J = 7.0 Hz, H12), 1.24-1.42 (m, H14), 1.61 (m, H13), 3.70 (q, J = 6.9 Hz, H11), 4.00 (q, $J \approx$ 8-9 Hz, H8), 4.90 (dd, J = 12.5, 9.0 Hz, H9), 6.36 (d, J = 12.5 Hz, H10), 6.95-7.06 (m, H5), 7.20-7.34 (m, H6, H7), 7.44 (d, J = 10.2 Hz, H4), 9.75 (broad s, OH); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 13.9 (C15), 14.7 (C12), 20.6 (C14), 37.6 (C13), 40.0 (C8), 64.9 (C11), 106.0 (C9), 121.4 (C7), 127.5 (C5), 135.8 (C6), 137.3 (C4), 144.6 (C3), 147.5 (C10), 169.0 (C1), 171.4 (C2).

[16] Relative integrals are not provided as the product was a mixture of two diastereomers.

Signals in the NMR spectra of the product that were assignable to ***cis*-3-(1-propyl-3-ethoxy)prop-2-enyltropolone (90)** were as follows: ^{1}H NMR (400 MHz, $CDCl_3$)[16] δ: 0.88 (t, J = 7.3 Hz, H15), 1.13 (t, J = 7.0 Hz, H12), 1.24-1.42 (m, H14), 1.61 (m, H13), 3.72 (q, J = 7.0 Hz, H11), 4.40 (q, $J \approx 8$ Hz, H8), 4.62 (dd, J = 8.9, 6.2 Hz, H9), 5.98 (d, J = 6.2 Hz, H10), 6.95-7.06 (m, H5), 7.20-7.34 (m, H6, H7), 7.50 (d, J = 10.3 Hz, H4), 9.75 (broad s, OH); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 13.9 (C15), 15.1 (C12), 20.8 (C14), 37.5 (C13), 38.1 (C8), 67.6 (C11), 108.3 (C9), 121.6 (C7), 127.2 (C5), 135.7 (C6), 138.0 (C4), 144.4 (C3), 145.7 (C10), 169.2 (C1), 171.3 (C2).

7.2.12 Synthesis of Derivatives of 3-(1-Formylmethyl)butyltropolone (92)

Preparation of 3-(1-carboxymethyl)butyltropolone (95)

$AgNO_3$ (1.0 g, 5.9 mmol) dissolved in water (6 ml) was treated with a solution of NaOH (0.26 g, 6.47 mmol) in water (3 ml). The mixture was stirred for 5 minutes and the precipitated Ag_2O was collected by filtration, washed with water and ethanol and dried. The Ag_2O (0.300 g, 1.29 mmol) was transferred to a reaction flask and covered with 10% aqueous NaOH solution (3 ml). 3-(1-Formylmethyl)butyltropolone (**92**) (0.252 g, 1.14 mmol) dissolved in ethanol was added dropwise to the reaction mixture which was preheated to 50°C. The reaction mixture was stirred for 3.5 hrs at 60°C. Metallic silver was removed by filtration and washed with ethanol and hot water. The filtrate and washings were combined and the mixture was acidified to pH *ca.* 1.5 with concentrated HCl. Ethanol was removed by evaporation under reduced pressure and the product extracted into ether. The two phases were separated and the ether layer washed with water, dried over $MgSO_4$ and the solvent removed to give **3-(1-carboxymethyl)butyltropolone (95)** as a yellow oil, (0.189 g, 71%). IR (NaCl, neat) cm^{-1}: 3200-3600, broad and 2500-2700, broad (ν O-H, COOH), 3171 (ν O-H tropolone), 1712 (ν C=O, COOH), 1614 (ν C=O, tropolone); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.85 (3H, t, J = 7.3 Hz, H11), 1.20 (2H, m, H10), 1.70 (2H, m, H9), 2.68 (1H, dd, J =16.0, 6.8 Hz, H12), 2.76 (1H, dd, J = 16.0, 7.6 Hz, H12'), 3.77 (1H, p, $J \approx 7.3$ Hz, H8), 7.02 (1H, ddd, J = 10.2, 9.4, 1.6 Hz, H5), 7.30 (overlapping with H7, dd, J = 10.2, 9.4 Hz, H6), 7.35 (dd, J = 10.2, 1.6 Hz, H7), (H6, H7, 2H), 7.44 (1H, d, J = 10.2 Hz, H4); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 13.9 (C11), 20.4 (C10), 36.0 (C9), 38.7 (C8), 39.3 (C12), 120.0 (C7), 127.6 (C5), 136.6 (C6), 138.0 (C4), 141.7 (C3), 169.1 (C1), 171.7 (C2), 177.4 (C13); MS (EI, GC/MS, TMSi derivative) *m/z*: 380

($M^{\cdot+}$, 0.5%), 365 (100%), 247 (6%), 233 (6%), 205 (28%), 73 (23%); High resolution MS (EI, DIP): found 236.1045 Da; $C_{13}H_{16}O_4$ requires 236.1049 Da.

Preparation of 3-(1-hydroxyethyl)butyltropolone (96)

To 3-(1-formylmethyl)butyltropolone (**92**) (0.250 g, 1.14 mmol) in 95% ethanol (3 ml) was added dropwise $NaBH_4$ (17 mg, 0.450 mmol) in water. The mixture was warmed to 45°C and stirred for 1.5 hrs. Ethanol was removed by evaporation under reduced pressure. Ether and 20% NaCl solution were added. The ether layer was separated, combined with ether washings of the NaCl layer, washed with water and dried over $MgSO_4$. The ether was removed to give **3-(1-hydroxyethyl)butyltropolone (96)** as a yellow oil (0.197 g, 78%). IR (NaCl, neat) cm^{-1}: 3100-3600, broad (ν O-H, alcohol and tropolone), 2957 and 2871 (ν C-H), 1616, (ν C=O, tropolone); ^1H NMR (400 MHz, $CDCl_3$) δ: 0.84 (3H, t, J = 7.3 Hz, H11), 1.19 (2H, m, H10), 1.56 (1H, m, H12), 1.66 (2H, q, $J \approx$ 7.6 Hz, H9), 2.08 (1H, m, H12'), 3.24 (1H, dt, J = 10.8, 4.1 Hz, H13), 3.51 (1H, td, J = 10.8, 5.0 Hz, H13'), 3.76 (1H, m, H8), 7.08 (1H, dd, J = 10.1, 9.8 Hz, H5), 7.31 (overlapping with H7, dd, $J \approx$ 10, 10 Hz, H6), 7.38 (d, J = 10.1 Hz, H7), (H6, H7, 2H), 7.46 (1H, d, J = 10.1 Hz, H4); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 13.8 (C11), 20.4 (C10), 36.4 (C12), 37.5 (C9), 39.3 (C8), 60.0 (C13), 121.3 (C7), 127.8 (C5), 136.0 (C6), 137.3 (C4), 144.1 (C3), 167.9 (C1), 173.1 (C2); MS (EI, GC/MS, TMSi derivative) *m/z*: 366 ($M^{\cdot+}$, 2%), 351 (100%), 250 (25%), 205 (41%), 179 (20%), 73 (35%); High resolution MS (EI, DIP): found 222.1257 Da; $C_{13}H_{18}O_3$ requires 222.1256 Da.

Preparation of 3-(1-ethyl)butyltropolone (97)

3-(1-Formylmethyl)butyltropolone (**92**) (0.50 g, 2.27 mmol), hydrazine hydrate (0.33 ml, 6.8 mmol), KOH (0.38 g, 6.8 mmol) and triethylene glycol (8 ml) were heated under reflux (150°C) for 2 hrs. Water and hydrazine hydrate were distilled until the reaction temperature reached *ca.* 200°C. The reaction mixture was heated under reflux for a further 4 hrs. The mixture was cooled, water was added and the pH was lowered to *ca.* 1.5. The aqueous phase was extracted with ether and the ether layer was washed with 5% $NaHCO_3$ solution, water, dried over $MgSO_4$ and the solvent removed to give crude 3-(1-ethyl)butyltropolone (**97**) as a black oil (0.19 g, 41%). The crude product was loaded onto a column of silica gel and eluted with a hexane:EtOAc gradient. Elution with 15:2 and 15:3 hexane:EtOAc gave **3-(1-ethyl)butyltropolone (97)** (0.071 g, 15%) as an orange oil. IR (NaCl, neat) cm^{-1}: 3182, broad,

(ν O-H, tropolone), 2960, 2930, 2872 (ν C-H), 1618 (ν C=O, tropolone); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.78 (t, *J* = 7.4 Hz, H13), 0.84 (t, *J* = 7.4 Hz, H11), (H13, H11 6H), 1.18 (2H, m, H10), 1.50-1.76 (4H, m, H9, H12), 3.48 (1H, m, H8), 7.01 (1H, t, *J* = 10.5 Hz, H5), 7.27 (1H, dt, *J* = 10.5 Hz, H6), 7.34 (overlapping with H4, dd, *J* = 10.5, 1.2 Hz, H7), 7.36 (d, *J* = 10.0 Hz, H4), (H7, H4, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 11.8 (C13), 14.2 (C11), 20.5 (C10), 28.3 (C12), 37.4 (C9), 42.0 (C8), 123.0 (C7), 127.4 (C5), 136.1 (C6), 137.0 (C4), 143.0 (C3), 170.1 (C1), 171.3 (C2); MS (EI, GC/MS) *m/z*: 206 ($M^{\cdot+}$, 18%), 177 (100%), 163 (46%), 149 (26%), 107 (40%), 91 (29%); MS (EI, GC/MS, TMSi derivative) *m/z*: 278 ($M^{\cdot+}$, 1%), 263 (100%), 249 (4%), 233 (7%), 219 (5%), 205 (24%); High resolution MS (EI, DIP): found 206.1301 Da; $C_{13}H_{18}O_2$ requires 206.1307 Da.

Preparation of the Cu(tropolone)$_2$ (101)

To tropolone (**48**) (1 g, 0.0082 mol) in water was added $Cu(CH_3COO)_2.H_2O$ (0.82 g, 0.0041 mol) in water. Cu(tropolone)$_2$ (**101**) precipitated and was collected by filteration and dried.

7.3 Antifungal Activity Assay Methods

7.3.1 Decay Fungi Assays

7.3.1.1 Brown Rot Fungus, *Coniophora puteana*

Preparation of the fungal inoculates was carried out under aseptic conditions in a laminar flow chamber. Malt agar medium was prepared from malt extract agar (10 g) and bacteriological agar (6 g) in 500 ml of deionised water. Following steam sterilisation and cooling the liquid agar was poured into sterile Petri dishes and allowed to solidify. Hyphae of *Coniophora puteana* were transferred to the agar media aseptically from laboratory cultures (provided courtesy of the Wood Mycology Group at Forest Research). The fungus was incubated at 25°C and 85% relative humidity for *ca.* 1 week. Sterilised antibiotic assay (AA) discs moistened with a mineral salt solution (see below) were transferred to fungal hyphae growing aerially above the surface of the agar. The plates were incubated at 25°C and 85% relative humidity for *ca.* 1 week.

Whatman 1 (9 cm) filter papers were dyed with Bayer violet direct dye and air dried. Dying of the papers enables the white fungal hyphae to be visualised. Compounds for testing were dissolved in CH_2Cl_2 or MeOH to give a range of treatment concentrations. Labelled filter papers were individually immersed in the test solutions until saturated and then allowed to air dry. Three replicates were prepared for each compound at each test concentration. Treatment loadings were calculated based on the concentration of the treatment solution and the average mass gain on treatment prior to drying. The average gain in mass was calculated from the mass of ten duplicate samples (for each solvent) before and after treatment with solvent blanks. Papers immersed in water, CH_2Cl_2 and MeOH and allowed to dry were used as controls. The treated filter papers were placed in heat sealed sterilisation tubing and sterilised with ethylene oxide gas for 1 day. Experiments were undertaken to confirm the ethylene oxide did not react with the compounds under investigation.

The sterilised filter papers were placed in labelled Petri dishes (8.5 cm diameter) and AA discs, inoculated with the fungus, were placed in the centre of the papers, which were moistened with 1 ml of the mineral salt solution. The samples were then incubated under the above conditions for 2 weeks. Periodically, mineral salt solution was added to the papers to ensure they remained moist. After two weeks of incubation the extent of the growth of the fungus was calculated from colony diameter measurements at three intervals spaced at 120°. The average radial growth of the fungus was calculated from these measurements. The mineral salt solution mentioned above contained: NH_4NO_3 (1.5 g/l), K_2HPO_4 (1.0 g/l), KH_2PO_4 (1.25g/l), $MgSO_4.7H_2O$ (1.0 g/l), thiamine pyrophosphate (10 μg/l).

7.3.1.2 White Rot Fungus, *Trametes versicolor*

The assay method for *Trametes versicolor* was identical to that used for *Coniophora puteana* with the exception that initially thermomechanical pulp (TMP) handsheets and later commercial newsprint paper were used as a test medium in place of filter paper. Mechanical pulp was obtained courtesy of Kathryn Anderson and Clinton Handcock of Forest Research. Handsheets were prepared using the standard Forest Research method (342). Commercial newsprint was obtained courtesy of Garth Weinberg of Forest Research. Samples of 8 cm diameter were cut from both paper sources and dyed as above.

7.3.1.3 Soft Rot Fungus, *Chatemonium globosum*

The assay method for *Chatemonium globosum* was identical to that used for *Coniophora puteana* with the exception that commercial newsprint paper was used as a test medium in place of filter paper.

7.3.2 *Sphaeropsis sapinea* Assay

7.3.2.1 Method Development

Preliminary trials with three staining fungi were undertaken to establish which of the following organisms would be most suitable for the above assay method. The test fungi included *Sphaeropsis sapinea, Ophiostoma piliferum* and *Alternaria alternata*. Staining fungi do not degrade wood components, hence it was necessary to add a readily assimilable carbon source to the mineral solution, which was used to support fungal growth. D-(+)-glucose was used for this purpose. Free sugars in unseasoned *Pinus radiata* have been estimated at *ca.* 6 g kg^{-1} (343). To achieve a similar concentration in the above filter paper samples a concentration of *ca.* 3.6 g l^{-1} of glucose was required in the mineral salt solution.

Onto steam sterilised filter paper and TMP papers, moistened with the mineral salt solution containing 3 g l^{-1} and 6 g l^{-1} glucose, was placed AA discs inoculated with *Sphaeropsis sapinea, Ophiostoma piliferum* and *Alternaria alternata*. The samples were incubated under the above conditions and growth rates were monitored. *S. sapinea* was the fastest growing, it showed consistent growth on filter paper and TMP handsheet samples treated with 6 g l^{-1} glucose. *O. piliferum* showed a moderate growth rate on TMP handsheets treated with 6 g l^{-1} glucose but irregular growth on the filter paper samples. *A. alternaria* was extremely slow growing relative to *S. sapinea*. Consequently *S. sapinea* was selected as the preferred staining fungus for the bioassays. *S. sapinea* was subsequently shown to grow well on newsprint paper moistened with the mineral salt solution containing 10 g l^{-1} glucose.

