Structural Characterisation and Absolute Stereochemistry of some Degraded Carotenoids from New Zealand Honeys

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Abstract

The structures and absolute stereochemistries of some degraded carotenoids from New Zealand native honeys were elucidated.

Determination of the absolute stereochemistry of 3,5,6-trihydroxy-β-ionone was attempted by an enantioselective synthesis. A racemic synthesis was achieved starting from β-ionone. The key steps involved regioselective hydroboration of 3,4-dehydro-β-ionone, followed by molybdenum-mediated stereoselective epoxidation of the resulting homoallylic alcohol. Ring opening of the epoxide afforded the required trihydroxy-ionone. Synthesis of both enantiomers of 3,5,6-trihydroxy-β-ionone *via* enantio- and regioselective functionalisation of 3,4-dehydro-β-ionone was not achieved. Methods of enantioselective enzyme-mediated kinetic resolution of cyclic homoallylic alcohols were investigated. A satisfactory procedure was not developed.

The absolute stereochemistry of 3,5,6-trihydroxy-β-ionone was determined by analysis of the diastereoisomeric proton chemical shifts of (*S*)- and (*R*)-α-methoxyphenylacetate (MPA) derivatives using the extended Mosher-Trost configurational model. This analysis indicated a 3*S*,5*R*,6*R* absolute configuration, the same configuration as 3,5,6-trihydroxy carotenoids. The natural product was extracted from thyme (*Thymus vulgaris*) honey using established procedures.

Diethyl ether extracts of kamahi (*Weinmannia racemosa*) honey were found to contain three diastereoisomers (kamahines) of an unusual degraded carotenoid with a 14 carbon skeleton. After acetylation, one of the isomers was fully characterised by multidimensional ^{1}H and ^{13}C NMR spectroscopy, and single-crystal X-ray crystallography, indicating the parent alcohol was 4,5-dihydro-1',5-dihydroxy-2',4,8',8'-tetramethylspiro[furan-2(*3*)*H*,7'[6']oxabicylco[3.2.1]oct[2']ene]-4'-one. Comparison of NMR-derived NOE and coupling constant data of kamahine A and B acetates with kamahine C acetate indicated the parent kamahines were C-4, C-5 epimers.

The absolute configurations of the kamahines were determined by NMR and a molecular modeling study (MacroModel) of the diastereoisomeric hemiacetal MPA esters. The results of the determination, coupled with a consideration of crystallographically-determined hemiacetal ester conformations obtained from a

search of the Cambridge Structural Database, supported the use of the Mosher-Trost model for determination of the absolute configurations of hemiacetals. Furthermore, this analysis suggested that the Mosher-Trost model should be amended to take into account a torsion angle of +25–50° between the methine proton and the carbonyl of hemiacetal MPA esters. Kamahines A-C were shown to have the absolute configurations 1'*R*,2*R*,4*R*,5*S*,5'*S*, 1'*R*,2*R*,4*S*,5*S*,5'*S* and 1'*R*,2*R*,4*R*,5*R*,5'*S*, respectively. The absolute configurations of kamahines A-C were consistent with an abscisic acid precursor.

Acknowledgements

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Table of Contents

List of Figures

List of Schemes

List of Tables

List of Abbreviations

ABA	abscisic acid
Ac	acyl
AMAA	arylmethoxyacetic acid or arylmethoxyacetate
ap	antiperiplanar
OAc	acetate
Ar	aryl
br.	broad
CC	*Candida cylindracea*
CD	circular dichroism
CDR	chiral derivatising reagent
CSA	chiral solvating agent
CSR	chiral shift reagent
CI	chemical ionisation
CO	carbonyl
COSY	correlation spectrosopy
$CDCl_3$	deuterochloroform
CD_3OD	deuteromethanol
CSD	Cambridge Scientific Database
1D	one-dimensional
2D	two-dimensional
DEPT	distortionless enhancement by polarisation transfer
$\Delta\delta$	differential chemical shift (ppm)
d	doublet
DCC	dicyclohexylcarbodiimide
DQFCOSY	double quantum filtered correlation spectroscopy
DMAP	4-dimethylaminopyridine
e.e.	enantiomeric excess
EIMS	electron impact mass spectrometry
FT	fourier transform
GB/SA	Generalised Born/surface area (solvation model)
GC	gas chromatography
GCMS	integrated gas chromatography/mass spectrometry
h	hour
HPLC	high performance liquid chromatography

HMBC	heteronuclear multiple bond coherence (long-range ^{1}H-^{13}C correlation)
HMQC	heteronuclear multiple quantum coherence (^{1}H-^{13}C correlation)
HSQC	heteronuclear single quantum coherence (^{1}H-^{13}C correlation)
IR	infrared
IUPAC	international union of pure and applied chamistry
J	coupling constant (Hz)
M^+	the ionised molecule
MCPBA	*m*-chloroperbenzoic acid
MDGC	multidimensional gas chromatography
Me	methyl
MM	molecular mechanics or molecular modeling
MM2*	MacroModel implimentation of the MM2 force field
m.p.	melting point
MPA	α-methoxyphenylacetic acid or α-methoxyphenylacetate
MS	mass spectrometry
MTPA	α-methoxy-α-trifluoromethylphenylacetic acid
m/z	mass/charge ratio
NMR	nuclear magnetic resonance
NOE	nuclear Overhauser effect
OMe	methoxyl
ORD	optical rotatory dispersion
PE	petroleum ether
Ph	phenyl
PLC	preparative layer chromatography
PPL	porcine pancreatic lipase
ppm	parts per million
q	quartet
RT	room temperature
s	singlet
S:N	signal-to-noise ratio
sp	synperiplanar
t	triplet
TLC	thin layer chromatography
UV	ultraviolet
XHCORR	^{13}C-^{1}H correlation spectroscopy

Chapter One

Introduction and Review

1.1. Honey Composition

The flavour and dietary value of honey have made it an important part of the diet of numerous civilisations in recorded history (Willson and Crane, 1975). The taste of honey has been ascribed to the content of sugars, gluconic acid and proline (Maeda *et al.*, 1962), and whilst honeys in general have a characteristic flavour, individual honeys are often very distinctive, each variety exhibiting unique flavour and aroma variations (White, 1975).

The major constituents of honey are carbohydrates and water. Honey also contains a diverse range of minor components including vitamins, minerals, organic and amino acids, and alkaloids, all of which contribute to the overall flavour of honey. Specific volatile constituents such as aromatic compounds, monoterpenes (Graddon *et al.*, 1979; Maga, 1983; Bonaga and Giumanini, 1986; Shimoda *et al.*, 1996), and degraded carotenoids (Tan, 1989; Häusler and Montag 1991; D'Arcy *et al.*, 1997) can contribute to the highly characteristic flavours and aromas of unifloral honeys. These constituents may arise directly from the plant nectar or by some modification of the plant constituents by the bee (Bonaga and Giumanini, 1986).

1.1.1. Extractable Organic Substances from Honeys

The composition of honey has been extensively investigated. Until approximately 1975, most research on the composition of honey was confined to analysis of the major non-volatile components. The determination of sugar, water, acids and enzyme activity in particular, have been necessary in establishing standards for the honey industry. Knowledge of the minor constituents in honey was largely confined to specific groups of compounds such as vitamins, minerals,

and amino acids. Reports of the detailed analyses of major and minor constituents were summarised in a comprehensive review (White, 1975). That review revealed that few studies had been directed towards identification of the volatile components of honeys. Technological limitations were the major reason for this restriction, particularly prior to the development of gas chromatography (GC) in the 1960s.

The use of GC has enabled separation and quantification of components from complex organic mixtures. Characterisation and accurate quantitative measurements improved significantly with the introduction of the capillary column in the early 1980s, and with the use of GC interfaced with mass spectrometry (GCMS) (Baldwin, 1995). The availability of high-field NMR techniques also enabled structural elucidation of components isolated from such complex mixtures. Those advances resulted in an upsurge of interest in the extractable organic components from honeys.

Many authors have investigated the volatile substances contributing to the variability of honey flavour and aroma (e.g. Dörrscheidt and Friedrich, 1962; Merz, 1963; Wootton *et al.*, 1978; Graddon *et al.*, 1979; Bonaga and Giumanini, 1986; Bouseta *et al.*, 1992). Techniques for isolation of volatiles vary from direct vapour sampling and simple solvent extraction, to steam distillation. Those studies identified various low molecular weight alcohols, carbonyls and esters, monoterpenes and aromatic compounds as contributing to the aroma of honey. Early GC work was reviewed by White (1975), Graddon *et al.* (1979), Maga (1983) and Tan (1989).

Total extractable organics were studied by Tan *et al.* (1988) who developed a procedure for the quantitative recovery of volatile and semi-volatile components (i.e. polar phenolic and acidic substances) from New Zealand native honeys. Diethyl ether extracts were obtained from aqueous honey solutions using a continuous liquid-liquid extractor, and were methylated prior to GCMS analysis. In a subsequent study, over 250 components were detected in several New Zealand unifloral honeys using this technique (Tan, 1989). Many components identified in that study had not been previously reported in honey. A number of more recent studies extend the list of extractable organics identified in New Zealand honeys (Wilkins *et al.*, 1993a; 1993b; 1995a; 1995b; Hyink, 1998). Other studies continue to add to the number of known organic compounds in honeys (e.g. Rowland *et al.*, 1995; D'Arcy *et al.*, 1997).