7.3.2.2 Antifungal Activity Assay

The antifungal assay was essentially the same as that used for *Coniophora puteana*, but with the following exceptions. First, commercial newsprint papers were used in place of filter

papers. Second, the mineral salt solution, described above, was supplemented with 10 g l^{-1} D-(+)-glucose. Third, *S. sapinea* grown on agar exhibited little in the way of aerial growth hence an alternative means of inoculating the AA disc was devised. Sterile AA were placed on newsprint papers moistened with the mineral salt/glucose solution and the newsprint papers were inoculated with an agar plug from a culture of *S. sapinea*. After 1 week incubation the fungal hyphae growing on the newsprint papers had inoculated the AA discs. These discs were then transferred to the treated test papers for antifungal testing as described above.

7.3.3 Determining Fungicidal or Fungistatic properties

When zero growth of the test fungus on a treated paper was observed the nature of the antifungal property was assessed by aseptic transfer of fibres from the base of the inoculated AA disc to freshly prepared malt agar plates. The plates were incubated for 2 weeks under the aforementioned conditions. Growth of fungal hyphae signified that the antifungal effect was of a fungistatic nature, no fungal growth indicated that the test compound was fungicidal.

If I have seen further, it is by standing on the shoulders of giants

Sir Isaac Newton

References

1 Hedley, M., 2001, Manufacturing Technologies, Forest Research, Rotorua, New Zealand, pers. commun.

2 Fengel, D. and Wegener, G., 1989, *Wood: Chemistry, Ultrastructure and Reactions*, Walter de Gruyter, Berlin, Germany, pp 26-181.

3 Sjostrom, E., 1981, *Wood Chemistry: Fundamentals and Applications*, Academic Press Inc., Orlando, USA, pp 49-87.

4 Hon, D. N. S. and Shiraishi, N., 1991, *Wood and Cellulosic Chemistry*, Marcel Dekker, New York, USA, pp 59-255.

5 Goring, D. A. I. and Timell, T., 1962, *Tappi*, **45**, 454-460.

6 Kirk, T. K., 1973, *The Chemistry and Biochemistry of Decay*, in "Wood Deterioration and its Prevention by Preservative Treatments", Vol. I, D. D. Nicholas (Ed.), Syracuse University Press, New York, USA, pp 149-181.

7 Scheffer, T. C., 1973, *Microbiological Degradation and the Casual Organisms*, in "Wood Deterioration and its Prevention by Preservative Treatments", Vol. I, D. D. Nicholas (Ed.), Syracuse University Press, New York, USA, pp 31-106.

8 Eaton, R. A. and Hale, M. D. C., 1993, *Wood: Decay, Pests and Protection*, 1st Edition, Chapman and Hall, London, UK, pp 76-110.

9 Wilcox, W. W., 1973, *Degradation in Relation to Wood Structure*, in "Wood Deterioration and its Prevention by Preservative Treatments", Vol. I, D.D. Nicholas (Ed.), Syracuse University Press, New York, USA, pp 107-148.

10 Eaton, R. A. and Hale, M. D. C., 1993, *Wood: Decay, Pests and Protection*, 1st Edition, Chapman and Hall, London, UK, pp 161-186.

11 Leise, W., 1970, *Ann. Rev. of Phytopath.*, **8**, 231-258.

12 Wilkinson, J. G., 1979, *Industrial Timber Preservation*, Associated Business Press, London, UK, pp 75-124.

13 Savory, J. G., 1954, *Annals of Applied Biology*, **41**, 336-347.

14 Corbett, N. H., 1965, *J. Inst. Wood Sci.*, **14**, 18-29.

15 Breuil, C., 1997, *Wood as a Nutritional Resource for Staining Fungi*, in "Biology and Prevention of Sapstain", Proceedings of Conference, Whistler, Canada, 25 May, Forest Products Society, pp 1-5.

16 Eaton, R. A. and Hale, M. D. C., 1993, *Wood: Decay, Pests and Protection*, 1st Edition, Chapman and Hall, London, UK, pp 130-159.

17 Morrell, J. and Xiao, Y., 1999, *Stain Research: Where are we going and how can we use where we've been to better purpose*, in "The 2nd New Zealand Sapstain Symposium", B. Kerber (Ed.), Proceedings of Symposium, Rotorua, New Zealand, 18-19 November, Forest Research Bulletin No. 215, pp 71-78.

18 Farrall, R. L., Hadar, E., Kay, S. J., Blanchette, R. A. and Harrington, T. C., 1997, *Survey of Sapstain Organisms in New Zealand and Albino Antisapstain Fungi*, in "Biology and Prevention of Sapstain", Proceedings of Conference, Whistler, Canada, 25 May, Forest Products Society, pp 57-62.

19 Pommer, E.-H., 1995, *Fungicides in Wood Preservation*, in "Modern Selective Fungicides - Properties, Applications, Mechanisms of Action", H. Lyr (Ed.), 2nd Edition, Gustav Fischer Verlag, New York, USA, pp 485-502.

20 Findlay, W. P. K. and Pettifor, C. B., 1937, *Forestry*, **11**, 40-52.

21 Eaton, R. A. and Hale, M. D. C., 1993, *Wood: Decay, Pests and Protection*, 1st Edition, Chapman and Hall, London, UK, pp 186-193.

22 Cowling, E. B. and Brown, W., 1969, *Structural features of cellulosic materials in relation to enzymatic hydrolysis*, in "Cellulases and their Applications", G. J. Hajny and E. T. Reese (Eds.), Advances in Chemistry Series 95, Amercian Chemical Society, Washington DC, USA, pp 157-187.

23 Reese, E. T., Siu, R. G. H. and Levinson, H. S., 1950, *J. Bacteriol.*, **59**, 485-497.

24 Shimada, M. and Takahashi, M., 1991, *Biodegradation of Cellulosic Materials*, in "Wood and Cellulosic Chemistry", D. N.-S. Hon and N. Shiraishi (Eds.), Marcel Dekker, New York, USA, pp 557-619.

25 Eriksson, K.-E. L., Blanchette, R. A. and Ander, P., 1990, *Microbial and Enzymatic Degradation of Wood and Wood Components*, Springer-Verlag, Berlin, Germany, pp 89-319.

26 Eriksson, K.-E. and Wood, T. M., 1985, *Biodegradation of Cellulose*, in "Biosynthesis and Biodegradation of Wood Components", T. Higushi (Ed.), Academic Press, Tokyo, Japan, pp 469-503.

27 Ayers, A. R., Ayers, S. B. and Eriksson, K.-E., 1978, *Eur. J. Biochem.*, **90**, 171-181.

28 Vaheri, M. P., 1982, *J. Appl. Biochem.*, **4**, 153-160.

29 Westermark, U. and Eriksson, K.-E., *Acta. Chem. Scand.*, 1974, **B28**, 209-214.

30 Dekker, R. F. H., 1985, *Biodegradation of the Hemicelluloses*, in "Biosynthesis and Biodegradation of Wood Components", T. Higuchi (Ed.), Academic Press, Tokyo, Japan, pp 505-533.

31 Fengel, D. and Wegener, G., 1989, *Wood: Chemistry, Ultrastructure and Reactions*, Walter de Gruyter, Berlin, Germany, pp 373-406.

32 Chen, C.-L. and Chang H. M., 1985, *Chemistry of Lignin Biodegradation*, in "Biosynthesis and Biodegradation of Wood Components", T. Higuchi (Ed.), Academic Press, Tokyo, Japan, pp 535-556.

33 Glenn, J. K., Morgan, M. A., Mayfield, M. B., Kuwahara, M. and Gold, M., 1983, *Biochem. Biophys. Res. Commun.*, **114**, 1077-1083.

34 Tien, M. and Kirk, T. K., 1983, *Science*, **221**, 661-663.

35 Shimada, M. and Higuchi, T., 1991, *Microbial, enzymatic and biometric degradation of lignin*, in "Wood and Cellulosic Chemistry", D. N.-S. Hon and N. Shiraishi (Eds.), Marcel Dekker, New York, USA, pp 557-619.

36 Koenigs, J. W., 1974, *Wood and Fiber*, **6**, 66-80.

37 Schmidt, C. J., Whittenden, B. K. and Nicholas, D. D., 1981, *Proc. Am. Wood Pres. Assoc.*, **77**, 157-164.

38 Hyde S. M. and Wood P. M., 1995, *Int. Res. Group on Wood Pres.*, Doc No. IRG/WP/95-10104.

39 Highley, T. L., 1982, *Can. J. For. Res.*, **12**, 435-438.

40 Levi, M. P. and Preston, R. D., 1965, *Holzforschung*, **19**, 183-190.

41 Butcher, J. A. and Nilsson, T., 1982, *Int. Res. Group on Wood Pres.*, Doc No. IRG/WP/1151.

42 Eaton, R. A. and Hale, M. D. C., 1993, *Wood: Decay, Pests and Protection*, 1st Edition, Chapman and Hall, London, UK, pp 231-307.

43 *Timber Preservation*, 1949, 4th Edition, The Timber Development Association, London, UK, pp 7.

44 Wilkinson, J. G., 1979, *Industrial Timber Preservation*, Associated Business Press, London, UK, pp 381-388.

45 Graham, R. D., 1973, *History of Wood Preservation*, in "Wood Deterioration and its Preventation by Preservative Treatments, Vol. I, Degradation and Protection of Wood", D. D. Nicholas (Ed.), Syracuse University Press, New York, USA, pp 1-31.

46 Eaton, R. A. and Hale, M. D. C., 1993, *Wood: Decay, Pests and Protection*, 1st Edition, Chapman and Hall, London, UK, pp 319-329.

47 Connell, M., 1990, *Industrial Wood Preservatives - The History, Development, Use, Advantages and Future Trends*, in "The Chemistry of Wood Preservation", R. Thompson (Ed.), The Royal Society of Chemistry, Cambridge, UK, pp 17-33.

48 Pizza, A., 1982, *J. Polymer Sci. Chem. Ed.*, **20**, 739-764.

49 Eaton, R. A. and Hale, M. D. C., 1993, *Wood: Decay, Pests and Protection*, 1st Edition, Chapman and Hall, London, UK, pp 344-372.

50 Tillott, R. J. and Coggins, C. R., 1981, *Rec. Brit. Wood Pres. Assoc. Ann. Conv.*, 32-46.

51 Clifton, N. C., 1991, *New Zealand Timbers, the complete guide to exotic and indigenous woods*, GP Publications Ltd, Wellington, New Zealand, pp 31-34.

52 Vinden, P. and Butcher J. A., 1991, *Wood Preservation: Strategies for the Future* in "The Chemistry of Wood Preservation", R. Thompson (Ed.), The Royal Society of Chemistry, Cambridge, UK, pp 117-135.

53 Kroese, H. W., Dawson B. S. W. and Franich R. A., 2001, *Holz als Roh- und Werkstoff*, **59**, 71-72.

54 Schweinfurth, H. and Ventur, D., 1991, *TBTO and TBTN - Safe and Effective Biocides for Wood Preservation*, in "The Chemistry of Wood Preservation", R. Thompson (Ed.), The Royal Society Of Chemistry, Cambridge, UK, pp 192-217.

55 Henshaw, B. G., Laidlaw, R. A., Orsler, R. J., Carey, J. K. and Savory, J. G., 1978, *Rec. Brit. Wood Pres. Assoc. Ann. Conv.*, 19-29.

56 Hilditch, E. A., 1991, *Organic Solvent Preservatives: Applications and Composition*, in "The Chemistry of Wood Preservation", R. Thompson (Ed.), The Royal Society of Chemistry, Cambridge, UK, pp 68-87.

57 Robinson, W. H., 1998, *Rec. Res. Dev. in Entomol.*, **2**, 9-17.

58 Valcke, A. R. and Goodwine, W. R., 1985, *Proc. Am. Wood Pres. Assoc.*, **81**, 196-202.

59 Valcke, A. R., 1989, *Int. Res. Group on Wood Pres.*, Doc. No. IRG/WP/3529.

60 Exner, O., 1991, *Int. Res. Group on Wood Pres.*, Doc. No. IRG/WP/3680.

61 Preston, A. F., Nicholas, D. D., Greenley, D. E., Walcheski, P. J. and McKraig, P. A., 1984, *6th Int. Biodeteriorat. Symp.*, Washington, USA, August, pp 100-107.

62 Hansen, J., 1984, *Int. Res. Group on Wood Pres.*, Doc. No. IRG/WP/3295.

63 Richardson, B. A., 1978, *Wood Preservation*, The Construction Press, Lancaster, UK pp 167-168.

64 Laks, P. E. and Woods, T. L., 1992, *Int. Res. Group on Wood Pres.*, Doc. No. IRG/WP/3712-92.

65 Wakeling, R. N., 1988, *The Control of Mould and Sapstain on Freshly Felled Timber*, M.Sc Literature Review, Portsmouth Polytechnic, UK, 120 p.

66 Freeman, M., 1991, *PQ-8*, in "Lumber Protection in the 90's", T. Byrne (Ed.), Proceeding of a meeting at Forintek Canada Corp., Vancouver, Canada, 31 January, pp 32-36.

67 Smith, R. S., 1991, *Lumber Protection Today*, in "Lumber Protection in the 90's", T. Byrne (Ed.), Proceedings of a meeting at Forintek Canada Corp., Vancouver, Canada, 31 January, pp 3-13.

68 Byrne, T., 1991, "Lumber Protection in the 90's", Proceedings of a meeting at Forintek Canada Corp., Vancouver, Canada, 31 January, pp 25-30, 37-45.

69 Williams, G. R. and Lewis D. A., 1991, *Performance of Alternative Antisapstain Compounds*, in "The Chemistry of Wood Preservation", R. Thompson (Ed.), The Royal Society of Chemistry, Cambridge, UK, pp 275-281.

70 Briscoe, P. A., Williams, G. R., Anderson, D. G. and Gadd, G. M., 1990, *Int. Res. Group on Wood Pres.*, Doc. No. IRG/WP/1464.

71 Suttie, E. D., 1997, *Chem. & Ind.*, 720-724.

72 Rowell, R. M., 1991, *Chemical Modification of Wood*, in "Wood and Cellulosic Chemistry", D. N.-S. Hon and N. Shiraishi (Eds.), Marcel Dekker Inc., New York, USA, pp 703-756.

73 Rowell, R. M., 1993, *Protection of wood against biodegradation by chemical modification*, in "Cellulosics: pulp, fibre and environmental aspects", J. F. Kennedy, G. O. Phillips and P. A. Williams (Eds.), Ellis Horwood Ltd, New York, USA, pp 473-478.

74 Eaton, R. A. and Hale, M. D. C., 1993, *Wood: Decay, Pests and Protection*, 1st Edition, Chapman and Hall, London, UK, pp 339-340.

75 Bravery, A. F., 1996, Timber Division, Building Research Establishment, Watford, UK, pers. commun.

76 Pittman, Jr., C. U., Kim, M. G., Nicholas, D. D, Wang, L., Ahmer Kabir, F. R., Schultz, T. P. and Ingram, Jr., L. L., 1994, *J. of Wood Chem. & Tech.*, **14**, 142-151.

77 Schneider, M. H., 1994, *Wood & Fiber Sci.*, **26**, 142-151.

78 Franich, R. A., 2002, Manufacturing Technologies, Forest Research, Rotorua, New Zealand, pers. commun.

79 Schultz, T. P., Fisher, T. H. and Nicholas, D. D., 1994, *Quantitive structure-activity relationships of stilbenols against wood-inhabiting fungi*, Proceedings of the 4th USDA Southern Station Chemical Science Meeting, USA, pp 58-68.

80 Suttie, E. D., Orsler, R. J. and Wood, P. M., 1996, *Int. Res. Group on Wood Pres.*, Doc. No. 96-10185.

81 Kersten, P. J., 1994, *Biochemical Approaches to Wood Preservation*, in "Wood Preservation in the 90's and Beyond", Proceeding from Conference, September 26-28, Georgia, USA, pp 153-157.

82 Wolkow, P. M., Sisler, H. D. and Vigil, E. L., 1983, *Phys. Plant Path.*, **23**, 55-71.

83 Baker, K. F., 1987, *Ann. Rev. Phytopathol.*, **25**, 67-85.

84 Morrell, J., 1999, *Global Perspective on Future Opportunities for Alternatives to Conventional Antisapstain Treatment*, in "The 2nd New Zealand Sapstain Symposium", B. Kerber (Ed.), Proceedings of Symposium, Rotorua, New Zealand, 18-19 November, Forest Research Bulletin No. 215, pp 7-11.

85 Fravel, D. R., 1988, *Ann. Rev. Phytopathol.*, **26**, 75-91.

86 Ricard, J. L., 1967, *Can. J. of Bot.*, **46**, 643-647.

87 Bruce, A. and King, B., 1983, *Mat. und Org.*, **18**, 171-181.

88 Bernier, R., Desrochers, M. and Jurasek, L., 1986, *J. Inst. Wood Sci.*, **10**, 214-216.

89 Behrendt, C. J., Blanchette, R. A. and Farrell, R. L., 1995, *Phytopathol.*, **85**, 92-96.

90 Uzunovic, A., Domanski, D. and Williams, D. P., 1999, *Biological Control of Bluestain in logs using Albino Bluestain fungi - Canadian Experience*, in "The 2nd New Zealand Sapstain Symposium", B. Kerber (Ed.), Proceedings of Symposium, Rotorua, New Zealand, 18-19 November, Forest Research Bulletin No. 215, pp 93-98.

91 Yang, D.-Q., 1999, *Biological and Integrated Control of Sapstain on Canadian Softwoods by using Gliocladium roseum*, in "The 2nd New Zealand Sapstain Symposium", B. Kerber (Ed.), Proceedings of Symposium, Rotorua, New Zealand, 18-19 November, Forest Research Bulletin No. 215, pp 87-92.

92 Jin, L. and Laks, P. E., 1994, *The Use of Natural Plant Products for Wood Protection*, in "Wood Preservation in the 90's and Beyond", Proceeding from Conference, September, Georgia, USA, 26-28, pp 153-157.

93 Laks, P. E., 1991, *Scottish Forestry*, **45**, 275-284.

94 Hart, J. H. and Hillis, W. E., 1974, *Phytopathol.*, **64**, 939-948.

95 Bultman, J. D. and Parrish, K. K., 1979, *Int. Biodeterior. Bull.*, **15**, 19-27.

96 Eslyn, W. E., Bultman, J. D. and Jurd, L., 1981, *Phytopathol.*, **71**, 521-524.

97 Kamdem, D. P., 1994, *For. Prod. J.*, **44**, 30-32.

98 Haluk, J.-P. and Roussel, C., 2000, *Ann. For. Sci.*, **57**, 819-829.

99 Nakayama, F. S., Chow, P., Bajwa, D. S., Youngquist, J. A., Muehl, J. H. and Krzysik, A. M., 2000, *Int. Res. Group on Wood Pres.*, Doc. No. IRG/WP 00-40154.

100 Eaton, R. A. and Hale, M. D. C., 1993, *Wood: Decay, Pests and Protection*, 1st Edition, Chapman and Hall, London, UK, pp 311-318.

101 Rao, P. S., 1982, *J. Ind. Acad. Wood Sci.*, **13**, 3-19.

102 Hillis, W. E., 1987, *Heartwood and Tree Exudates*, Springer-Verlag, Berlin, Germany, pp 180-208.

103 Hawley, L. F., Fleck, L. C. and Richards, C. A., 1924, *Ind. Eng. Chem.*, **16**, 699-706.

104 Da Costa, E. W. B. and Rudman, P., 1957, *Austr. J. Biol. Sci.*, **11**, 45-57.

105 Anderson, A. B., 1962, *Tappi*, **45**, 40A-62A.

106 Rudman, P., 1963, *Holzforschung*, **17**, 54-57.

107 Rudman, P., 1965, *Holzforschung*, **19**, 52-57.

108 Scheffer, T. C. and Cowling, E. B., 1966, *Ann. Rev. of Phytopath.*, **4**, 147-170.

109 Hart, J. H. and Shrimpton, D. M., 1979, *Phytopathol.*, **69**, 1138-1143.

110 Clark, J. W. and Scheffer, T. C., 1983, *For. Prod. J.*, **33**, 15-20.

111 Smith, A. L., Campbell, C. L., Douglas, B. W. and Hanover, J. W., 1989, *Holzforschung*, **43**, 293-296.

112 Schultz, T. P., Harms, W. B., Fisher, T. H., McMurtrey, K. D., Minn, J. and Nicholas, D. D., 1995, *Holzforschung*, **49**, 29-34.

113 Fengel, D. and Wegener, G., 1989, *Wood: Chemistry, Ultrastructure and Reactions*, Walter de Gruyter, Berlin, Germany, pp 182-214.

114 Rennerfelt, E. and Nacht, G., 1955, *Sv. Bot. Tidskr.*, **49**, 419-432.

115 Rennerfelt, E. and Nacht, G., 1945, *Sv. Bot. Tidskr.*, **39**, 311-318.

116 Celimene, C. C., Micales, J. A., Ferge, L. and Young, R. A., 1999, *Holzforschung*, **53**, 491-497.

117 Reyes-Chilpa, R., Gomez-Garibay, F., Moreno-Torres, G., Jimenez-Estrada, M. and Quiroz-Vasquez, R. I., 1998, *Holzforschung*, **52**, 459-462.

118 Rudman, P., 1962, *Holzforschung*, **16**, 56-61.

119 Anderson, A. B., 1961, *J. Inst. Wood Sci.*, **8**, 14-29.

120 Scalbert, A., 1991, *Phytochem.*, **30**, 3875-3883.

121 Laks, P. E., McKaig, P. A. and Hemingway, R. W., *Holzforschung*, **42**, 299-306.

122 Hillis, W. E., 1987, *Heartwood and Tree Exudates*, Springer-Verlag, Berlin, Germany, pp 95-105.

123 Rudman, P., 1965, *Holzforschung*, **19**, 57-58.

124 Kim, Y. G., Watanabe, N., Sano, Y. and Uraki, Y., 1998, *Res. Bull. Hokkaido Uni. For.*, **55**, 63-73.

125 Manners, G. D., and Jurd, L., 1977, *J. Agric. Food Chem.*, **25**, 726-730.

126 Guiraud, P., Steiman, R., Campos-Takaki, G.-M., Seigle-Murandi, F. and de Buochberg, M. S., 1994, *Planta Med.*, **60**, 373-374.

127 Fitzgerald, M. G. and Line, M. A., 1990, *Holzforschung*, **44**, 335-338.

128 Jurd, L., King, A. D., Mihara, K. and Stanley, W. L., 1971, *Appl. Microbiol.*, **21**, 507-510.

129 Hendrickson, J. B., 1965, *The Molecules of Nature*, W. A. Benjamin Inc., New York, USA, pp 34-39.

130 Bramley, P. M., 1997, *Isoprenoid Metabolism*, in "Plant Biochemistry", P. M. Dey and J. B. Harborne (Eds.), Academic Press, London, UK, pp 417-437.

131 Rohmer, M., Knani, M., Simonin, P., Sutteer, H. and Sahm, H., 1993, *Biochem. J.*, **295**, 517-524.

132 Anderson, A. B., Scheffer, T. C. and Duncan, C. G., 1963, *Holzforschung*, **17**, 1-5.

133 Erdtman, H., and Rennerfelt, E., 1949, *Acta Chem. Scand.*, **3**, 906-911.

134 Barton, G. M., 1976, *Wood and Fiber*, **8**, 172-176.

135 Maatooq, G. T., Stumpf, D. K., Hoffmann, J. J., Hutter, L. K. and Timmkermann, B. N., 1996, *Phytochem.*, **41**, 519-524.

136 Maatooq, G. T. and Hoffmann J. J., 1996, *Phytochem.*, **43**, 67-69.

137 Kondo, R., and Imamura, H., 1986, *Mokuzai Gakkaishi*, **32**, 213-217.

138 Chang, S.-T., Wu, C.-L., Wang, S.-Y. and Su, Y.-C., 1998, *For. Prod. Industries*, **17**, 287-304.

139 Shieh, J.-C. and Sumimoto, M., 1992, *Mokuzai Gakkaishi*, **38**, 482-489.

140 Richardson, D. P., Messer, A. C., Greenberg, S., Hagedorn, H. H. and Meinwald, J., 1989, *J. Chem. Ecol.*, **15**, 731-747.

141 Chowdhry, L., Khan, Z. K. and Kulshrestha, D. K., 1996, *Mycoses*, **39**, 449-452.

142 Jain, P. and Khan, Z. K., 1999, *J. de Mycol. Med.*, **9**, 217-220.

143 Singh, J. and Tripathi, N. N., 1999, *Flav. and Frag. J.*, **14**, 1-4.

144 Aoyama, M. and Shuichi, D., 1992, *Mokuzai Gakkaishi*, **38**, 101-105.

145 Aoyama, M., Yoneyama, S., Togashi, I. and Shuichi, D., 1991, *Antifungal Activity of (+)-Juvabione and (+)-Todomatuic acid against True Fungi*, in "Appita 6th International Symposium on Wood and Pulping Chemistry Proceedings", Vol. 2, Melbourne, pp 263-266.

146 Kemp, M. S. and Burden, R. S., 1986, *Phytochem.*, **25**, 1261-1269.

147 Bendall, J. G. and Cambie, R. C., 1995, *Aust. J. Chem.*, **48**, 883-917.

148 Cambie, R. C. and Mander, L. N., 1962, *Tetrahedron*, **18**, 465-475.

149 Grant, P. K., Munro, M. H. G. and Hill, N. R., 1965, *J. Chem. Soc.*, 3846-3850.

150 Chang, S.-T., Wang, S.-Y., Wu, C.-L., Su, Y.-C. and Kuo, Y.-H., 1999, *Holzforschung*, **53**, 487-490.

151 Gottstein, D. and Gross, D., 1992, *Trees*, **6**, 55-68.

152 Burden, R. S. and Kemp, M. S., 1984, *Phytochem.*, **23**, 383-385.

153 Overeem, J. C. and Elgersma, D. M., 1970, *Phytochem.*, **9**, 1949-1952.

154 Burden, R. S. and Kemp, M. S., 1983, *Phytochem.*, **22**, 1039-1040.

155 Cousins, D. J. (Ed.), 1995, *Plants with Antimicrobial Properties,* CAB international, Wallingford, UK, 109 p.

156 Kurita, N., Miyaji, M., Kurane, R. and Takahara, Y., 1981, *Agric. Biol. Chem.*, **45**, 945-952.

157 Thompson, D. P., 1989, *Mycologia*, **81**, 151-153.

158 Muller-Riebau, F., Berger, B. and Yengen, O., 1995, *J. Agric. Food Chem.*, **43**, 2262-2266.

159 Didry, N., Dubreuil, L. and Pinkas, M., 1993, *Pharmazie*, **48**, 301-304.

160 Sivropoulou, A., Papanikolaou, E., Nikolaou, C., Kokkini, S., Lanaras, T. and Arsenakis, M., 1996, *J. Agric. Food Chem.*, **44**, 1202-1205.

161 Karpouhtsis, I., Pardali, E., Feggou, E., Kokkini, S., Scouras, Z. G. and Mavragani-Tsipidou, P., 1998, *J. Agric. Food Chem.*, **46**, 1111-1115.

162 Buchanan, R. L. and Shepherd, A. J., 1981, *J. Food Sci.*, **46**, 976-977.

163 Asada, T., Ishimoto, T., Sakai, A. and Sumiya, K., 1989, *Mokuzai Gakkaishi*, **35**, 851-855.

164 Hill, R. A., Holland, P. T., Rohitha, B. H., Parker, S. and Cooney, J., 1997, *Use of Natural Products in Sapstain Control*, in "Strategies for Improving Protection of Logs and Lumber", B. Kreber (Ed.), Proceeding of Symposium, Rotorua, New Zealand, 21-22 November, FRI-Bullet No. 204., p 39-42.

165 Holland, P. T., 1999, Cawthron Institute, Nelson, New Zealand, pers. comm.

166 Holland, P. T., Trower, T. M., Cooney, J., Rohitha, B. H. and Hill, R. A., 1996, *Proceedings 2nd Natural Products Workshop*, Palmerston North, New Zealand, 6-7 November.

167 Tripathi, R. D., Banerji, R., Sharma, M. L., Balasubrahmanyam, V. R. and Nigam, S. K., 1985, *Agric. Biol. Chem.*, **49**, 2277-2282.

168 Saito, K., Okabe, T., Inamori, Y., Tsujibo, H., Miyake, Y., Hiraoka, K. and Ishida, N., 1996, *Mokuzai Gakkaishi*, **42**, 677-680.

169 Kurita, N., Miyaji, M., Kurane, R., Takahara, Y. and Ichimura, K., 1979, *Agric. Biol. Chem.*, **43**, 2365-2371.

170 Mori, M., Aoyama, M. and Doi, S., 1997, *Holz als Roh- und Werkstoff*, **55**, 275-278.

171 Ohasi, H., Asai, T. and Kawai, S., 1994, *Holzforschung*, **48**, 193-198.

172 Chalchat, J. C., Garry, R. P. and Michet, A., 1985, *Planta Med.*, **51**, 285.

173 Kubo, I., Muroi, H. and Himejima, M., 1992, *J. Agric. Food Chem.*, **40**, 245-248.

174 Kubo, I. and Taniguchi, M., 1988, *J. Nat. Prod.*, **51**, 22-29.

175 Jacyno, J. M., Montemurro, N., Bates, A. D., and Cutler, H. G., 1991, *J. Agric. Food Chem.*, **39**, 1166-1168.

176 Picman, A. K., 1986, *Biochem. Sys. Ecol.*, **14**, 255-281.

177 Franich, R. A. and Cambie, R. C., 1999, *J. Essent. Oil Res.*, **11**, 507-510.

178 Ying, X. and Eden D., 1996, unpublished data.

179 Dewar, M. J. S., 1945, *Nature*, **155**, 50.

180 Buchanan G. L. and Raphael, R. A., 1956, *Monocyclic Quasi-Aromatic Compounds*, in "Chemistry of Carbon Compounds", Vol. III, E. H. Rodd (Ed.), Elsevier Publishing Company, Amsterdam, Netherlands, pp 1221-1248.

181 Pietra, F., 1973, *Chem. Rev.*, **73**, 293-364.

182 Pauson P. L., 1955, *Chem. Rev.*, **55**, 9-136.

183 Nozoe, T., 1959, *Tropones and Tropolones*, in "Non-benzenoid Aromatic Compounds", D. Ginsburg (Ed.), Interscience, New York, USA, pp 339-464.