Five major categories of extractable organic constituents are found in honeys. These are the hydrocarbons and fatty acids, aliphatic acids, aromatic derivatives, monoterpenes, and degraded carotenoids (also referred to as *nor*-isoprenoids).

Hydrocarbons and Fatty acids

The hydrocarbon fraction of honeys generally consists of odd numbered n-alkanes (C-21–C-33) and even numbered alkanes at lower concentrations. The major components of the fatty acid fraction include palmitic acid (16:0), lignoceric acid (24:0), oleic acid (18:1) and α-linolenic acid (18:3). Hydrocarbons and fatty acids are believed to be derived from beeswax which has not been separated from the honey during harvest and processing (Graddon *et al.*, 1979; Bonaga and Giumanini, 1986; Tan *et al.*, 1988). Consequently, the composition of this fraction shows little variation amongst honey samples.

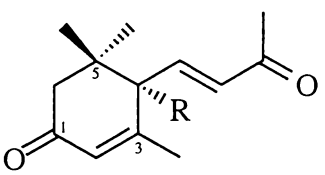
Aliphatic acids, Aromatics and Monoterpenes

Numerous aliphatic monoprotic and diprotic acids constitute the acidic fraction of honey extractives. Some diacids are intermediates in the Krebs cycle and are probably present in the plant nectar. Long chain diacids, identified as part of the pheromone system of the honeybee, have also been detected in honey extractives (Tan *et al.*, 1988). Aromatic carboxylic acids found in honeys, are believed to arise from phenylpropanoid metabolism (Steeg and Montag, 1988; Tan, 1989). Other aromatic compounds include neutral aromatic substances such as alkyl benzenes, benzaldehyde and acetophenone derivatives.

Monoterpenes identified in honeys include pinene and limonene (Bouseta *et al.*, 1992; 1996), and various oxygenated derivatives of limonene, terpinene and menthene (Tsuneya *et al.*, 1974; Blank *et al.*, 1989). Hydroxylated linalool derivatives and lilac alcohols and aldehydes are monoterpenoid constituents of some honeys (Wilkins *et al.*, 1993b). Monoterpenes and aromatics are commonly found in the essential oils of plants (Simonsen, 1953).

Degraded Carotenoids

Prior to the mid 1980s, carotenoid-derived isoprenoids were not recognised as constituents of honey. A significant advance in this type of research was made with the discovery of a range of 3,5,5-trimethylcyclohex-2-en-1-one derivatives (**1-12**) (Figure 1.1) in New Zealand ling heather (*Calluna vulgaris*) honey (Tan, 1989; Tan *et al.*, 1989a; Sun, 1995). Quantities of the dominant components **1** (100-180 μg/g honey) and **2** (27-36 μg/g) were sufficient for structural elucidation by high-field one- and two-dimensional NMR spectroscopy. The structures possessing thirteen and nine carbon atoms bear similarity to the well known plant hormone abscisic acid (ABA), the *trans,cis* **(11)** and *trans,trans* **(12)** isomers of which were also detected in that honey (< 5 μg/g). These degraded carotenoids were suggested to contribute to the distinctive flavour of heather honey.



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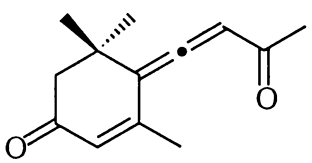

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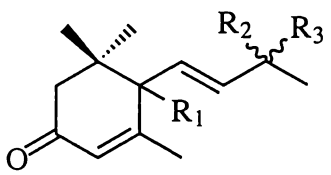
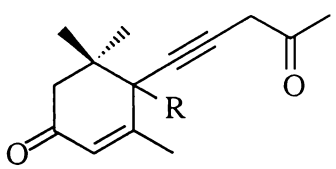


(3) $R_1 = R_2 = OH$, $R_3 = H$
(4) $R_1 = R_3 = OH$, $R_2 = H$
(5) $R_1 = R_2 = H$, $R_3 = OH$



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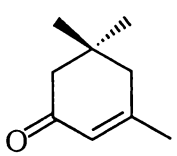

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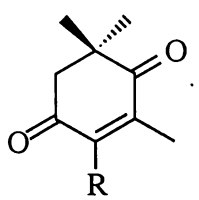

(9) R = H
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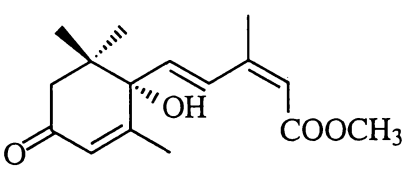

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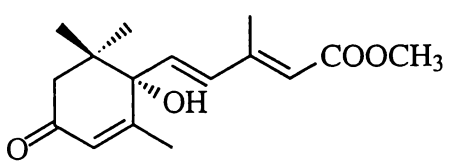


(12)

Figure 1.1. Degraded carotenoids identified in New Zealand ling heather honey.

A concurrent study (Häusler and Montag, 1989) also demonstrated the presence of (*S*)-(+)-dehydrovomifoliol ((*S*)-(+)-(**1**)) in German and French heather (*Calluna*) honeys, in relatively high concentrations. Degraded carotenoids with a 3,5,5-trimethylcyclohex-2-en-1-one ring structure (e.g. **3/4**) have been identified in Asian longan (*Euphoria longana*) honey (Ichimura, 1994).

The distribution of the ubiquitous plant hormone ABA in honeys appears to be dependent on the floral source. ABA isomers (**11**) and (**12**) were found at relatively high levels (40-100 μg/g) in New Zealand willow (*Salix*) honey (Tan *et al.*, 1990), in constrast with low concentrations (< 5 μg/g) found in other New Zealand unifloral honeys (Tan, 1989). Ferreres *et al.* (1996) found ABA isomers in high concentrations (25-166 μg/g) in the phenolic fraction of Portuguese heather honey. It was suggested that Portuguese heather honey (produced from *Erica spp.*) accumulated ABA, while New Zealand heather (*Calluna spp.*) honey accumulated other chemically related derivatives, with ABA isomers as minor constituents. Different extraction techniques were used in those two studies.

Australian native honeys are also a rich source of degraded carotenoids. Tan *et al.* (1989a) noted compounds **8**, **9** and **13** had previously been detected by Graddon *et al.* (1979) in several unifloral Australian honeys, although the structures were incorrectly assigned. More recently, those components, in addition to **1**, were identified in the extractives of Australian leatherwood (*Eucryphia lucida*) honey (Rowland *et al.*, 1995). In that study, they were described as *nor*-isoprenoids. Dehydrovomifoliol (**1**) was also found in an unripe honey sample, consistent with it being a flavour precursor. Vitispiranes and theaspirans (Figure 1.7), flavour components found in quince and vanilla fruits, grapes and fermented plant products like tea and tobacco, are thought to be derived from **1** (Häusler and Montag, 1991). Neither of these groups of compounds have been detected in New Zealand honeys.

Australian blue gum (*Eucalyptus leucoxylon*) and yellow box (*Eucalyptus melliodora*) honeys were reported to contain a number of C-13 *nor*-isoprenoids which had not been previously identified in honey (Sun, 1995; D'Arcy *et al.*, 1997) (Figure 1.2). These included the diastereoisomeric 3,4-dihydro-3-oxoactinidols (**14-17**), 3-oxoretro-α-ionols (**18/19**), megastigma-4-ene-3,9-dione (**20**) and 8,9-dehydrotheaspirone (**21**). Dehydrovomifoliol (**1**) and vomifoliol (**3/4**) were also detected, although they were well below levels which characterise heather honeys (Tan *et al.*, 1989a; Häusler and Montag, 1989; 1991).

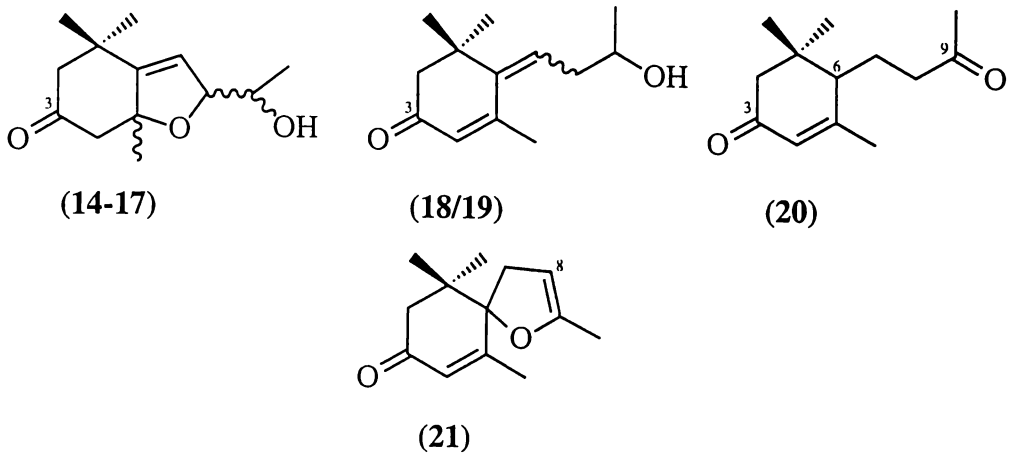


Figure 1.2. C-13 *nor*-isoprenoids identified in Australian native honeys.

The degraded carotenoid, 3,5,6-trihydroxy-5,6-dihydro-β-ionone (**22**), characterises New Zealand thyme (*Thymus vulgaris*) honey (Tan, 1989; Tan *et al.*, 1989b; Tan *et al.*, 1990) (Figure 1.3). The diastereoisomeric epoxides **23/24** were also detected in the extractives and were suggested precursors (one or both) to triol **22**.

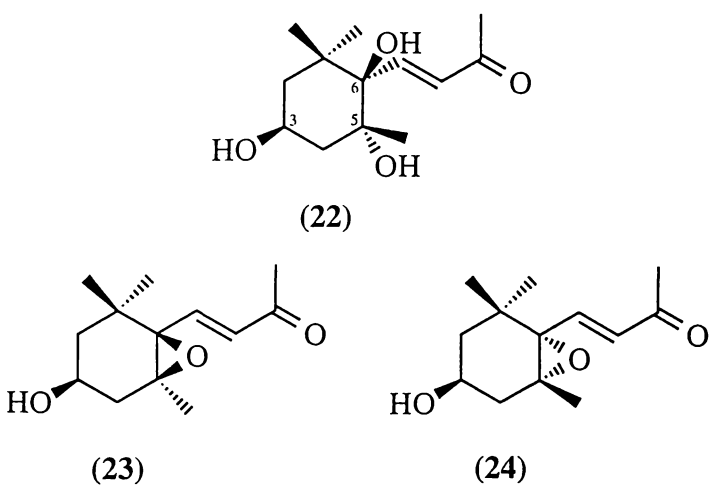
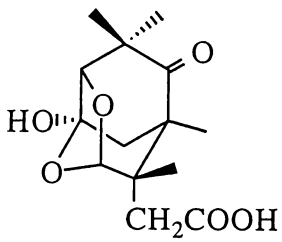


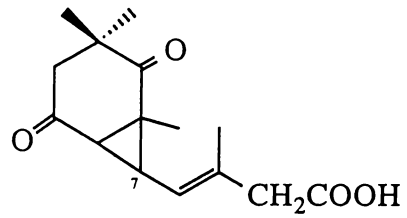
Figure 1.3. Degraded carotenoids found in New Zealand thyme honey.

Several components detected in the extractives of New Zealand kamahi (*Weinmannia*) honey were suggested to be degraded carotenoids (Tan, 1989). Those components exhibited some similarities in mass spectral data to the degraded carotenoids already identified in heather honey. Structural elucidation of one of those components (kamahine (**25**)) is the subject of the present investigation (Chapters Four and Five). Meliracemoic acid (**26**) (Ede *et al.*, 1993) is another degraded carotenoid-like substance found in this honey. More recently, ericinic acid (**27**) and its C-7 epimer have been identified as constituents of New Zealand

erica (*Ericaceae*) honey (Hyink, 1998). Meliracemoic acid (**26**) and ericinic acid (**27**) are unique degraded carotenoids in that they appear to have undergone side-chain migration and rearrangement. Meliracemoic (**26**) acid has a fourteen carbon skeleton.



(**26**)



(**27**)

1.1.2. Origins of Extractable Organic Substances in Honeys

The origin of extractable organic substances in honeys is unclear, as few studies have addressed this aspect of honey research. Some preliminary studies have sought to correlate the chemical constituents of nectar with honey composition (Section 1.2.1). Compounds present in the nectar may undergo enzymatic or chemical transformations during honey maturation. The dependency of some honey constituents on bee physiology (Tan *et al.*, 1988), microbiological activity (Bouseta *et al.*, 1992), or processing and storage conditions (Wootton *et al.*, 1978), has been reported.

Degraded Carotenoids

Degraded isoprenoids constitute a large group of compounds in nature, many possessing highly potent aroma properties. The formation of these compounds has been attributed to the degradation of higher molecular weight terpenoids in plant tissues (Weeks, 1986). Honeys contain a number of *nor*-isoprenoid compounds thought to be derived from cyclic carotenoids.

Carotenoids are a widely distributed group of natural pigments, responsible for many of the brilliant colours in fruit, vegetables, fish, Crustacea, and other plants and animals. These pigments have a vital role as attractants or deterrents in plants and animals, and have other physiological functions which include protection against potentially harmful light or singlet oxygen (Schrott, 1985), and

the transportation of oxygen in the photosynthetic process (Cogdell, 1985; Young, 1993). Plants and microorganisms have the ability to biosynthesise carotenoids, whilst animals obtain these pigments from their diet (Davies, 1985).

The fundamental carotenoid structure consists of a central chain of conjugated bonds, which may be modified by the presence of acyclic or cyclic end-groups at one or both ends. Carotenoid end-groups include the acyclic (ψ) type, and cyclohexene (β and ε), methylenecyclohexene (γ), cyclopentane (κ), and aryl (ϕ and χ) cyclic types, and their derivatives. Examples of these carotenoid end-groups are shown in Figure 1.4. The possibility of geometrical isomerism of the polyene chain in addition to different permutations of end-groups, including oxygen functionalities and stereochemistry, introduces the possibility of a wide range of carotenoid structures. The ability of animals to metabolise carotenoids (Davies, 1985) contributes to the diversity in structures found. Over 600 carotenoids have been isolated from plant and animal sources (Britton, 1996). Several extensive reviews report advances in carotenoid chemistry and biochemistry (e.g. Britton, 1989; 1991; Britton *et al.*, 1995a; 1995b; 1996).

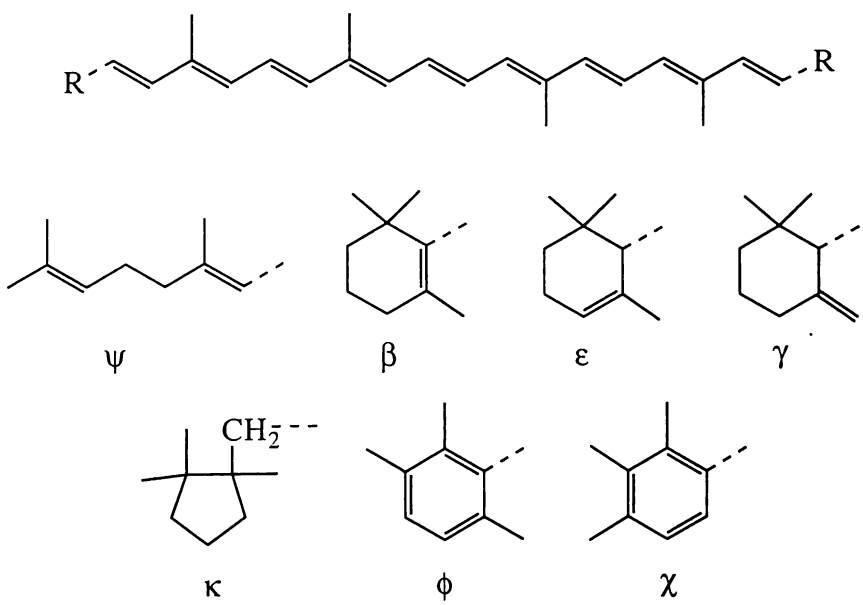
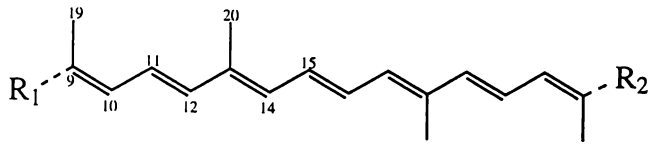
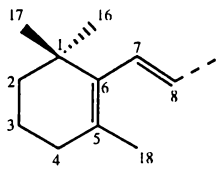


Figure 1.4. The fundamental structure of carotenoids includes a central chain of conjugated double bonds modified by acyclic or cyclic end-groups (R=ψ,β,ε,γ,κ,ϕ,χ).

Carotenoids possessing the β and ε types are the most ubiquitous in nature. β-Carotene (**28**), its dihydroxy derivatives lutein (**29**) and zeaxanthin (**30**), and epoxy derivatives violaxanthin (**31**) and neoxanthin (**32**) (Figure 1.5) are commonly found in the green tissues of plants (Khachik *et al.*, 1991). The

interconversion of zeaxanthin and violaxanthin is believed to be linked to the photosynthetic process, *via* the violaxanthin cycle (Yamamoto, 1979; Young, 1993).





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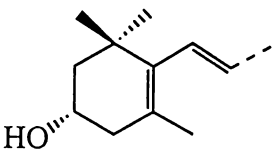

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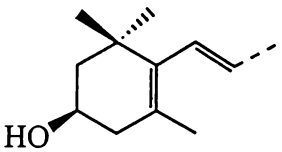

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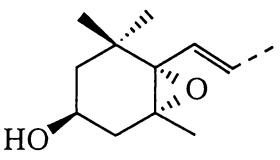

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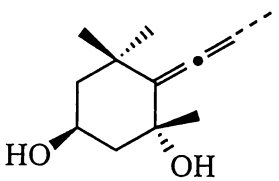


e

(**28**) β-carotene: $R_1 = R_2 = a$

(**29**) lutein: $R_1 = c$, $R_2 = b$

(**30**) zeaxanthin: $R_1 = R_2 = c$

(**31**) violaxanthin: $R_1 = R_2 = d$

(**32**) neoxanthin: $R_1 = d$, $R_2 = e$

Figure 1.5. Carotenoids commonly encountered in nature. The IUPAC numbering system of carotenoids is shown (Weedon and Moss, 1995).