184 Gardner, J. A. F., 1962, *The Tropolones*, in "Wood extractives and their Significance to the Pulp and Paper Industry", W. E. Hillis (Ed.), Academic Press, New York, USA, pp 317-330.

185 Erdtman, H. and Gripenberg, J., 1948, *Acta Chem. Scand.*, **2**, 625-638.

186 Erdtman, H. and Gripenberg, J., 1948, *Nature*, **161**, 719.

187 Zavarin, E., Smith, L. V. and Bicho, J. G., 1967, *Phytochem.*, **6**, 1387-1394.

188 Doi, K. and Saito, O., 1972, *Mokuzai Gakkaishi*, **18**, 251-252.

189 Ohira, T., Terauchi, F. and Mitsuyoshi, Y., *Holzforschung*, **48**, 308-312.

190 Nozoe, T., 1950, *Sci. Repts. Tohoku Univ. First Ser.*, **34**, 199; *Chem. Abstr.*, **45**, 10238 (1951).

191 Nozoe, T., 1957, *Experientia Suppl.* No. **7**, 306-327.

192 Carlsson, B., Erdtman, H., Frank, A. and Harvey, W. E., 1952, *Acta Chem. Scand.*, **6**, 690-696.

193 Anderson, A. B., Scheffer, T. C. and Duncan, C., 1962, *Science*, **137**, 859-860.

194 Gardner, J. A. F., Barton, G. M. and MacLean, H., 1957, *Can. J. Chem.*, **35**, 1039-1048.

195 Sakai, K., Yamaguchi, T. and Itose, R., 1997, *Mokuzai Gakkaishi*, **43**, 696-698.

196 Yamaguchi, T., Itose, R. and Sakai, K., 1997, *J. Fac. Agr. Kyushu Univ.*, **42**, 131-138.

197 Fujita, K., Yamaguchi, T., Itose, R. and Sakai, K., 2000, *J. Plant Physiol.*, **156**, 462-467.

198 MacLean, H. and Gardner, J. A. F., 1956, *Forest Prods. J.*, **6**, 510-516.

199 Nault, J., 1988, *Wood Sci. Tech.*, **22**, 73-80.

200 DeBell, J. D., Morrell, J. J. and Gartner, B. L., 1997, *Wood Fiber Sci.*, **29**, 364-369.

201 Anderson, A. B. and Sherrard, E. C., 1933, *J. Am. Chem. Soc.*, **55**, 3813-3819.

202 Buckland, D. C., 1946, *Can J. Res. C*, **24**, 158-181.

203 Scheffer, T. C., 1957, *J. For.*, **55**, 434-442.

204 Rennerfelt, E., 1948, *Physiologia Plantarum*, **1**, 245-254.

205 Roff, J. W. and Whittaker, E. I., 1959, *Can. J. Bot.*, **37**, 1132-1134.

206 Rudman, P., 1962, *Holzforschung*, **16**, 74-77.

207 Grohs, B. M., Wegen, H.-W. and Kunz, B., 1999, *Holz als Roh- und Werkstoff*, **57**, 277-281.

208 Sholberg, P. L. and Shimizu, B. N., 1991, *Can. Inst. Sci. Technol. J.*, **24**, 273-277.

209 Lyr, H., 1962, *Nature*, **195**, 289-290.

210 Jin, L., van der Kamp, B. J., Wilson, J. and Swan, E. P., 1988, *Can. J. For. Res.*, **18**, 782-786.

211 Trust, T. J. and Coombs, R. W., 1973, *Can. J. Microbiol.*, **19**, 1341-1346.

212 Saito, K., Okabe, T., Fukui, T. and Inamori, Y., 1997, *Mokuzai Gakkaishi*, **43**, 882-891.

213 Inamori, Y., Shinohara, S., Tsujibo, H., Okabe, T., Morita, Y., Sakagami, Y., Kumeda, Y. and Ishida, N., 1999, *Bio. Pharm. Bull.*, **22**, 990-993.

214 Becker, V. G., 1963, *Holzforschung*, **17**, 19-20.

215 Inamori, Y., Sakagami, Y., Morita, Y., Shibata, M., Sugiura, M., Kumeda, Y., Okabe, T., Tsujibo, H. and Ishida, N., 2000, *Bio. Pharm. Bull.*, **23**, 995-997.

216 Arndt, V. U., 1968, *Holzforschung*, **22**, 104-109.

217 Bryant, B. E., Fernelius, W. C. and Douglas, B. E., 1953, *J. Amer. Chem. Soc.*, **75**, 3784-3786.

218 Bryant, B. E., Fernelius, W. C. and Douglas, B. E., 1954, *J. Amer. Chem. Soc.*, **76**, 1696-1697.

219 Bohme, G., Schonfeld, P., Kuster, U., Kunz, W. and Lyr, H., 1980, *Acta Biol. Med. Ger.*, **39**, 1153-1163.

220 Lyr, H., 1960, *Flora*, **150**, 227-242.

221 Raa, J. and Goksoyr, J., 1965, *Physiologia Plantarum*, **18**, 159-176.

222 Kahn, V. and Andrawis, A., 1985, *Phytochem.*, **24**, 905-908.

223 Tanaka, T., Takeuchi, M. and Ichishima, E., 1989, *Agric. Biol. Chem.*, **53**, 557-558.

224 Sakai, K., Kusaba, K., Tsutsumi, Y. and Shiraishi, T., 1994, *Mokuzai Gakkaishi*, **40**, 1-5.

225 Madar, Z., Gottlieb, H. E., Cojocaru, M., Riov, J., Solel, Z. and Sztejnberg, A., 1995, *Phytochem.*, **38**, 351-354.

226 Richardson, M. L. (Ed.), 1992, *The Dictionary of Substances and Their Effects*, Royal Society of Chemistry, Clays Ltd, St Ives, UK, Vol. 1 pp 410, 693, Vol. 2 pp 505, 652, Vol. 6 pp 30, 292, 449, 508, 753, Vol. 7 pp 277, 449.

227 Kang, S.-K. and Yoon, S.-K., 2002, *J. Chem. Soc., Perkin Trans. 1*, 459-461.

228 Dolhem, E., Barhdadi, R., Folest, J.-C., Nedelec, J. Y. and Troupel, M., 2001, *Tetrahedron*, **57**, 525-529.

229 Hudlicky, M., 1984, *Reductions in Organic Chemistry*, Ellis Horwood Ltd, Chichester, UK, pp 112-113.

230 Huang-Minlon, 1946, *J. Am. Chem. Soc.*, **68**, 2487-2488.

231 Staude, E. and Patat, F., 1967, *Cleavage of the C-O-C bond*, in "The Chemistry of the Ether Linkage", S. Patai (Ed.), Interscience Publishers, London, UK, pp 21-80.

232 Horner, L. and Schmitt, E., 1978, *Liebigs. Ann. Chem.*, 1617-1633.

233 Hudlicky, M., 1984, *Reductions in Organic Chemistry*, Ellis Horwood Ltd, Chichester, UK, pp 28-29.

234 Risinger, G. E., Mach, E. E. and Barnett, K. W., 1965, *Chem. Ind.*, 679.

235 Martin, E. L., 1936, *J. Am. Chem. Soc.*, **58**, 1438-1442.

236 Fieser, L. F. and Fieser, M. F., 1968, *Reagents for Organic Synthesis*, John Wiley and Sons, Inc., New York, USA, pp 149, 1287-1289.

237 Wightman, R. H. and Malaiyandi, M., 1983, *Environ. Sci. Technol.*, **17**, 256-61.

238 Birch, A. J. and Chamberlain, K. B., 1988, *Org. Syn. Coll. Vol.*, **6**, W. E. Norland (Ed. in Chief), John Wiley & Sons, New York, USA, 996-1000.

239 Birch, A. J., 1944, *J. Am. Chem. Soc.*, **66**, 430-436.

240 March, J., 1985, *Advanced Organic Chemistry: Reactions, Mechanisms and Structure*, 3rd Edition, John Wiley and Sons, New York, USA, pp 700-702.

241 Hine, J., 1966, *J. Org. Chem.*, **31**, 1236-1244.

242 Fieser, L. F. and Fieser, M. F., 1968, *Reagents for Organic Synthesis*, John Wiley and Sons, Inc., New York, USA, pp 54-56.

243 Dryden, Jr., H. L., Weber, G. M., Burtner, R. P. and Cella, J. A., 1961, *J. Org. Chem.*, **26**, 3237-3245.

244 Varo, P. T. and Heinz, D. E., 1970, *J. Agr. Food Chem.*, **18**, 239-242.

245 Birch, A. J. and Rao, G. S. R., 1970, *Aust. J. Chem.*, **23**, 1641-1649.

246 Missen, A. W., 1971, *Ring C transformations of Podocarpic acid*, Ph.D thesis, Auckland University, Auckland, New Zealand, pp 11-14.

247 Birch, A. J. and Smith, H., 1958, *Chem. Soc. Quart. Rev.*, **12**, 17-33.

248 Badger, A. M., Schwartz, D. A., Picker, D. H., Dorman, J. W., Bradley, F. C., Cheeseman, E. N., DiMartino, M. J., Hanna, N. and Mirabelli, C. K., 1990, *J. Med. Chem.*, **33**, 2963-2970.

249 Soffer, M. D. and Williston, A. C., 1957, *J. Org. Chem.*, **22**, 1254.

250 Soffer, M. D. and Jevnik M. A., 1955, *J. Amer. Chem. Soc.*, **77**, 1003-1006.

251 Wildman, W. C., Wildman, R. B., Norton, W. T. and Fine, J. B., 1953, *J. Amer. Chem. Soc.*, **75**, 1912-1915.

252 House, H. O., 1972, *Modern Synthetic Reaction*, 2nd Edition, W. A. Benjamin Inc., Menlo Park, USA, pp 643-645.

253 Graham, D. E. and Nashu, A. M., 1967, US Patent 3322619.

254 Cahn, R. S., Ingold, C. and Prelog, V., 1966, *Angew. Chem. Int. Ed. Engl.*, **5**, 385-415.

255 Adams, R., 1995, *Identification of Essential Oil Components by Gas Chromatography/Mass Spectroscopy*, Allured Publishing Corporation, Carol Stream, USA, 469 p.

256 Swern, D., 1979, *Peroxides*, in "Comprehensive Organic Chemistry: The Synthesis and Reaction of Organic compounds, Vol. 1: Stereochemistry, Hydrocarbons, Halo compounds, Oxygen compounds", J. F. Stoddart (Ed.), Pergamon Press, Oxford, UK, pp 909-924.

257 March, J., 1985, *Advanced Organic Chemistry: Reactions, Mechanisms and Structure*, 3rd Edition, John Wiley and Sons, New York, USA, pp 633-635.

258 Hudlicky, M., 1984, *Reductions in Organic Chemistry*, Ellis Horwood Ltd, Chichester, UK, pp 130-131.

259 Condon, F. E., 1948, *J. Am. Chem. Soc.*, **70**, 2265-2267.

260 Francis, A. W., 1948, *Chem. Rev.*, **43**, 257-269.

261 March, J., 1985, *Advanced Organic Chemistry: Reactions, Mechanisms and Structure*, 3rd Edition, John Wiley and Sons, New York, USA, pp 447-458, 479-484.

262 Olah, G. A., 1963, *Friedel-Crafts and Related reactions Vol. 1 General Aspects*, Interscience, New York, USA, pp 34-49, 70.

263 McOmie, J. F. W. and Watts, M. L., 1963, *Chem. Ind.*, 1658.

264 Carnduff, J. and Leppard, D. G., 1976, *J. Chem. Soc., Perkin Trans. 1*, 2570-2573.

265 Kornblum, N., Smiley, R. A., Blackwood, R. K. and Iffland, D. C., 1955, *J. Am. Chem. Soc.*, **77**, 6269-6280.

266 Kornblum, N. and Lurie, A. P., 1958, *J. Am. Chem. Soc.*, **81**, 2705-2715.

267 Kornblum, N., Berrigan, P. J. and Le Noble, W. J., 1963, *J. Am. Chem. Soc.*, **85**, 1141-1147.

268 Kornblum, N., Seltzer, R. and Haberfield, P., 1963, *J. Am. Chem. Soc.*, **85**, 1148-1154.

269 Carnduff, J. and Leppard, D. G., 1967, *Chem. Commun.*, 829-830.

270 Schwarz, H. and Schiebel, H.-M., 1983, *Mass Spectroscopy of Organic Peroxides*, in "The Chemistry of Peroxides", S. Patai (Ed.), John Wiley and Sons, New York, USA, pp 113-115.

271 van Lier, J. E. and Smith, L. L., 1971, *J. Org. Chem.*, **36**, 1007-1009.

272 March, J., 1985, *Advanced Organic Chemistry: Reactions, Mechanisms and Structure*, 3rd Edition, John Wiley and Sons, New York, USA, pp 68-69.

273 Brady, P. A. and Carnduff, J., 1974, *J. Chem. Soc., Chem. Commun.*, 816-817

274 Devon, T. K. and Scott, A. I., 1972, *Handbook of Naturally Occuring Compounds, Vol. II Terpenes*, Academic Press, New York, USA, pp 55-184.

275 Berry, K. M., Perry, N. B. and Weavers, R. T., 1985, *Phytochem.*, **24**, 2893-2898.

276 Damodaran, N. P. and Dev. S., 1968, *Tetrahedron*, **24**, 4113-4122.

277 Nabeta, K., Katayama, K., Matsubara, M., Hatakeyama, C., Shimada, T., Tazaki, H., Okuyama, H. and Miyake, M., 1992, *Mokuzai Gakkaishi*, **38**, 936-971.

278 Yatagai, M. and Sato, T., 1986, *Biochem. Sys. Ecol.*, **14**, 469-478.

279 Yatagai, M., Sato, T. and Takahashi, T., 1985, *Biochem. Sys. Ecol.*, **13**, 377-385.

280 Analytical Methods Committee, 1984, *Analyst*, **109**, 1343-1359.

281 Weavers, R., 1999, Department of Chemistry, University Of Otago, Dunedin, New Zealand, pers. commun.

282 Clarke, D. B., 1992, *Natural Products Chemistry*, Ph.D thesis, University of Otago, Dunedin, New Zealand.

283 Lorimer, S. D. and Weavers, R. T., 1987, *Phytochem.*, **26**, 3207-3215.

284 Davies, N. W., 1990, *J. Chrom.*, **503**, 1-24.

285 Dellar, J. E., Cole, M. D., Gray, A. I., Gibbons, S. and Waterman, P. G., 1994, *Phytochem.*, **36**, 957-960.

286 Garg, S. C. and Dengre, S. L., 1992, *Flav. Frag. J.*, **7**, 125-127.

287 Ulubelen, A., Topcu, G., Eris, C., Sonmez, U., Kartal, M., Kurucu, S. and Bozok-Johansson, C., 1994, *Phytochem.*, **36**, 971-974.