A variety of degradative oxygenations produce shorter chain isoprenoid compounds from carotenoids. In plants, this is believed to occur at the onset of senescence following loss of chlorophyll (Weeks, 1986), and upon ripening (Winterhalter, 1996). *In vivo* cleavage of the carotenoid chain is considered to be catalysed by dioxygenase systems (Enzell, 1985; Wahlberg and Enzell, 1987; Winterhalter, 1996). Site-specific enzymes such as soybean lipoxygenase effect the conversion of β-carotene (**28**) and violaxanthin (**31**) to the corresponding ionones, *via* cleavage of the respective C-9–C-10 bonds. Alternatively, non-site specific enzymes or singlet oxygen may attack any one of the polyene bonds of a carotenoid, the site and rate of attack, to some extent, influenced by ring functionalities.

Degradative oxygenations involve cleavage of C-9–C-10, C-8–C-9, C-7–C-8, and C-6–C-7 bonds of the polyene chain to produce cyclic compounds containing 13, 11, 10, and 9 carbon atoms, respectively (Figure 1.6). Oxidative studies of carotenoids indicate all polyene double bonds are probably vulnerable to attack, although there is a preference for attack on the C-9–C-10 double bond. Support for this observation is the prevalence of C-13 degraded carotenoids in nature (e.g. Wahlberg and Enzell, 1987).

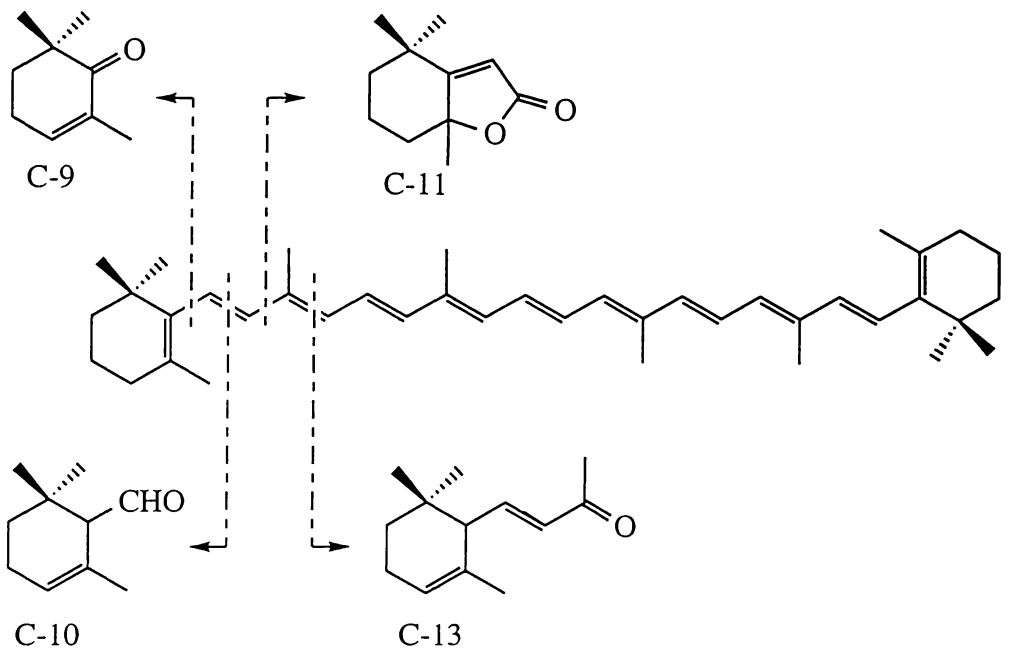
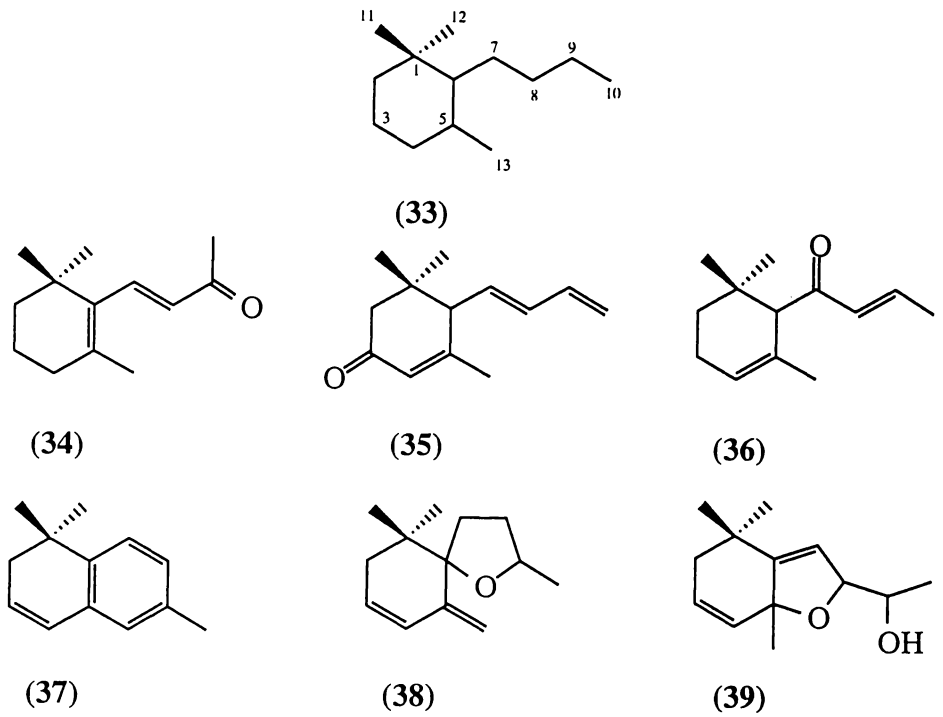


Figure 1.6. The oxidation of β-carotene (**28**) to produce smaller volatile compounds (Weeks, 1986); C-9 cyclohexenone, C-10 cyclocitral, C-11 dihydroactinidiolide and C-13 ionone derivatives.

C-15 plant hormones and C-20 retinoids are other classes of carotenoid derivatives, assumed to be formed by regioselective attack of dioxygenases (Winterhalter, 1996). In higher plants, the degradation of violaxanthin (**31**) and neoxanthin (**32**) is established as a source of the sesquiterpene, ABA (**11/12**) (e.g. Dewick, 1995). Isoprenoids with similar ring substitution to ABA are often referred to as sesquiterepenes (C-15) and *nor*-sesquiterpenes (C-14/C-13) (e.g. Faga, 1994; 1995; 1998).

C-13 degraded carotenoids in particular, exhibit a variety of structures involving both megastigmane (**33**) and rearranged skeletal types. These compounds include the ionones (**34**), megastigmatrienones (**35**), damascones

(**36**), decalines (**37**), vitispiranes (**38**) and actinidols (**39**). Representatives of these types are shown in Figure 1.7. Degraded carotenoids, such as damascone and ionone derivatives, are important aroma constituents which are commercially useful and of considerable economic importance (Weeks, 1986; Winterhalter, 1996).



(**33**) (**34**) (**35**) (**36**) (**37**) (**38**) (**39**)

Figure 1.7. Above: The megatigmane skeleton (**33**) showing numbering characteristic of cyclic carotenoids. Below: Examples of C-13 degraded carotenoid skeletons often encountered in nature: β-ionone (**34**), 4,7,9-meagastigmatrien-3-one (**35**), α-damascone (**36**), 1,1,6-trimethyl-1,2-dihydronaphthalene (**37**), vitispirane (**38**) and actinidol (**39**).

Many of the above carotenoid metabolites can be viewed as arising from C-13 methyl ketones (e.g. (**34**)) formed by initial cleavage of a carotenoid (primary degradation products), by simple reactions such as oxidation, reduction, dehydration, acid-catalysed rearrangement and in some cases, loss of carbon (Enzell, 1985; Weeks, 1986; Wahlberg and Enzell, 1987; Winterhalter, 1996). These reactions may be enzymatic processes occurring in the plant tissues and/or chemcial processes following breakdown of the cell wall. The formation of many non-megastigmanes (e.g. decalines, vitispiranes, theaspiranes, actinidols) is attributed to acid-catalysed cyclisation and rearrangement reactions (Strauss *et al.*,

1986; Sefton *et al.*, 1989 and cited references). These compounds therefore, do not have a direct enzyme biogenesis.

Degraded carotenoids are widespread in nature and previously unknown compounds of these types are regularly reported. A majority have been found in tobacco leaves, or wine and grapes, as these two products have been extensively studied due to their economic importance. More than 80 degraded carotenoids have been found in tobacco, with over 50 of these being C-13 derivatives (Wahlberg and Enzell, 1987). C-13 degraded carotenoids, which are also referred to as C-13 *nor*-isoprenoids, are common constituents of wine (Strauss *et al.*, 1986; Sefton *et al.*, 1989) and other fruits such as apricot purees (Bolzoni *et al.*, 1990) and passionfruit (Winterhalter, 1990). Individual *nor*-isoprenoids are not always unique to particular food products, such as wine or fruit tissues, however collectively, they result in characteristic profiles. New C-13–C-15 *nor*-isoprenoids identified from plant and animal sources are regularly reviewed (e.g. Faga, 1995; 1998; Grayson, 1994, 1997).

The biological role of a majority of carotenoid metabolites remains unknown. Some have been isolated as glycosides suggesting they are true plant metabolites. C-9 ionol glycosides, for example, have been isolated from various plant sources (e.g. Faga, 1994), demonstrating that glycosidation must occur after cleavage of the C-9–C-10 bond in the precursor. Gycosidation is believed to provide a means of transportation within the plant (Enzell, 1985; Wahlberg and Enzell, 1987). Glycosides of C-13 *nor*-isoprenoids (Strauss *et al.*, 1987a; 1987b; Sefton *et al.*, 1989) and monoterpenes (Engel and Tressl, 1983) are well-known components of natural products such as fruits and grape juice.