288 Yano, S., Nakamura, T., Uehara, T. and Furuno, T., 1995, *Mokuzai Gakkaishi*, **41**, 573-579.

289 Gunasena, G. H., Vinson, S. B., Williams, H. J. and Stipanovic, R. D., 1988, *J. Econ. Entomol.*, **81**, 93-97.

290 Stipanovic, R. D., Elissalde, M. H., Altman, D. W. and Norman, J. O., 1990, *J. Econ. Entomol.*, **83**, 737-741.

291 Therbtaranonth, C., Therbtaranonth, Y., Wanauppathamkul, S. and Yuthavong, Y., 1995, *Phytochem.*, **40**, 125-128.

292 Toyota, M., Tanaka, M. and Asakawa, Y., 1999, *Spectroscopy*, **14**, 61-66.

293 Chang, S.-T., Wang, S.-Y., Wu, C.-L., Chen, P.-F. and Kuo, Y.-H., 2000, *Holzforschung*, **54**, 241-245.

294 Yatagai, M., Sato, T., Yamaguchi, Y. and Takahashi, T., 1984, *Mokuzai Gakkaishi*, **30**, 240-243.

295 McDaniel, C. A., 1989, *Mat. und Org.*, **24**, 1-15.

296 Vallejo, I., Rebordinos, L., Collado I. G. and Cantoral Fernandez, J. M., 2001, *J. Phytopath.*, **149**, 113-118.

297 Wassmuth-Wagner, I., Kalinowski, H. O. and Jork, H., 1995, *Planta Med.*, **61**, 196-197.

298 Tkku, D. K., Roshchin, V. I., Malysheva, O. N. and Solov'-ev, V. A., 1987, *Khimiya Drevesiny*, 103-104.

299 March, J., 1985, *Advanced Organic Chemistry: Reactions, Mechanisms and Structure*, 3rd Edition, John Wiley and Sons, New York, USA, pp 310-311, 382-385.

300 Olson, C. E. and Bader, A. R., 1963, *Org. Syn. Coll. Vol.*, **4**, N. Rabjohn (Ed. in Chief), John Wiley & Sons, New York, USA, pp 898-899.

301 Danheiser, R. L. and Helgason, A. L., 1994, *J. Am. Chem. Soc.*, **116**, 9471-9479.

302 Johnson, E. L. and Cserjesi, A. J., 1980, *For. Prod. J.*, **30**, 52-53.

303 Ozawa, H., Seto, S., Murai, S. and Ohizumi, Y., 1971, *Yakugaku Zasshi*, **91**, 550-559; *Chem. Abstr.*, **75**, 61884m, (1971).

304 Haworth, R. D., Moore, B., and Pauson, P., 1948, *J. Chem. Soc.*, 1045-1051.

305 Haworth, R. D. and Hobson, J. D., 1951, *J. Chem. Soc.*, 561-568.

306 Evans, T. W. and Dehn, W. M., 1930, *J. Amer. Chem. Soc.*, **52**, 3649.

307 Budzikiewwicz, H., Djerassi, C. and Williams, D. H., 1967, *Mass spectroscopy of Organic Compounds*, Holden-Day Inc., San Francisco, pp 539-546.

308 Smith, G. G. and Kelly, F. D., 1971, *Prog. Phys. Org. Chem.*, **8**, 150-153.

309 Crow, W. D., Haworth, R. D. and Jefferies, P. R., 1952, *J. Chem. Soc.*, 3705-3713.

310 Smith, M., Moffat, J. G. and Khorana, H. G., 1958, *J. Am. Chem. Soc.*, **80**, 6204-6212.

311 March, J., 1985, *Advanced Organic Chemistry: Reactions, Mechanisms and Structure*, 3rd Edition, John Wiley and Sons, New York, USA, pp 290-295, 333-334, 347-351.

312 Nozoe, T., Kitahara, Y., Doi, K., Masamune, S., Endo, M., Mutusuo, I. and JeKyun, S., 1954, *Sci. Repts. Tohoku Univ. First Ser.*, **38**, 257-259; *Chem. Abst.*, **50**, 2532c, (1956).

313 Crawford, M. and Little, W. T., 1959, *J. Chem. Soc.*, 722-728.

314 Nozoe, T., Kitahara, Y. and Doi, K., 1953, *Proc. Japan. Acad.*, **29**, 203-206; *Chem. Abst.*, **49**, 2415f, (1955).

315 Sutherland, I. O., 1979, *Esters*, in "Comprehensive Organic Chemistry: The Synthesis and Reaction of Organic compounds, Vol. 2: Nitrogen compounds, Carboxylic acids, Phosphorus compounds", I. O. Sutherland (Ed.), Pergamon Press, Oxford, UK, pp 907-909.

316 Sommer, T. J., 2000, *Chem Innov.*, 25-32.

317 Tarbell, D. S., Williams, K. I. H. and Sehm, E. J., 1959, *J. Amer. Chem. Soc.*, **81**, 3443-3446.

318 Scott, A. I., McCapra, F., Buchanan, R. L., Day, A. C. and Young, D. W., 1965, *Tetrahedron*, **21**, 3605-3631.

319 Cohen, T. and Schambach, R. A., 1969, *J. Am. Chem. Soc.*, **92**, 3189-3190.

320 Critchlow, A., Haworth, R. D. and Pauson, P. L., 1951, *J. Chem. Soc.*, 1318-1325.

321 Collier, P. D., 1969, *J. Chem. Soc. (C)*, 612-621.

322 Horner, L. and Weber, K.-H., 1965, *Chem. Ber.*, **98**, 1246-1251.

323 Karrer, P. and Schneider, L., 1947, *Helv. Chim. Acta*, **30**, 859-861.

324 Fernholz, H., 1951, *Chem. Ber.*, **84**, 110-122.

325 Skau, E. L. and Saxton, B., 1930, *J. Am. Chem. Soc.*, **52**, 335-336.

326 Lloyd, D., 1984, *Non-benzenoid Conjugated Carboxylic Compounds*, Elsevier Science Publishers, New York, USA, pp 101-119.

327 Harrison, R. M., Hobson, J. D., and Midgley, A. W., 1973, *J. Chem. Soc., Perkin Trans. I,* 1960-1966.

328 Harrison, R. M. and Hobson, J. D., 1973, *J. Chem. Soc., Perkin Trans. I,* 1958-1960.

329 Yamato, M., Hashigaki, K., Kokubu, N. and Nakato, Y., 1984, *J. Chem. Soc., Perkin Trans. I,* 1301-1304.

330 Yamato, M., Hashigaki, K., Kokubu, N., Tsuruo, T. and Tashiro, T., 1984, *J. Med. Chem.*, **27**, 1749-1753.

331 Stevens, H. C., Reich, D. A., Brandt, D. R., Fountain, K. R. and Gaughan, E. J., 1965, *J. Amer. Chem. Soc.*, **87**, 5257-5259.

332 Minns, R. A., 1988, *Org. Syn. Coll. Vol.*, **6**, W. E. Norland (Ed. in Chief), John Wiley & Sons, New York, USA, 1037-1040.

333 Zhu, H.-J., Wang, J.-T., Xu, F. and Kong, A.-W., 2000, *Jingxi Huagong*, **17**, 559-561.

334 Klein, J. and Bergmann, E. D., 1957, *J. Am. Chem. Soc.*, **79**, 3452-3454.

335 Alexopoulos, C. J. and Mims, C. W., 1979, *Introductory Mycology*, 3rd Edition, John Wiley and Sons, New York, USA, pp 10-11.

336 Elliott, M., 1974, *Chem. Ind.*, 978-980.

337 Elliott, M., 1980, *Pestic. Sci.*, **11**, 119-128.

338 Laks, P. E., 1989, Condensed Tannins as a Source of Novel Biocides, in "Chemistry and Significance of Condensed Tannins", R. W. Hemingway and J. J. Karchesy (Eds.), Plenum, New York, USA, pp 503-515.

339 Pouchert, C. J. and Behnke, J., 1993, The Aldrich Library of ^{13}C and ^{1}H FT NMR Spectra, Volumes I and II, Aldrich Chemical Company Inc., USA, Vol. I pp1195 and Vol. II pp 175.

340 Krapcho, J., 1967, US Patent 3326963.

341 Horner, L. and Weber, K.-H., 1962, *Chem. Ber.*, **95**, 1227-1236.

342 Appita, 1993, AS/NZS 1301.203s.

343 Kreber, B., Fernandez, M. and McDonald, A. G., 1998, *Holzforschung*, **52**, 441-446.

344 Tressl, R., Friese, L., Fendesack, F. and Koppler, H., 1978, *J. Agric. Food Chem.*, **26**, 1422-1430.

345 Ramaswami, S. K., Briscese, P., Gargiullo, R. J. and von Geldern T., 1986, *Sesquiterpene hydrocarbons: From Mass Confusion to Orderly Line-Up*, in "Flavors and Fragrances: A World Perspective, Proceedings of the 10th International Congress of Essential Oils, Flavors and Fragrances", B. M. Lawrence, B. D. Mookherjee and B. J. Willis (Eds.), November 16th-20th, Washington DC, USA, p 951-981.

346 Parker, D., 1991, *Chem. Rev.*, **91**, 1441-1457.

347 Sanders, J. K. M. and Hunter, B. K., 1993, *Modern NMR spectroscopy*, Oxford University Press, Oxford, UK, pp 303-307.

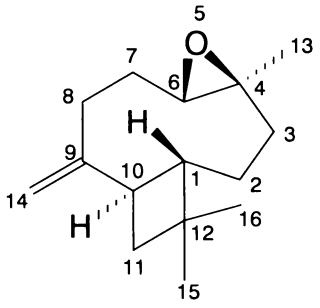
You cannot teach a man anything, you can only help him find it within himself

Galileo Galilei

Appendices

Appendix A: Spectral Data of Identified Sesquiterpenoid Epoxides from *Podocarpus totara* Foliage Oil

Caryophyllene oxide (35)



RI (Ultra-2) = 1618 (literature value = 1581 (255));

RI (EC^{TM}-WAX) = 1982 (literature value = 2000 (284), 1966 (344)).

^{1}H NMR (300 MHz, $CDCl_3$)[1] δ: 0.92 (m, H3), 0.98 (s, H16), 0.99 (s , H15), 1.19 (s, H13), 1.31 (m, H7), 1.40 (m, H2), 1.60 (dd, *J* = 19.0, 10.7 Hz, H11), 1.61 (m, H2'), 1.65 (d, *J* = 10.7 Hz, H11'), 1.75 (t, *J* = 10.0 Hz, H1), 2.06 (m, H3'), 2.08 (m, H8), 2.22 (m, H7'), 2.32 (m, H8'), 2.60 (dd, *J* = 18.8, 9.3 Hz, H10), 2.85 (dd, *J* =10.6, 4.2 Hz, H6), 4.83 (d, *J* = 0.95 Hz, H14), 4.95 (d, *J* = 0.95 Hz, H14').

^{13}C NMR (100 MHz, $CDCl_3$) δ: 16.8 (C13), 21.4 (C15), 26.9 (C2), 29.5 (C8), 29.7 (C16), 30.0 (C7), 33.8 (C12), 38.9 (C3), 39.5 (C11), 48.5 (C10), 50.4 (C1), 59.5 (C4), 63.4 (C6), 112.6 (C14), 151.4 (C9).

MS (EI, GC/MS) *m/z*: 220 (1%), 205 (4%), 177 (8%), 161 (10%), 149 (14%), 138 (17%), 121 (29%), 109 (56%), 93 (84%), 79 (100%), 69 (65%), 55 (50%).

[1] Relative integrals not provided as the ^{1}H NMR spectrum was of a mixture of sesquiterpenoids.

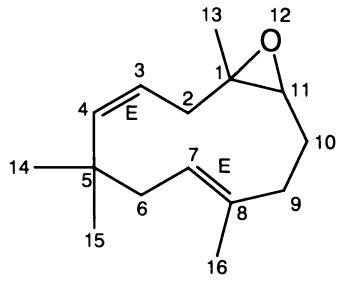
Table A1: gCOSY correlations observed for caryophyllene oxide (**35**) in *Podocarpus totara* essential oil distillation and chromatography fraction, TF8f2.

^{1}H	δ (ppm)	Correlated ^{1}H signals
H3	0.92	H2', H3'
H7	1.31	H6, H7', H8, H8'
H2	1.40	H1, H2', H3'
H11	1.60	H10
H2'	1.61	H3'
H11'	1.65	H10
H1	1.75	H10
H8	2.08	H7', H8'
H7'	2.22	H6, H8'
H14	4.83	H14'
H14'	4.95	H10

Table A2: HMQC and HMBC correlations observed for caryophyllene oxide (**35**) in *Podocarpus totara* essential oil distillation and chromatography fraction, TF8f2.

^{13}C	δ (ppm)	HMQC correlations, δ (ppm)	HMBC correlations
C13	16.8	H13, 1.19	H3
C15	21.4	H15, 0.99	H1, H11, H11', H16
C2	26.9	H2, 1.40; H2', 1.61	H3, H10
C8	29.5	H8, 2.08; H8', 2.32	H7, H7', H10, H14, H14'
C16	29.7	H16, 0.98	H1, H11, H15
C7	30.0	H7, 1.31; H7', 2.22	H6, H8, H8'
C12	33.8		H1, H11, H11', H15, H16
C3	38.9	H3, 0.92; H3', 2.06	H1, H2, H2', H13
C11	39.5	H11, 1.60; H11', 1.65	H10, H15, H16
C10	48.5	H10, 2.60	H1, H11, H11', H14, H14'
C1	50.4	H1, 1.75	H10, H11', H15, H16
C4	59.5		H3, H13
C6	63.4	H6, 2.85	H8, H8', H13
C14	112.6	H14, 4.83; H14', 4.95	H8, H8', H10
C9	151.4		H1, H8, H8', H10, H11, H14 , H14'

***Humulene epoxide II* (36)**



RI (Ultra-2) = 1646 (literature value = 1606 (255));

RI (EC^{TM}-WAX) = 2037 (literature value = 2011 (344)).

^{1}H NMR (300 MHz, $CDCl_3$)[2] δ: 1.06 (s, H16), 1.09 (s, H14), 1.29 (s, H13), 1.34 (m, H10), 1.53 (s, H15), 1.61 (m, H2), 1.84 (dd, *J* = 13.6, 5.5 Hz, H6), 1.98 (dd, *J* = 13.6, 9.1 Hz, H6'), 2.07 (m, H9), 2.13 (m, H10'), 2.22 (m, H9'), 2.50 (dd, *J* = 10.4, 3.9 Hz, H11), 2.54 (m, H2'), 4.97 (m, H7), 5.12 (d, *J* = 15.9 Hz, H4), 5.25 (ddd, *J* = 15.9, 10.1, 5.4 Hz, H3).

^{13}C NMR (100 MHz, $CDCl_3$) δ: 14.8 (C15), 16.9 (C13), 24.5 (C10), 25.6 (C14), 29.1 (C16), 36.2 (C5), 36.4 (C9), 40.0 (C6), 42.3 (C2), 61.6 (C11), 62.9 (C1), 121.8 (C3), 125.5 (C7), 131.5 (C8), 142.8 (C4).

MS (EI, GC/MS) *m/z*: 220 (2%), 205 (1%), 177 (1%), 150 (5%), 138 (81%), 123 (28%), 109 (100%), 96 (90%), 93 (41%), 81(36%), 67 (75%), 55 (36%).

Table A3: gCOSY correlations observed for humulene epoxide II (**36**) in *Podocarpus totara* essential oil distillation and chromatography fraction, TF8f2.