Carotenoids were not detected in New Zealand honeys found to contain degraded carotenoid-like substances (Tan, 1989; Tan *et al.*, 1989a; 1990). It was suggested that the degraded carotenoids, or their immediate precursors, were of biosynthetic origin and were present in the nectar. However, future studies are required to confirm the absence of carotenoids in these honeys.

Absolute Configuration of Degraded Carotenoids

Optical activity in nature is a consequence of enzyme enantioselectivity. In early investigations of carotenoid chirality, it was found that a particular carotenoid always had the same chirality, irrespective of its source. However, mixtures of

enantiomers, diastereoisomers and racemates are now known to exist amongst carotenoids. This suggests that different enzymes are operative in plants catalysing the stereospecific functionalisation of the parent carotenoid end-groups. The absolute stereochemistries of primary degraded carotenoids examined are identical with their presumed parent carotenoids (Winterhalter, 1996). However, assignment of configuration of a degraded carotenoid, based on the chirality of the assumed carotenoid precursor, may be erroneous.

The enantiomeric composition of natural aroma compounds (secondary metabolites) is known to reflect the enantioselectivity of their biosynthesis. Elucidation of the chiral composition of C-13 degraded carotenoids (*nor*-isoprenoids) provides information about the enzymatic transformations of primary carotenoid degradation products. In many cases, enantiospecific analyses of plant volatiles have shown that these compounds are produced as specific mixtures of optical isomers. This information has implications for biotechnological studies aimed at production of natural flavours. The stereochemistry of a flavour compound can determine its sensory properties as well as its aroma intensity (Winterhalter, 1996).

Stereochemical analysis of C-13 volatiles has become a progressive area of research, particularly since enantiospecific analysis by chromatographic methods has been made possible (Chapter Three). These analyses require preparation of optically pure reference samples, and research has been initiated in this direction (Winterhalter, 1996). Methods of stereoselective synthesis (Chapter Two) and chiral recognition (Chapter Three) are vital to this area of research.

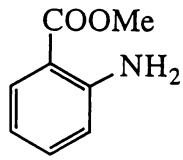
1.2. Floral Source Determination of Honeys

1.2.1. Chemical Means for Floral Source Identification of Honeys

Unifloral honeys possess highly characteristic flavours and aromas due to the presence of specific volatile components. These variables have been investigated as indicators of the geographical and floral origins of honeys.

The floral dependency of certain components in honeys was recognised as early as the 1930s. Before the introduction of gas-liquid chromatography, Nelson

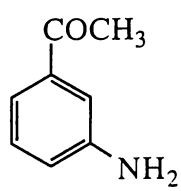
(1930) identified methyl anthranilate (**40**) in orange honey, and noted that it contributed to the distinctive aroma of that honey. Two years later Lothrop (1932) suggested that its presence provided a specific test for orange honey.



(**40**)

It was not until the advent of modern analytical techniques such as GC and GCMS that significant progress was made in this area of research. Dörrscheidt and Friedrich (1962) were first to report the application of GC to analysis of volatile components of honeys. Six honey samples were analysed by direct vapour sampling, and of the 31 components detected, only four appeared to be common. Although none of the components were positively identified, it was suggested that chromatograms might serve as 'fingerprints' for identification of honey type. Various other studies of that nature demonstrated that the extractives of unifloral honeys were chemically different from one another (Wootton *et al.*, 1978; Graddon *et al.*, 1979; Bicchi *et al.*, 1983). However, these studies provided only limited data of the natural honey volatiles and identified few significant volatiles such as potential floral source descriptors.

Bonaga and Giumanini (1986) suggested that an important aroma component of unifloral chestnut honey, 3-aminoacetophenone (**41**), was unique to that floral source. Those authors noted that approximately 40% of the volatiles identified had not been previously reported as present in other honey samples; however similar studies had employed different sample preparation techniques (such as simple solvent extraction or distillation), making speculation as to the reasons for differences between studies premature. It was concluded that the next step in this type of research would be to correlate floral source with the presence of certain compounds, originating either in the nectar or by some modification by the bees.



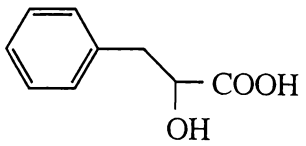
(**41**)

A series of investigations identifying extractable organic constituents was carried out with the objective of floral source discrimination of New Zealand unifloral honeys (Tan 1989; Wilkins *et al.*, 1993a; 1993b; 1995a; 1995b). GCMS analysis of the extractives of over 200 honey samples, collected over four flowering seasons, showed that the distribution and relative concentrations of volatile and semi-volatile components were sufficiently diagnostic to identify the contributing floral sources.

Analyses of honey volatiles obtained by head space analyses or simple solvent extraction indicate that highly volatile components are not sufficiently diagnostic of floral source. Bouseta *et al.* (1992) analysed 84 unifloral honeys (from 14 unifloral sources in 10 countries) by dynamic head space analysis. Those authors concluded that analysis of less volatile compounds, including sesquiterpenes and oxygenated derivatives, was required in order to differentiate honeys.

The investigations of New Zealand unifloral honeys demonstrated that the floral origins could be reliably inferred from substances present in the honey (floral markers), such as aromatics, monoterpenes and degraded carotenoids (Figure 1.8/Table 1.1). For example, manuka (*Leptospermum scoparium)* honey was characterised by high levels of extractable aromatic substances, such as 2-hydroxy-3-phenylpropionic acid (**42**), and lesser levels of 4-hydroxy-3,5-dimethoxybenzoic acid (**43**), acetophenone (**44**) and 2'-methoxyacetophenone (**45**) (Tan *et al.*, 1988; Wilkins *et al.*, 1993a). 1,4-Dihydroxybenzene (**46**) was proposed as a floral marker for vipers bugloss (*Echium vulgare*) honey (Wilkins *et al.*, 1995b). Clover (*Trifolium repens*) honey is characterised by relatively low concentrations of extractable organic components.

Monoterpenes such as hydroxylated linalool derivatives (**47-50**) and lilac aldehydes (**51**) and alcohols (**52**) characterise nodding thistle (*Carduus nutans*) honey (Wilkins *et al.*, 1993b). Rewarewa (*Knightea excelsa)* honey is dominated by acidic substances such as 2-methoxybutanedioic acid (**53**) and 4-hydroxy-3-methyl-*trans*-2-pentenedioic acid (**54**), which are proposed as floral markers for this honey (Wilkins *et al.*, 1995a). Various degraded carotenoid-like substances are indicative of honeys derived from ling heather, thyme and kamahi floral sources (Section 1.1.1). Table 1.1 summarises the characteristic constituents of New Zealand native honeys.



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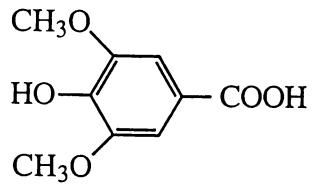

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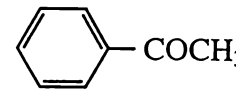

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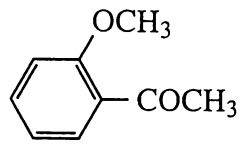

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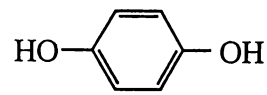

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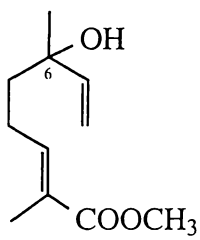

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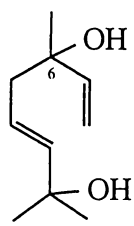

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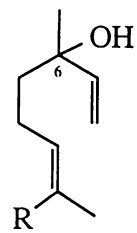

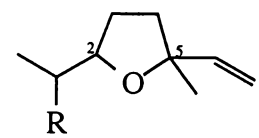

(49) R = CHO (51) R = CHO

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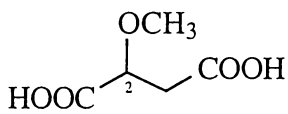

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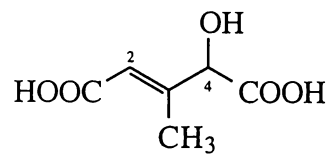


(54)

Figure 1.8. Structures of compounds which characterise some New Zealand unifloral honeys. Degraded carotenoids which characterise other New Zealand honeys are shown in Figures 1.1 and 1.3 (Section 1.1.1).

Table 1.1. Summary of the characteristic extractable organic constituents of native New Zealand honeys.