^{1}H	**δ (ppm)**	**Correlated ^{1}H signals**
H10	1.34	H11
H6	1.84	H6'
H10'	2.13	H11
H7	4.97	H6, H6'
H4	5.12	H3
H3	5.25	H2, H2'

[2] Relative integrals not provided as the ^{1}H NMR spectrum was of a mixture of sesquiterpenoids.

Table A4: HMQC and HMBC correlations observed for humulene epoxide II (**36**) in *Podocarpus totara* essential oil distillation and chromatography fraction, TF8f2.

^{13}C	δ (ppm)	HMQC Correlations, δ (ppm)	HMBC Correlations
C15	14.8	H15, 1.53	H4
C13	16.9	H13, 1.29	
C10	24.5	H10, 1.34; H10', 2.13	H9', H11'
C14	25.6	H14, 1.09	H4
C16	29.1	H16, 1.06	H7
C5	36.2		H3, H4, H6', H7, H14, H15
C9	36.4	H9, 2.07; H9', 2.22	H16
C6	40.0	H6, 1.84; H6', 1.98	H14, H15
C2	42.3	H2, 1.61; H2', 2.54	H3, H4, H13
C11	61.6	H11, 2.50	H5', H13
C1	62.9		H2, H2', H4, H13
C3	121.8	H3, 5.25	H2, H2', H4
C7	125.5	H7, 4.97	H6, H6', H16
C8	131.5		H6, H6', H16
C4	142.8	H4, 5.12	H2, H2', H3, H14, H15

Appendix B: Identified Sesquiterpenes from Totara, Rimu and Kahikatea Foliage Essential Oils

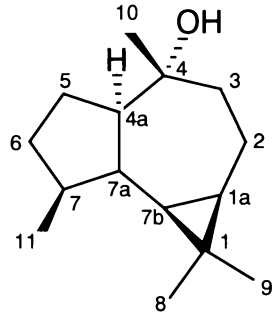
Table B1: Comparison of RI values for identified sesquiterpene hydrocarbons from totara (i), rimu (ii) and kahikatea (iii) foliage essential oils on polar and nonpolar GC capillary columns.

Sesquiterpene Hydrocarbon	RI_{ULTRA2}	$RI_{LIT.}$		$RI_{EC\text{-}WAX}$	$RI_{LIT.}$	
		1	2		2	3
β-elemene [(i,ii)]	1410	1391	1400[3]	1592	1591	1608
cis-caryophyllene [(i)]	1431	1404		1572		
longifolene [(ii)]	1436	1402	1398	1563	1574	1643
trans-caryophyllene [(i,ii)]	1446	1418	1410	1596	1598	
aromadendrene [(i)]	1466	1439	1455	1603	1654	1650
α-humulene [(i,ii)]	1482	1454	1437	1665	1672	1707
alloaromadendrene [(ii)]	1489	1461	1452	1638	1648	1660
9-epi-caryophyllene [(ii)]	1495	1467		1667		
β-selinene [(i, ii, iii)]	1515	1485	1477	1712	1727	1756
α-murrolene [(iii)]	1520	1490	1495[3]	1716	1727	1753
α-selinene [(i, ii, iii)]	1522	1494	1484	1717	1729	1759
γ-cadinene [(iii)]	1537	1513	1510	1751	1763	1766
δ-cadinene [(iii)]	1543	1524	1510	1751	1763	1761
trans-calamenene [(iii)]	1546	1532	1502	1825	1837	1839
α-cadinene [(iii)]	1559	1538		1784		
α-calacorene [(iii)]	1567	1542		1908	1916	1926
cadalene [(iii)]	1700	1674	1646	2218		2203

[1] Adams (255), [2] Ramaswami *et. al* (345), [3] Davies (284).

Appendix C: Spectral Data of Identified Oxygenated Sesquiterpenes from *Dacrycarpus dacrydioides* Foliage Oil

Globulol (37)



RI (Ultra-2) = 1616 (literature value = 1583 (255));

RI (EC^{TM}-WAX) = 2066 (literature value = 2104 (284)).

^{1}H NMR (300 MHz, $CDCl_3$)[3] δ: 0.53 (m, H7b), 0.60 (m, H1a), 0.90 (m, H2), 0.91 (d, J = 7.0 Hz, H11), 0.98, 1.0 (2 × s, H8 and H9), 1.1 (s, H10), 1.22 (m, H7a), 1.24 (m, H6), 1.42 (m, H5), 1.52 (m, H3), 1.67 (m, H6'), 1.73 (m, H3'), 1.75 (m, H5'), 1.80 (m, H2'), 1.90 (m, H4a), 2.01 (m, H7).

^{13}C NMR (100 MHz, $CDCl_3$) δ: 15.8 (C8 or C9), 16.0 (C11), 19.3 (C1), 20.1 (C2), 20.2 (C10), 26.1 (C5), 26.7 (C1a), 28.3 (C7b), 28.6 (C8 or C9), 34.6 (C6), 36.3 (C7), 39.6 (C7a), 44.5 (C3), 57.0 (C4a), 72.4 (C4).

MS (EI, GC/MS) *m/z*: 222 (7%), 204 (49%), 189 (44%), 175 (14%), 161 (83%), 147 (36%), 135 (35%), 121 (58%), 109 (81%), 93 (68%), 81 (80%), 69 (77%), 55 (46%), 43 (100%).

Table C1: gCOSY correlations observed for globulol (**37**) in *Dacrycarpus dacrydioides* essential oil distillation and chromatography fraction, Kf6.

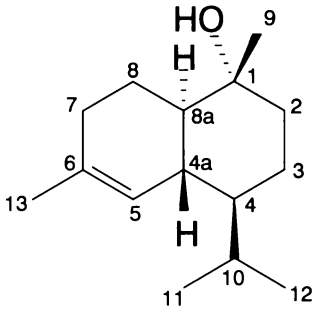
^{1}H	δ (ppm)	Correlated ^{1}H signals
H7b	0.53	H7a
H2	0.90	H1, H2'
H11	0.91	H7
H6	1.24	H5, H6'
H3	1.52	H10

[3] Relative integrals not provided as the ^{1}H NMR spectrum was of a mixture of sesquiterpenoids.

Table C2: HMQC and HMBC correlations observed for globulol (**37**) in *Dacrycarpus dacrydioides* essential oil distillation and chromatography fraction, Kf6.

^{13}C	δ (ppm)	HMQC Correlations, δ (ppm)	HMBC Correlations
C8 or C9	15.8	H8 or H9, 0.98	1.0 ppm (H8 or H9)
C11	16.0	H11, 0.91	H6, H6'
C1	19.3		H8, H9
C2	20.1	H2, 0.90; H2', 1.80	H3, H3'
C10	20.2	H10, 1.1	-
C5	26.1	H5, 1.42; H5', 1.75	H4a, H6'
C1a	26.7	H1a, 0.60	H3, H3', H7b, H8, H9
C7b	28.3	H7b, 0.53	H2, H2', H4a, H7a, H8, H9
C8 or C9	28.6	H8 or H9, 1.0	H7b, 1.0 ppm (H8 or H9)
C6	34.6	H6, 1.24; H6', 1.67	H5', H11
C7	36.3	H7, 2.01	H5, H5', H6, H6', H11
C7a	39.6	H7a, 1.22	H4a, H5', H6, H6', H11
C3	44.5	H3, 1.52; H3', 1.73	H2, H2', H10
C4a	57.0	H4a, 1.90	H3, H3', H5, H7a, H10
C4	72.4		H2, H2', H3, H3', H4a, H5, H7a, H10

α-Cadinol (**38**)[4]



RI (Ultra-2) = 1682 (literature value = 1653 (255));

RI (EC^{TM}-WAX) = 2229 (literature value = 2224 (284)).

^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.73 (3H, d, J = 6.9 Hz, H11), 0.88 (3H, d, J = 6.9 Hz, H12), 1.02 (overlapping with H3, m, H4), 1.04 (overlapping with H9, m, H3), 1.06 (s, H9), (H4, H3, H9, 5H), 1.18 (2H, m, H8, H8a), 1.39 (1H, dt, J = 12.4, 4.0 Hz, H2), 1.56 (1H, m, H3'), 1.62 (overlapping with H4a, s, H13), 1.67 (m, H4a), (H13, H14a, 4H), 1.77 (1H, td, J = 12.4, 3.3 Hz, H2'), 1.94 (overlapping with H8', m, H7), 1.97 (m, H8'), (H8', H7, 3H), 2.12 (1H, m, H10), 5.40 (1H, s, H5).

^{13}C NMR (100 MHz, $CDCl_3$) δ: 15.1 (C11), 20.7 (C9), 21.5 (C12), 21.9 (C3), 22.6 (C8), 23.8 (C13), 25.9 (C10), 30.9 (C7), 39.8 (C4a), 42.1 (C2), 46.6 (C4), 49.9 (C8a), 72.4 (C1), 122.3 (C5), 134.9 (C6).

MS (EI, GC/MS) *m/z*: 222 (7%), 204 (59%), 189 (11%), 161 (55%), 149 (13%), 137 (17%), 121 (83%), 109 (36%), 95 (100%), 81 (34%), 71 (26%), 55 (16%), 43 (57%).

Table C3: gCOSY correlations observed for α-cadinol (**38**) from *Araucaria imbricata.*

^{1}H	δ (ppm)	Correlated ^{1}H signals
H8	1.18	H8', H7
H2	1.39	H2', H3, H3', H9
H3'	1.56	H2', H3
H4a	1.67	H4, H5, H8a
H10	2.12	H11, H12

Table C4: HMQC and HMBC correlations observed for α-cadinol (**38**) from *Araucaria imbricata.*

^{13}C	δ (ppm)	HMQC Correlations, δ (ppm)	HMBC Correlations
C11	15.1	H11, 0.73	H10, H12
C9	20.7	H9, 1.06	H2, H8a
C12	21.5	H12, 0.88	H4, H10, H11
C3	21.9	H3, 1.04; H3', 1.56	H2, H2', H10
C8	22.6	H8, 1.18; H8', 1.97	H7, H8a
C13	23.8	H13, 1.62	H5
C10	25.9	H10, 2.12	H11, H12
C7	30.9	H7, 1.94	H5, H8, H8', H13
C4a	39.8	H4a, 1.67	H5, H8', H8a
C2	42.1	H2, 1.39; H2', 1.77	H3', H9
C4	46.6	H4, 1.02	H2, H2', H3, H3', H5, H11, H12
C8a	49.9	H8a, 1.18	H2, H2', H5, H7, H8, H8', H9
C1	72.4		H2, H2', H8, H8', H9
C5	122.3	H5, 5.40	H4a, H7, H13
C6	134.9		H4a, H7, H8' , H13

[4] Spectral data obtained from pure α-cadinol (**38**) (from *Araucaria imbricata*)

Appendix D: 2D ^{1}H-^{1}H and ^{13}C-^{1}H NMR Spectroscopy Correlations of 4-Nonylcyclohexanone (20)

Table D1: Selected gCOSY correlations observed for 4-nonylcyclohexanone (**20**).

^{1}H	δ (ppm)	Correlated ^{1}H signals
H3/H5	1.30-1.40	H4, H3'/H5', H2/H6
H4	1.66	H3'/H5'
H3'/H5'	2.02	H2/H6

Table D2: HMQC and HMBC correlations observed for 4-nonylcyclohexanone (**20**).

^{13}C	δ (ppm)	HMQC Correlations, δ (ppm)	HMBC Correlations
C15	14.1	H15, 0.85	
C14	22.6	H14, 1.24	H13, H15
C8	27.3	H8, 1.28	H7, H9
C9-C12	29.3-29.7	H9-H12, 1.20-1.30	H8-H13
C13	31.9	H13, 1.24	H14, H15
C3, C5	32.7	H3/H5, 1.30-1.40; H3'/H5', 2.02	H2/H6, H5/H3
C7	35.5	H7, 1.28	
C4	35.9	H4, 1.66	H2/H6, H3/H5
C2, C6	40.8	H2/H6, 2.25-2.38	H3'/H5'
C1	212.5		H2/H6, H3'/H5'

Appendix E: ^{1}H-^{1}H and ^{13}C-^{1}H NMR Correlations for 4-Alkyltropolones

Table E1: Selected gCOSY, HMQC and HMBC correlations observed for 3-carboxy-4-(2-hydroxynonyl)tropolone (**58**).

Signal	δ (ppm)	Correlated ^{1}H signals, δ (ppm)	^{13}C, δ (ppm)	Correlated ^{1}H signals
H9	2.74	H9'	C9	H5
H10	3.95	H9, H9', H11	C10	H9
H6	7.37	H5, H7	C7	H5
			C5	H7, H9, H9'
C11	38.4	H11, 1.55	C6	H5
C9	46.5	H9, 2.74, H9', 2.92	C3, 137.5	H5, H9, H9'
C10	71.8	H10, 3.95	C4, 148.2	H6, H9, H9'
C7	117.4	H7, 7.25	C1, 167.0	H6, H7
C5	132.8	H5, 7.22	C2, 174.2	H7
C6	135.7	H6, 7.37		

Table E2: gCOSY, HMQC and HMBC correlations observed for 4-heptyltropolone (**63**).

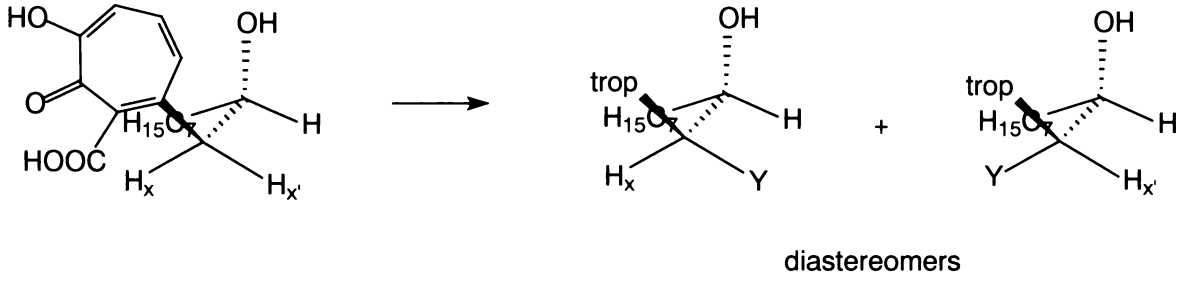
Signal	δ (ppm)	Correlated ^{1}H signals, δ (ppm)	^{13}C, δ (ppm)	Correlated ^{1}H signals
H14	0.88	H13, H12	C14	H13
H9	1.63	H8, H10	C13	H14
H6	7.28	H5, H7	C10-C11	H9, H12
			C9	H8
C14	14.0	H14, 0.88	C12	H9, H11, H13
C13	22.6	H13, 1.32	C8	H3, H5, H9
C10-C11	29.0	H10-H11, 1.35	C7	H5
C9	31.3	H9, 1.63	C3	H5, H8
C12	31.6	H10, 1.30	C5	H3, H7, H8
C8	41.3	H8, 2.61	C6	H7
C7	122.2	H7, 7.21	C4, 154.7	H6, H8
C3	125.3	H3, 7.29	C1-C2, 170.9	H3, H6, H7
C5	129.5	H5, 6.92		
C6	137.0	H6, 7.28		

Table E3: gCOSY, HMQC and HMBC correlations observed for 4-pentyltropolone (**64**).