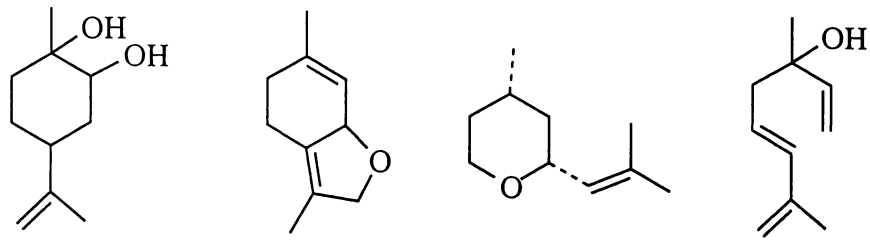
Honey type	Honey characteristics or unique compound(s)	Common range (μg/g)
white clover [a]	low extractable organic substances	total < 50
manuka [b]	2-hydroxy-3-phenylpropionic acid (**42**)	420-1120
	4-hydroxy-3,5-dimethoxybenzoic acid (**43**)	30-46
	acetophenone (**44**)	2-25
	2'-methoxyacetophenone (**45**)	10-40
vipers bugloss [c]	1,4-dihydroxybenzene (**46**)	16-28
nodding thistle [d]	(*E*)-2,6-dimethyl-6-hydroxy-2,7-octadienoic acid (**47**)	6-30
	(*E*)-2,6-dimethyl-3,7-octadiene-2,6-diol (**48**)	1-6
	(*Z*)-2,6-dimethyl-6-hydroxy-2,7-octadienal (**49**)	2-11
	(*Z*)-2,6-dimethyl-2,7-octadiene-1,6-diol (**50**)	2-12
	α,5-dimethyl-5-ethenyl-2-tetrahydrofuran-acetaldehydes (lilac aldehydes) (**51**)	1-5
	β,5-dimethyl-5-ethenyl-2-tetrahydrofuran-ethanols (lilac alcohols) (**52**)	1-3
rewarewa [e]	2-methoxybutanedioic acid (**53**)	2-3
	4-hydroxy-3-methyl-*trans*-2-pentenedioic acid (**54**)	1-4
ling/heather [f]	4-hydroxy-4-(3-oxo-1-butenyl)-3,5,5-trimethylcyclohex-2-en-1-one (**1**)	100-180
	4-(3-oxo-1-butenylidene)-3,5,5-trimethyl-cyclohex-2-en-1-one (**2**)	27-36
	4-hydroxy-4-(3-hydroxy-1-butenyl)-3,5,5-trimethylcyclohex-2-en-1-one (**3/4**)	30-60
	4-hydroxy-4-(3-oxo-1-butynyl)-3,5,5-trimethylcyclohex-2-en-1-one (**6**)	1-2
	4-(3-oxo-1-butynyl)-3,5,5-trimethylcyclohex-2-en-1-one (**7**)	1-2

Table 1.1 continued

thyme [g]	3,5,6-trihydroxy-5,6-dihydro-β-ionone (**22**)	25-110
	3-hydroxy-5,6-epoxy-5,6-dihydro-β-ionone (**23/24**)	1-5
	3-hexanoic acid	4-8
	3'-aminoacetophenone (**41**)	1-5
willow [g]	*trans-cis*-abscisic acid (**11**)	100
	trans-trans-abscisic acid (**12**)	40
	2-methoxy-3,5,5-trimethylcyclohex-2-en-1,4-dione (**10**)	1
kamahi [h]	kamahine (3 diastereoisomers) (**25a**)-(**26c**) [i]	10-150
	meliracemoic acid (**26**) [j]	0.5-10
	unknown (*m/z* 266, 239, 185, 137, 125, 99, 83)	1-10
	unknown (*m/z* 170, 152, 137, 125, 109, 83, 69)	2-10
erica [k]	ericinic acid (**27**)	120

[a] Tan *et al.*, 1988; [b] Tan *et al.*, 1988; Wilkins *et al.*, 1993a; [c] Wilkins *et al.*, 1995b; [d] Wilkins *et al.*, 1993b; [e] Wilkins *et al.*, 1995a; [f] Tan *et al.*, 1989a; [g] Tan *et al.*, 1990; [h] Tan, 1989; [i] Chapters Four and Five of this thesis; [j] Ede *et al.*, 1993; [k] Hyink, 1998.

Various other investigators have reported floral markers for different honey types. As discussed previously, Häusler and Montag (1989; 1991) identified (*S*)-(+)-dehydrovomifoliol ((*S*)-(+)-(**1**)) as a diagnostic constituent of European heather (*Calluna*) honeys, whilst Ferreres *et al.* (1996) reported Portuguese heather (*Erica spp.*) honey was characterised by high levels of *cis*- and *trans*-ABA (**11**)/(**12**). Source-specific monoterpenes have been proposed for linden (*Tilia sp*) honey such as 8-*p*-menthene-1,2-diol (**55**) (Tsuyena *et al.*, 1974), linden ether (**56**) and *cis*-rose oxide (**57**) (Blank *et al.*, 1989) (Figure 1.9). Rowland *et al.* (1995) reported hotrienol (**58**), a principal aroma component in Australian leatherwood (*Eucryphia lucida*) honey, was not found in other unifloral honeys. 8,9-Dehydrotheaspirone (**21**) (Figure 1.2) and 3-oxo-α-ionone (**13**) (Figure 1.1) are indicative of Australian blue gum honey (D'Arcy *et al.*, 1997).



(55) (56) (57) (58)

Figure 1.9. Floral descriptors proposed for different honeys.

Some honey constituents may reflect microbiological activity and processing and storage conditions, rather than the floral contributions of the honeys (e.g. Bouseta *et al.*, 1992). Thus, various attempts have been made to correlate the chemical constituents of the plant to those of the corresponding honey. Tan *et al.* (1988) extracted manuka flowers but did not detect a relationship between those extractives and the manuka honey extract. However, Blank *et al.* (1989) were able to identify the odorant compounds of linden honey in the extracts of linden blossoms.

Rowland *et al.* (1995) showed that the major component in leatherwood honey, hotrienol (**58**), in addition to some of the aromatic components detected in that honey, originated in the nectar. Moreover, comparison of unripe and mature honeys indicated that modification of plant components does take place in the hive and/or during postharvest processing. The occurrence of ABA in both heather honey and floral nectar was reported by Ferreres *et al.* (1996).

Other reports indicate the presence of compounds which probably originate directly from the floral source. Terpenes commonly found in plant essential oils were found amongst volatiles of Georgian (Tschogowadse *et al.*, 1973) and Hungarian (Tóth *et al.*, 1987) honeys. Some plant toxins have been identified in honey, such as atropine from *Datura stramonium* and scopolomine from Egyptian henbane (*Datuna metem*) (White, 1975). Echium honey was shown to contain pyrrolizidine alkaloids found in *Echium plantagineum* (Culvenor *et al.*, 1981).

The above studies demonstrate the potential of chemical analysis, particularly that of volatile and semi-volatile components, for authenticating the floral origins of honeys. As a result, identification of floral markers is one of the primary objectives of the apiculture industry (Bouseta *et al.*, 1996). Similarly,

analysis of volatile flavour components is being investigated as a possible means of characterising wine varieties (Rapp, 1995).

Various other methods of chemical fingerprinting honeys have been proposed. GC analysis of honey amino acids was reported as a means of identifying the geographical origins of honeys but not floral origins (Davies, 1975, 1976; Bosi and Battaglini, 1978; Gilbert *et al.*, 1981). Flavonoid analysis has been used as a tool for studying the geographical origins of honeys and is currently being investigated as a means of identifying floral contributions (Ferreres *et al.*, 1991; Martos *et al.*, 1997). Conte *et al.* (1998) demonstrated that chemometric evaluation of chemical parameters including free amino acid composition, sugar content and pH allowed discrimination of honey floral sources.

1.2.2. Floral Source Determination of New Zealand Commercial Honeys

Identification of honey type is usually based on assessment of flavour, colour, aroma and on the season and location of production. Until recently, melissopalynology, the pollen analysis of honey (Maurizio, 1975), was the only objective method available for determining the geographical and floral origin of honeys.

In contrast to European practice, pollen analysis was not used in New Zealand until the mid 1980s. This was due to the negligible volumes of honey exported, and the dominance of clover honey in the domestic market (Moar, 1985). As interest in export of characteristic New Zealand honeys increased, it became clear that customer requirements, including those of product quality, needed to be satisfied. Pollen analysis provided apiarists with guidelines for monitoring the quality of their product, as well as meeting the requirements for microscopic examination occasionally demanded by importing countries. New Zealand annual honey production exceeds 9000 tonnes, of which about 3000 tonnes are exported. Major importing countries include Japan, Germany, Europe and the United States. For the year 1996-1997, export earnings from all honeys (excluding honeydew and beeswax) exceeded NZ $15 M (Reid, M; Ministry of Agriculture and Forestry, Hamilton, New Zealand; personal communication, 1998).

A detailed discussion on the use of pollen microscopy for determination of floral origins of honeys is reported by Maurizio (1975). This method affords the

percentage floral purity of a honey based on pollen type. Identification and quantification of pollen grains in honey sediment allow conclusions to be drawn as to the sources of the raw material because most of the pollen present in honey originates in the nectar and is characteristic of its origin.

The percentages of different pollens in the honey sediment do not necessarily reflect the proportions of nectar contributed to the resultant honey by the individual plant species. Differences in floral structure and biology, the water content of the nectar, distance between hive and forage source, and beekeeping practice are factors which may influence the number of pollen grains found in a unit volume of honey (Maurizio, 1975). In an attempt to correct for these factors, estimates are made of the number of pollen grains in a unit volume of nectar, and a correction factor is applied to the results of pollen counts in the honey sediment (Maurizio, 1975).

In New Zealand, standards have been established that define the minimum frequency of the predominant pollen considered to qualify a honey as being a unifloral honey of that species. A unifloral honey is derived mainly from one species, but not exclusively so (Louveaux *et al.*, 1978). In most unifloral honeys, the principal pollen type is predominant and therefore presents in frequencies of 45% or more. Over-represented honeys however, exhibit considerably higher frequencies whilst under-represented honeys, lower frequencies. Samples are then compared against these standards to determine whether the honeys being tested may be considered unifloral (Moar, 1985).

An element of uncertainty remains, in that the standards themselves may not be unifloral. Moar (1985) suggested that further determinations of 'unifloral' honeys be carried out, particularly for under-represented (citrus and rewarewa) and over-representred (kamahi and manuka) honeys. Ideally, correction factors as used in other countries could be determined experimentally, but to date such factors have not been determined. More recently, an exporter of New Zealand unifloral honeys (Airbourne honeys, Christchurch) has set standards, such as colour, conductivity, pH, and pollen counts, for these honeys (Wilkins A. L., Waikato University, Hamilton, New Zealand; personal communication, 1998).

Although pollen analysis is generally a reliable method when used by experts with the necessary experience and judgement, the technique is tedious and alternative methods for characterising honeys have been sought for some time (Tan *et al.*, 1989c). Chemical analyses represent potentially useful alternatives, able to

recognise the presence of floral sources contributing significant amounts of nectar but relatively little pollen (i.e. under-represented honeys). An objective method of honey certification is particularly important in the case of unifloral honeys which command high market premiums, such as New Zealand thyme honey (Tan *et al.*, 1990).

Floral markers are proposed as a means of identifying honey floral source within a given geographical area. Chemical discrimination of honeys is based on the recognition of substances dependent on the floral source (floral markers), which can be quantitatively identified from extractives of the honey samples (e.g. Tan 1989). At present, calculation of the percentage contribution from individual floral sources by this chemical procedure is not possible; nevertheless generalisations as to the level of floral input (high, medium or low) can be made. Future applications of the GC technique for New Zealand native honeys are expected to yield a catalogue of substances against which floral source integrity can be assessed (Table 1.1) (Tan *et al.*, 1989a; 1990; Wilkins *et al.*, 1993a; 1993b; 1995a; 1995b; Sun, 1995; Hyink, 1998).

The GC technique outlined by Tan (1989) could solve, at least in part, the complications arising from pollen analysis of New Zealand honeys discussed previously, particularly in the case of those which exibit under- or over-represented pollen grain counts. For under-represented honeys such as nodding thistle, heather, willow and thyme, the chemical approach utilising floral marker components (Table 1.1) would give an improved indication of the contributing nectar sources. Similarly, chemical analyses are being investigated as a means of authenticating lavender (*Lavandula angustifolia*), citrus (*Citrus spp.*) and rosemary (*Rosmarinus officinalis*) honeys, for which the pollen yields are low (Bouseta *et al.*, 1992). For over-represented honeys such as manuka for which pollen counting is reliable, floral marker compounds would be less useful.

Although the detailed interpretation of chromatograms is no easier than analysis of organoleptic or pollen profiles, the chemical procedure is a reliable procedure for the discrimation of high purity (unifloral) and low purity honeys (Tan *et al.*, 1989c). A further advantage of the chemical procedure is the detection of 5-hydroxymethyl-2-furfural and ethyl ester components recovered in the honey extractives, which reflect the state of honey in terms of excessive heating in processing and storage or, fermentation, respectively (Tan, 1989).

1.3. Aims of Present Study

In order to confirm the reliability of a chemical technique for the identification of floral source as outlined previously, it is necessary to structurally characterise particular marker compounds, and establish that they are indeed characteristic for each floral source. The general utilisation of organic extractive profiles for characterising the floral source of honeys requires the location of a peak or peaks in the chromatogram that are associated with a particular floral source. Different compounds can have the same retention times under the same standard conditions; therefore structural elucidation of these compunds is required before GC can be utilised for this purpose.

Degraded carotenoids (or *nor*-isoprenoids), in particular, are potentially useful as floral markers (Tan 1989; Tan *et al.*, 1989a; 1990). To be used in this manner, identification must be complimented by an understanding of their means of entry into the honeys containing them. Determination of the source of these compounds requires structural and stereoochemical characterisation. Stereochemical information may help determine whether these compounds have a direct enzyme biosynthesis or, are the products of chemical processes within the honey.

Tan (1989) detected a large number of components in New Zealand honeys and identified most by their mass spectra. However, for a number of compounds, the mass spectra did not correspond with those of any previously reported compounds in honey or to compounds recorded in the NBS Mass Spectral Database. Some of these compounds were isolated from bulk extracts of the honey by preparative layer chromatography (PLC) and were characterised by other techniques such as 1- and 2D NMR, and in one case, X-ray crystallography. However, the structures of a number of components, some of which were potential floral markers, still remained uncertain.

The present study is an extension of the work described by Tan (1989), and centres on the structural and stereochemical elucidation of some of the unique components in New Zealand honeys. The first aim of this study was to determine of the absolute stereochemistry of the degraded carotenoid 3,5,6-trihydroxy-5,6-dihydro-β-ionone (**22**). Development of an enantioselective synthesis was planned, and Chapter Two of this thesis evaluates synthetic routes to this compound.

Chapter Three discusses elucidation of the absolute stereochemistry of carotenoids and their derivatives, and determines the absolute stereochemistry of ionone **22** using the NMR method and chiral derivatives (Dale and Mosher, 1973; Trost *et al.*, 1986).

The structural characterisation of three unknown components from kamahi honey, by NMR and X-ray crystallography, is described in Chapter Four, and determination of the absolute stereochemistry of these compounds is discussed in Chapter Five.

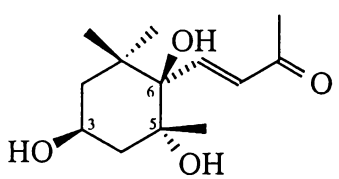
Chapter Two

Synthetic Studies Towards 3,5,6-Trihydroxy-5,6-dihydro-β-ionone

2.1. Introduction

The general utility of organic extractive profiles for characterising floral sources of New Zealand honeys was discussed in Chapter One. The basis for this method is the identification of components in the diethyl ether extractives of honeys, which are recognised to be associated with one floral type. Amongst the components which are of interest as floral markers for these honeys are a range of degraded carotenoids.

Tan *et al.* (1989b; 1990) reported the isolation and characterisation by NMR and X-ray crystallography, of the novel degraded carotenoid **22**, recovered from the ether extracts of thyme honey. Although a variety of dihydro-β-ionone derivatives possessing 3-hydroxy-, 3-hydroxy-5,6-epoxy- and 5,6-dihydroxy-substitution had been isolated from various sources (e.g.Wahlberg and Enzell, 1987), this was the first report of a 3,5,6-trihydroxy-substituted derivative.

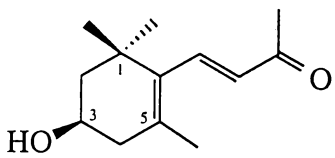


(22)

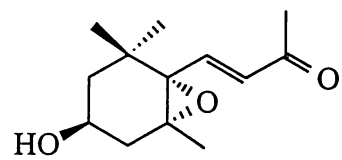
The occurence of triol **22** is confined to honey samples which include a thyme component. Tan *et al.* (1989b; 1990) indicated that the floral integrity of New Zealand thyme honey could be verified by identification and quantification of **22** in the ether extracts of that honey. Thyme honey commands a substantial premium in the market place, and it is not unknown for honeys of uncertain floral integrity to be described as thyme honey (Tan *et al.*, 1990). The use of a chemical

procedure alongside pollen microscopy is advantageous, particularly when applied to honeys such as thyme, which exhibit under-represented pollen counts (Moar, 1985).

A possible biosynthetic route for triol **22** proceeds *via* the 3-hydroxy- or 3-hydroxy-5,6-epoxy-5,6-dihydro-β end-group of a carotenoid (structures **59** and **24**). Carotenoids such as zeaxanthin (**30**) and violaxanthin (**31**) which possess those end-groups are widely distributed in nature (Chapter One). Carotenoids with trihydroxy end-groups, although less common, have been found in flowers and fruit (Eugster, 1985).



(**59**)



(**24**)

The absolute configurations of many carotenoids have been determined by chemical, chiroptical (circular dichroism), NMR and X-ray studies (Chapter Three). Those studies confirm xanthophylls with 3-hydroxy-, 3-hydroxy-5,6-epoxy- and 3,5,6-trihydroxy-5,6-dihydro-β end-groups occur predominantly as single enantiomers. However, epimers, diastereoisomers, racemates and meso forms are more widespread than previously thought, particularly in the animal kingdom (Eugster, 1995). Thus, determination of the absolute configuration of degraded carotenoid **22** was of interest in the present study.

In a previous study, the triol **22** crystallised in the optically active space group $P2_12_12_1$, although it was not possible to determine the absolute configuration from the crystallographic data (Tan *et al.*, 1990). To assist in determining the absolute stereochemistry, an enantiomeric synthesis was planned. This Chapter evaluates synthetic routes to both enantiomers of **22**.

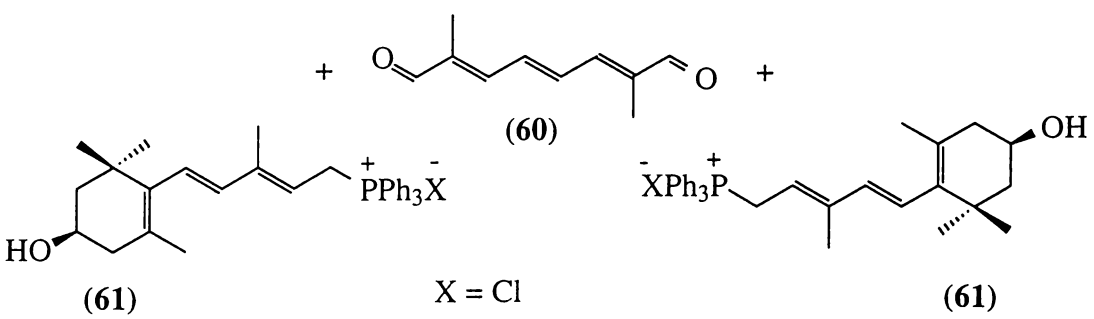
2.1.1. Synthesis of Cyclic Carotenoids

Over 600 naturally occurring carotenoids have been identified to date, many of which possess chiral groups and occur in optically active forms (Britton *et al.*, 1995a). The total synthesis of optically active carotenoids in particular, has presented a challenge to the skill and ingenuity of the synthetic organic chemist.