Signal	**δ (ppm)**	**Correlated ^{1}H signals, δ (ppm)**	**^{13}C, δ (ppm)**	**Correlated ^{1}H signals**
H12	0.91	H11, H10	C12	H11
H9	1.66	H8, H10	C11	H10, H12
H6	7.29	H5, H7	C9	H8, H10
			C10	H9, H11, H12
C12	13.9	H12, 0.91	C8	H3, H5, H9
C11	22.4	H11, 1.35	C7	H5
C9	31.0	H9, 1.66	C3	H5, H8
C10	31.2	H10, 1.35	C5	H3, H7, H8
C8	41.3	H8, 2.63	C6	H7
C7	122.2	H7, 7.23	C4, 154.7	H6, H8
C3	125.3	H3, 7.30	C1-C2, 170.9	H3, H6, H7
C5	129.5	H5, 6.93		
C6	137.0	H6, 7.29		

Appendix F: Non-equivalence of Methylene Protons in Chiral or Prochiral Molecules

For chiral or prochiral compounds which contain methylene protons it is likely that these protons are in non-equivalent environments, regardless of whether rapid bond rotation can occur. This non-equivalence leads to different chemical shifts for the two protons of the methylene group. The subject has been recently reviewed by Parker (346) and Sanders and Hunter (347). To show that superficially identical protons of a methylene group in a chiral compound are formally different a simple substitution test can be applied (347). Using 3-carboxy-4-(2-hydroxynonyl)tropolone (**58**) as an example, the molecule is depicted from the point of view of the methylene carbon (below). The two geminal protons, labelled H_x and $H_{x'}$, are replaced separately with a Y group. In this case the two new molecules generated are diastereomers, hence H_x and $H_{x'}$ are said to be diastereotopic and will have different chemical shifts in the ^1H NMR spectrum. The substitution test can be applied to any molecule containing a methylene group as a test for non-equivalence of methylene protons.



Appendix G: ^{1}H-^{1}H and ^{13}C-^{1}H NMR Correlations for 3-Alkyltropolones

Table G1: gCOSY and HMQC correlations observed for *cis*- and *trans*-3-(1-phenyl-3-ethoxy)prop-2-enyl-6-isopropyltropolone (**84**, **85**).

***Trans* isomer (85)**			***Cis* isomer (84)**		
Signal	**δ (ppm)**	**Correlated ^{1}H signals, δ (ppm)**	**Signal**	**δ (ppm)**	**Correlated ^{1}H signals, δ (ppm)**
H14, H15	1.24	H13	H12	1.17	H11
H12	1.25	H11	H14, H15	1.24	H13
H9	5.18	H8, H10	H9	4.86	H8, H10
H5	6.89	H4, H7	H5	6.89	H4
C12	14.7	H12, 1.25	C12	15.2	H12, 1.17
C14, C15	23.4	H14, H15, 1.24	C14, C15	23.4	H14, H15, 1.24
C13	38.4	H13, 2.84	C13	38.4	H13, 2.84
C8	45.3	H8, 5.37	C8	42.6	H8, 5.79
C11	65.1	H11, 3.76	C11	67.9	H11, 3.79
C9	104.9	H9, 5.18	C9	106.8	H9, 4.86
C7	120.8	H7, 7.30	C7	121.0	H7, 7.18-7.30[1]
C5	126.3	H5, 6.89	C5, C4'	126.0, 126.1	H5, 6.89 & H4', 7.18-7.30[1]
C4'	126.4	H4', 7.18	C2', C6'	127.7	H2', H6', 7.18-7.30[1]
C3', C5'	128.1	H3', H5', 7.21-7.29	C3', C5'	128.2	H3', H5', 7.18-7.30[1]
C2', C6'	128.3	H2', H6', 7.21-7.29	C4	138.4	H4, 7.47
C4	138.5	H4, 7.37	C10	146.0	H10, 6.13
C10	148.8	H10, 6.29			

[1] Signals unresolvable due to overlap with signals of *trans* isomer

Table G2: HMBC correlations observed for *cis*- and *trans*-3-(1-phenyl-3-ethoxy)prop-2-enyl-6-isopropyltropolone (**84**, **85**).

***Trans* isomer (85)**			***Cis* isomer (84)**		
Signal	**δ (ppm)**	**Correlated ^{1}H signals, δ (ppm)**	**Signal**	**δ (ppm)**	**Correlated ^{1}H signals, δ (ppm)**
C12	14.7	H11	C12	15.2	H11
C14, C15	23.4	H13, H14, H15	C14, C15	23.4	H13, H14, H15
C13	38.4	H5, H7, H14, H15	C13	38.4	H14, H15
C8	45.3	H4, H9, H10, H2', H6'	C8	42.6	H4, H8
C11	65.1	H10, H12	C11	67.9	H10, H12
C9	104.9	H8, H10	C9	106.8	H8, H10
C7	120.8	H5, H13	C7	121.0	H5, H13
C5	126.3	H6, H7, H13	C5	126.0	H13
C4'	126.4	H3', H5'	C2', C6'	127.7	H8
C3', C5'	128.1	H4'	C4	138.4	H8
C2', C6'	128.3	H8, H6', H2'	C3	141.3	H4, H8
C4	138.5	H8	C1'	143.6	H8
C3	141.7	H4, H5, H8	C10	146.0	H8, H9
C1'	143.1	H8, H2', H6'	C6	158.1	H4
C10	148.8	H8, H9	C2	170.6	H4, H8
C4	158.3	H4, H7, H13, H14, H15			
C1	168.2	H7			
C2	170.7	H4, H7, H8			

Table G3: HMBC correlations observed for *cis*- and *trans*-3-(1-propyl-3-ethoxy)prop-2-enyl-6-isopropyltropolone (**86**, **87**).

***Trans* isomer (87)**			***Cis* isomer (86)**		
Signal	**δ (ppm)**	**Correlated ^{1}H signals**	**Signal**	**δ (ppm)**	**Correlated ^{1}H signals**
C15	14.0	H13	C15	14.0	H13
C12	14.7	H11	C12	15.1	H11
C14	20.7	H8, H13, H15	C14	20.8	H8, H13, H15
C17, C18	23.4	H16, H18, H17	C17, C18	23.4	H16, H17, H18
C13	37.7	H8, H9, H15	C13	37.5	H8, H9, H15
C16	38.4	H5, H7, H17, H18	C8	37.9	H4, H10
C8	39.8	H4, H10, H13	C16	38.4	H5, H7, H17, H18
C11	65.0	H10, H12	C11	67.6	H10, H12
C9	106.2	H8, H10, H13	C9	108.5	H8, H10
C7	121.7	H5, H16	C7	121.9	H5, H16
C5	126.8	H7, H16	C5	126.6	H7, H16
C4	137.1	H8	C4	137.7	H8
C3	142.1	H5, H6, H8, H13	C3	141.8	H5, H6, H8
C10	147.5	H8, H9, H11	C10	145.7	H8, H9, H11
C6	158.1	H4, H7, H16, H17, H18	C6	157.9	H4, H7, H16, H17, H18
C1	169.3	-	C1	169.6	-
C2	169.8	H4, H7, H8	C2	169.6	H4, H7, H8

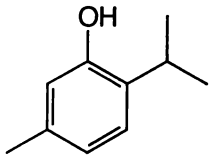
Table G4: gCOSY, HMQC and HMBC correlations observed for 3-(1-ethyl)butyltropolone (**97**).

Signal	**δ (ppm)**	**Correlated ^{1}H signals, δ (ppm)**	**^{13}C, δ (ppm)**	**Correlated ^{1}H signals**
H13	0.78	H12	C13	H12
H11	0.84	H10	C11	H10
H8	3.48	H9, H12	C10	H9, H11
H5	7.01	H4, H6	C12	H8, H13
			C9	H8, H10, H11, H12
C13	11.8	H13, 0.78	C8	H4, H9, H12, H13
C11	14.2	H11, 0.84	C7	H5
C10	20.5	H10, 1.18	C5	H7
C12	28.3	H12, 1.55 & 1.71	C6	H4
C9	37.4	H9, 1.57 & 1.63	C4	H6, H8
C8	42.0	H8, 3.48	C3, 143.0	H5, H8, H9, H12
C7	123.0	H7, 7.34	C1, 170.1	H6, H7
C5	127.4	H5, 7.01	C2, 171.3	H4, H7, H8
C6	136.1	H6, 7.27		
C4	137.0	H4, 7.36		

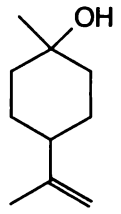
Appendix H: ^{1}H-^{1}H and ^{13}C-^{1}H NMR Correlations for 3-Carboxy-4-styryltropolone (65)

Table H1: gCOSY, HMQC and HMBC correlations observed for 3-carboxy-4-styryltropolone (**65**).

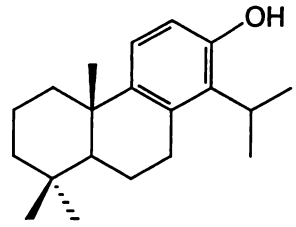
Signal	δ (ppm)	Correlated ^{1}H signal, δ (ppm)	^{13}C, δ (ppm)	Correlated ^{1}H signal
H7	7.28	H5, H6	C7	H5, H6
H9	7.34	H10	C5	H7, H9
H6	7.49	H5	C9	H5, H10
H2' & H6'	7.61	H4', H3' & H5'	C2' & C6'	H4', H10
			C3' & C5'	H5' & H3'
C7	118.3	H7, 7.28	C4'	H2' & H6'
C5	126.8	H5, 7.62	C3, 134.9	H5, H9
C9	127.6	H9, 7.34	C1', 136.7	H3', H5', H9
C2' & C6'	127.7	H2' & H6', 7.61	C10	H2', H6', H9
C3' & C5'	129.3	H3' & H5', 7.45	C4, 143.8	H6, H10
C4'	129.6	H4', 7.40	C1, 168.0	H6, H7
C6	136.4	H6, 7.49	C2, 172.5	H7
C10	137.6	H10, 7.48		



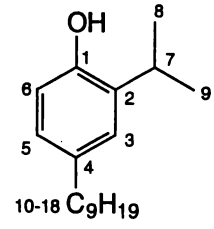
1



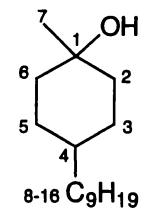
2



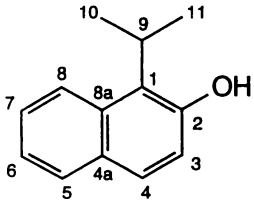
3



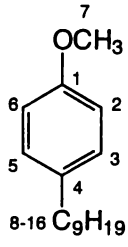
4



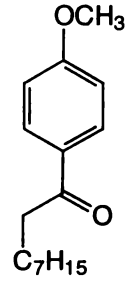
5



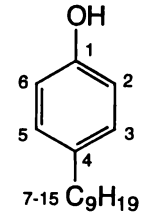
6



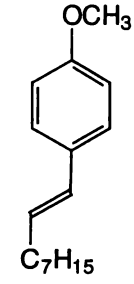
7



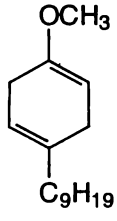
8



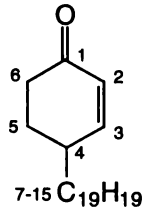
9



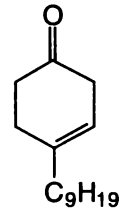
10



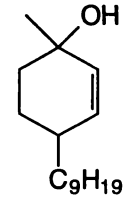
11



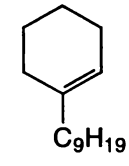
12



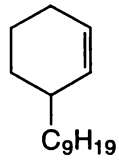
13



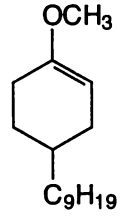
14



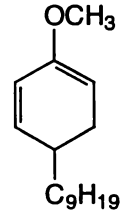
15



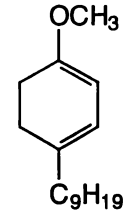
16



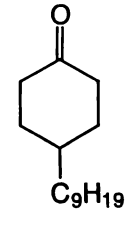
17



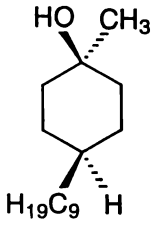
18



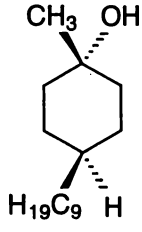
19



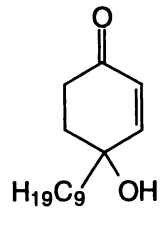
20



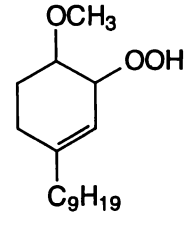
21



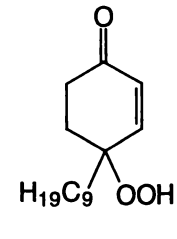
22



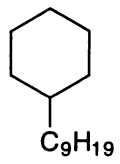
23



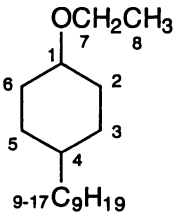
24



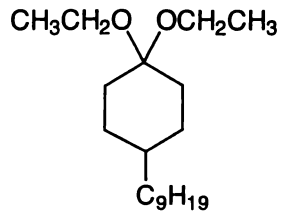
25



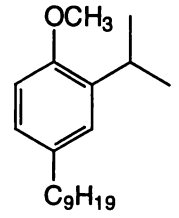
26



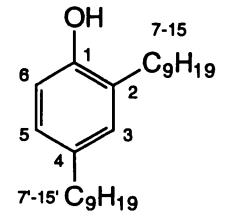
27



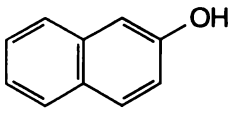
28



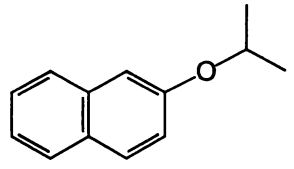
29



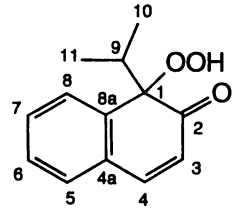
30



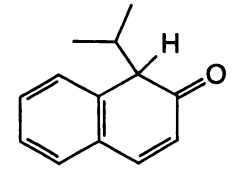
31



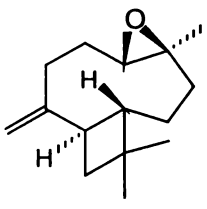
32



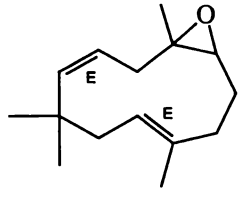
33



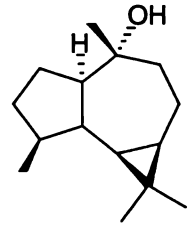
34



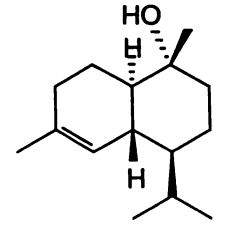
35



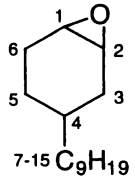
36



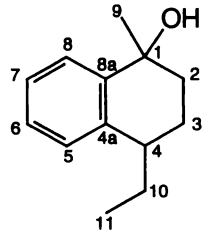
37



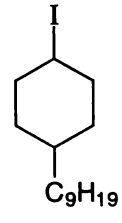
38



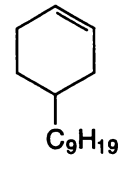
39



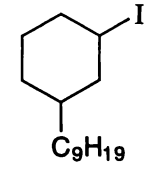
40



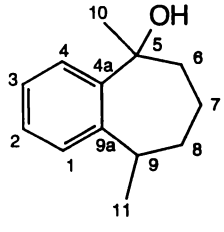
41



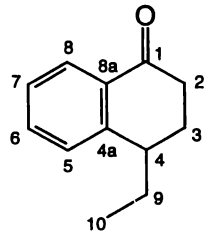
42



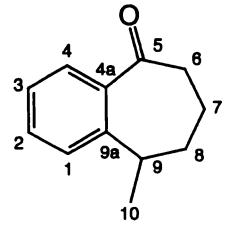
43



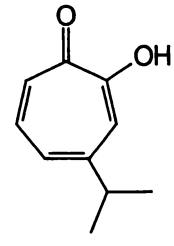
44



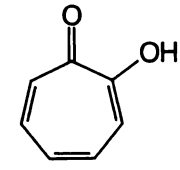
45



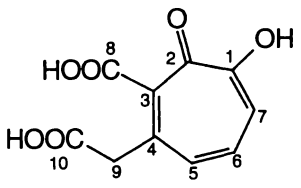
46



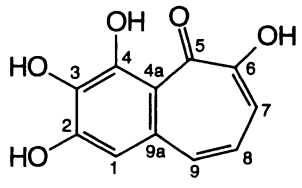
47



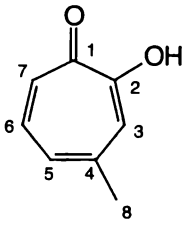
48



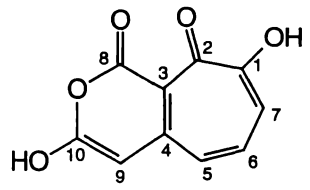
49



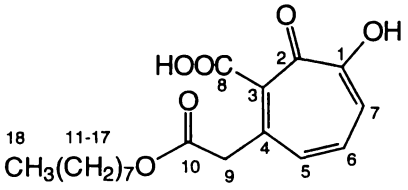
50



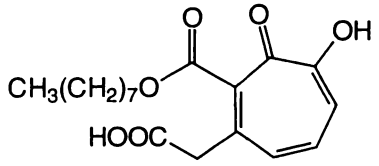
51



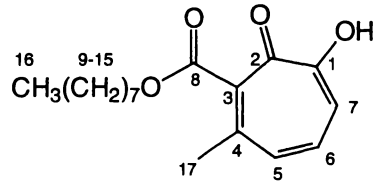
52



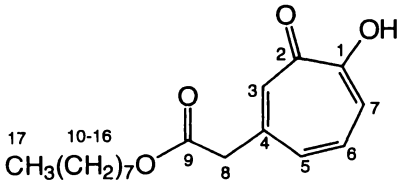
53



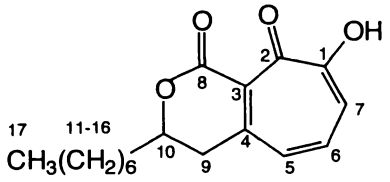
54



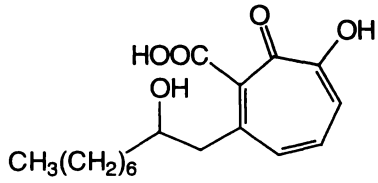
55



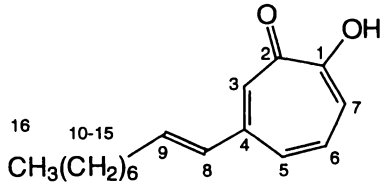
56



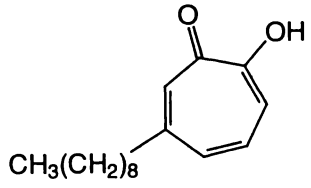
57



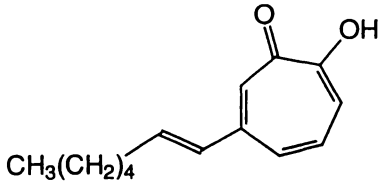
58



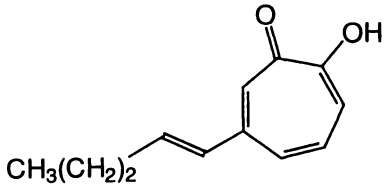
59



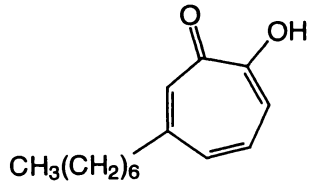
60



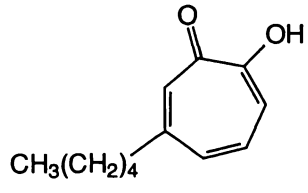
61



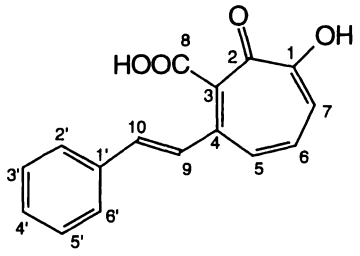
62



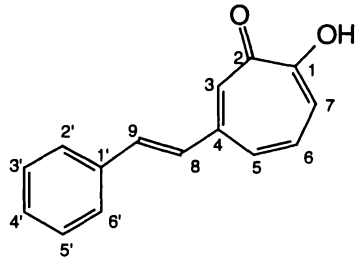
63



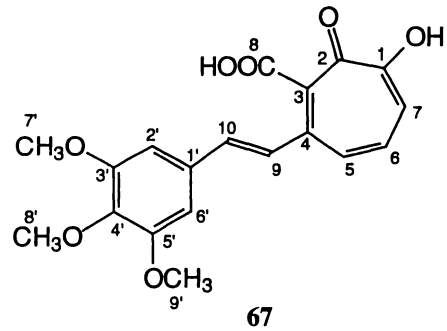
64



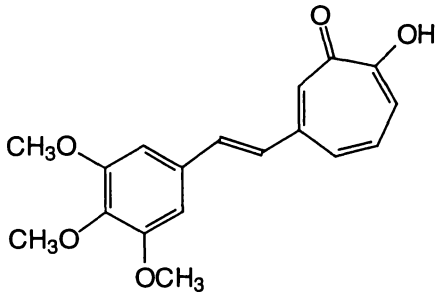
65



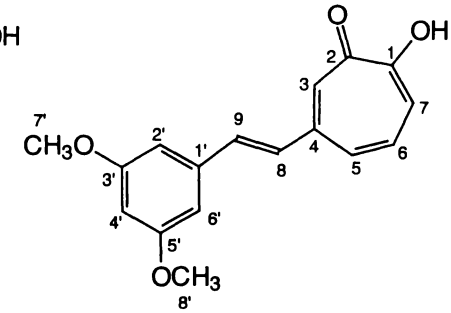
66



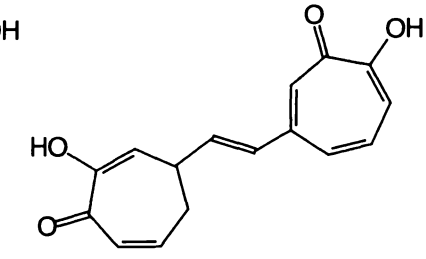
67



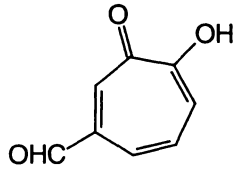
68



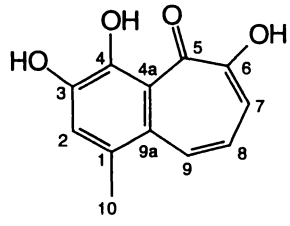
69



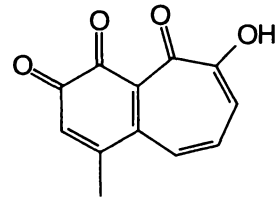
70



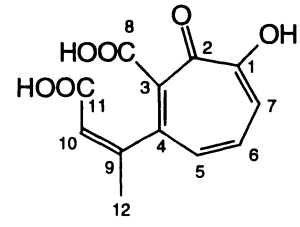
71



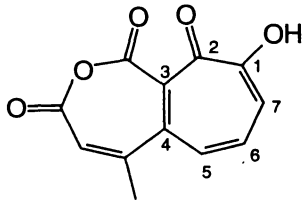
72



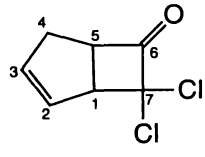
73



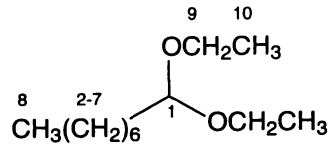
74



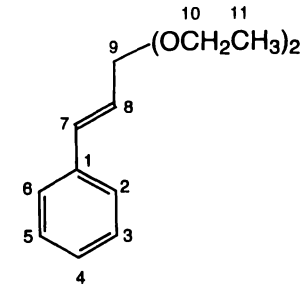
75



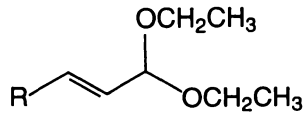
76



77

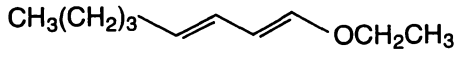


78

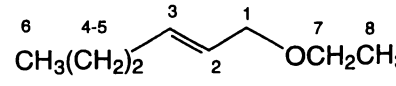


79 R = $CH_3(CH_2)_2$

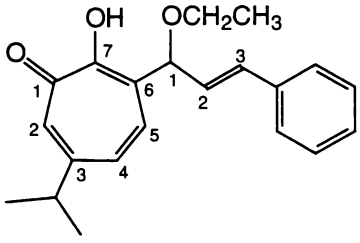
80 R = $CH_3(CH_2)_4$



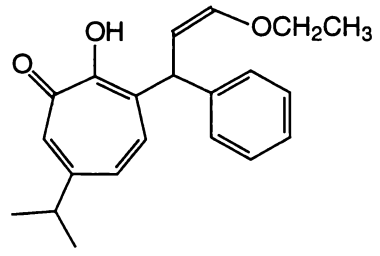
81



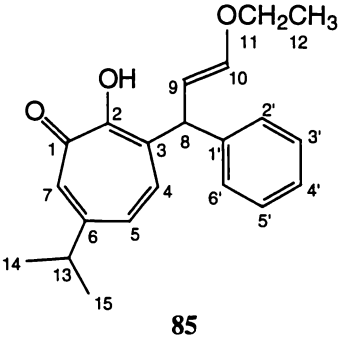
82



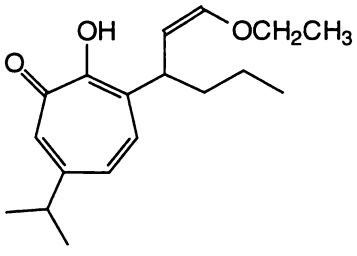
83



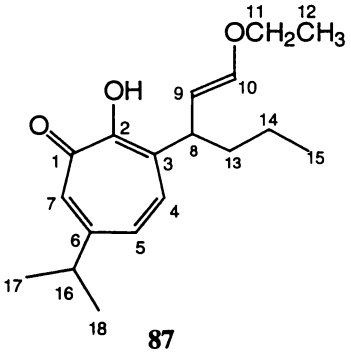
84



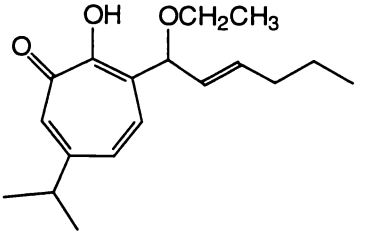
85



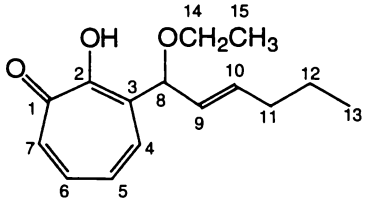
86



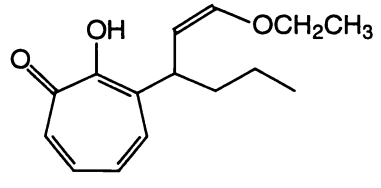
87



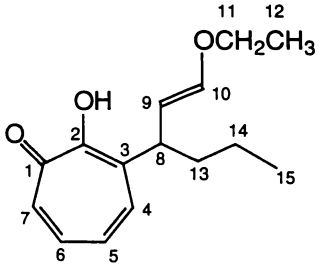
88



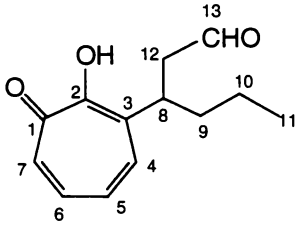
89



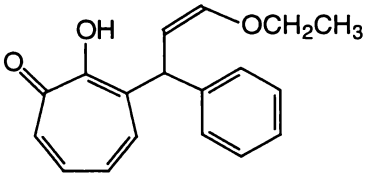
90



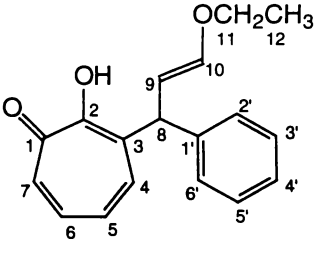
91



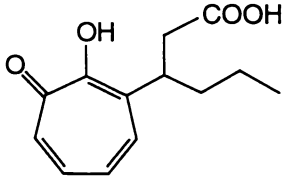
92



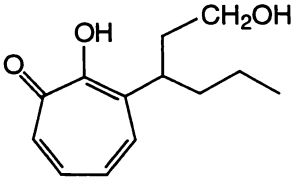
93



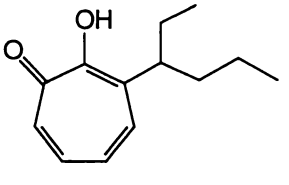
94



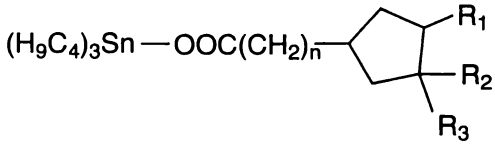
95



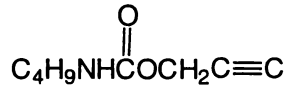
96



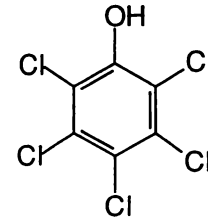
97


(H9C4)3Sn—OOC(CH2)n
R1
R2
R3

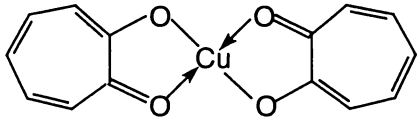
98


O
C4H9NHCOCH2C≡Cl

99


OH
Cl
Cl
Cl
Cl
Cl

100


O
O
Cu
O
O

101