Methods to construct the polyene chain and to couple end-groups with a central building block are well established, the main challenge being the design of routes to new end-groups exhibiting defined chirality.

The introduction and control of chirality during a synthesis, especially with molecules possessing more than one chiral centre, is an important but also difficult task. Here, the application of new methods and modern physical techniques, areas which have progressed significantly in recent years, have become increasingly important. Reviews which cover all aspects of carotenoid research, including synthesis are regularly published (Britton, 1989; 1991; Britton *et al.*, 1995a; 1995b; 1996).

A number of different strategies for the syntheses of optically active cyclic carotenoids have been published. Most syntheses involve preparation of chiral components (usually C-9 or C-10) possessing the absolute configuration of the target molecule, which are then connected to the reactive polyene chain at the end of the synthesis. Functionalisation of a ring already bearing an olefinic chain fragment usually results in poor yields, due to the chemically labile polyene chain, and the neccessity to purify products by extensive chromatography (Widmer, 1985; Soukup *et al.*, 1996). A common approach to synthesis of the carotenoid skeleton is the double Wittig condensation (or alternatively, the Julia sulphone coupling), according to the C-15 + C-10 + C-15 principle (Scheme 2.1). This route makes use of the readily available C-10-dialdehyde **60**.



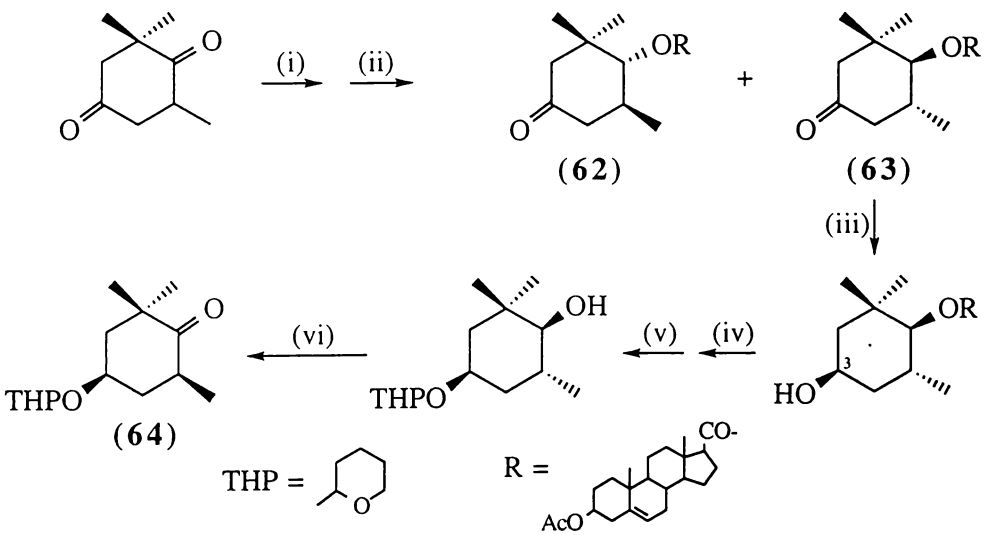
Scheme 2.1. The double Wittig condensation demonstrates the C-15 + C-10 + C-15 principle which is commonly adopted for synthesis of C-40 carotenoids.

Enantiomeric precursors (C-9 or C-10) are prepared either from natural sources, by optical resolution of diastereoisomeric derivatives, enzyme-catalysed processes or asymmetric syntheses. In many syntheses, these cyclic precursors are then transformed to C-15 phosphonium salts (e.g **61**) *via* C-13 ionones. In the

following discussion, syntheses of carotenoid end-groups (ionones) which are structurally related to triol **22** are described.

Preparation of Chiral 3-Oxygenated and 3,5,6-Trioxygenated-β End-Groups

Cyclic end-groups with oxygenation at C-3[1] are precursors to 3-hydroxy-, 3-hydroxy-5,6-epoxy- and 3,5,6-trihydroxy-5,6-dihydro-β-substituted carotenoids. Various approaches have been adopted to introduce chirality at C-3. The first reported synthesis of an optically active 3-hydroxy-β end-group featured a resolution step (Mori, 1974) (Scheme 2.2). A steroidal acid, 3β-acetoxy-etienic acid (RCl), was employed to give the mixture of diastereoisomeric ketones **62** and **63**. Subsequent stereospecific reduction of diastereoisomer **63** using LiAlH(*t*-BuO)$_3$ introduced the chiral hydroxyl group at C-3. However, the resolution step, which involved derivatisation in low yield, led to poor yields of precursor **64**.

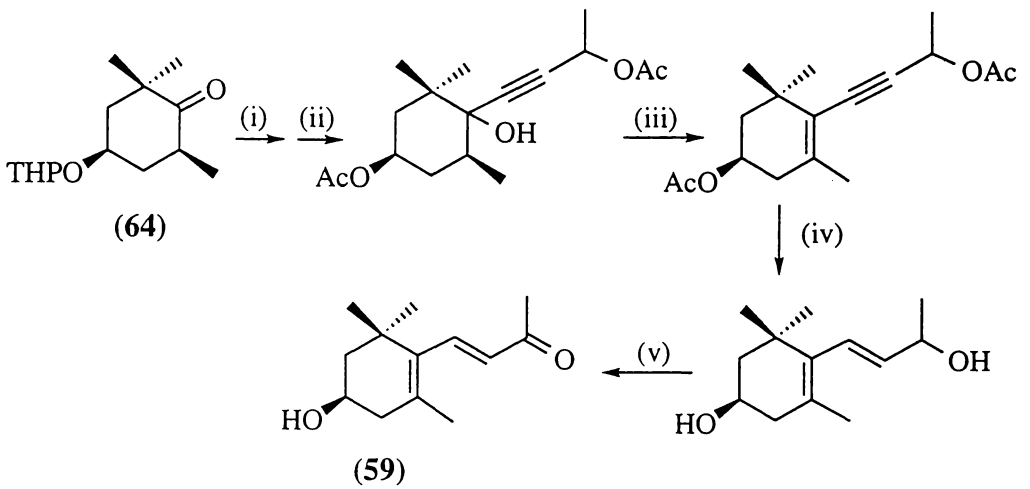


Reagents: i) H_2/PtO_2, 76%; ii) RCl, 40%(total); iii) LiAlH(*t*-BuO)$_3$, 78%; iv) DHP/TsOH, 75%; v) $LiAlH_4$, 98%; vi) CrO_3/Al_2O_3, 67%.

Scheme 2.2. Introduction of chirality at C-3 *via* resolution and subsequent stereospecific reduction.

[1] To avoid confusion, the numbering of all carotenoid precursors herein corresponds to IUPAC carotenoid nomenclature (for the most recent publication of carotenoid nomenclature, see Weedon and Moss (1995).

Mori (1974) prepared (*3R*)-3-hydroxy-β-ionone (**59**) from the C-9 precursor (**64**) in five steps (Scheme 2.3). Addition of but-3-yn-2-ol THP ether was achieved in good yield under Grignard conditions. Analogous methods of chain lengthening have been described, in addition to various step-wise routes. In general, the latter approaches require a larger number of steps and are a less efficient means of chain lengthening. Methods of chain lengthening have been extensively reviewed (Mayer, 1979; Widmer, 1985; Britton *et al.*, 1996).



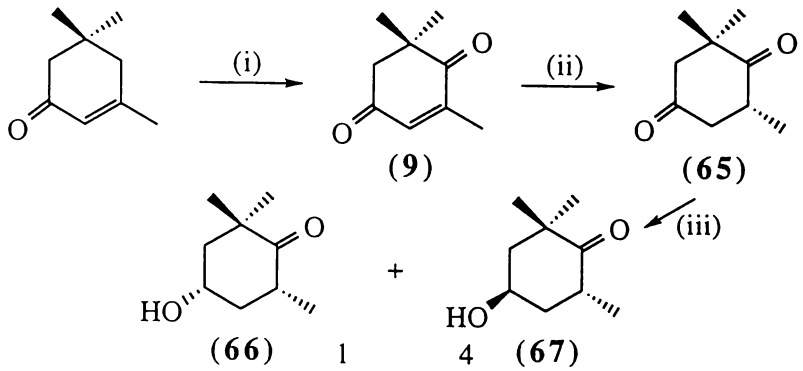
(**64**)

(**59**)

Reagents and Conditions: (i) $HCCCH(CH_3)OTHP$/EtMgBr, *p*-TsOH/MeOH, 100%; (ii) Ac_2O/pyridine, 97%; (iii) $POCl_3$, 37%; (iv) $LiAlH_4$, 97%; (v) DDQ, 44%.

Scheme 2.3. Synthesis of (*3R*)-3-hydroxy-β-ionone (**59**).

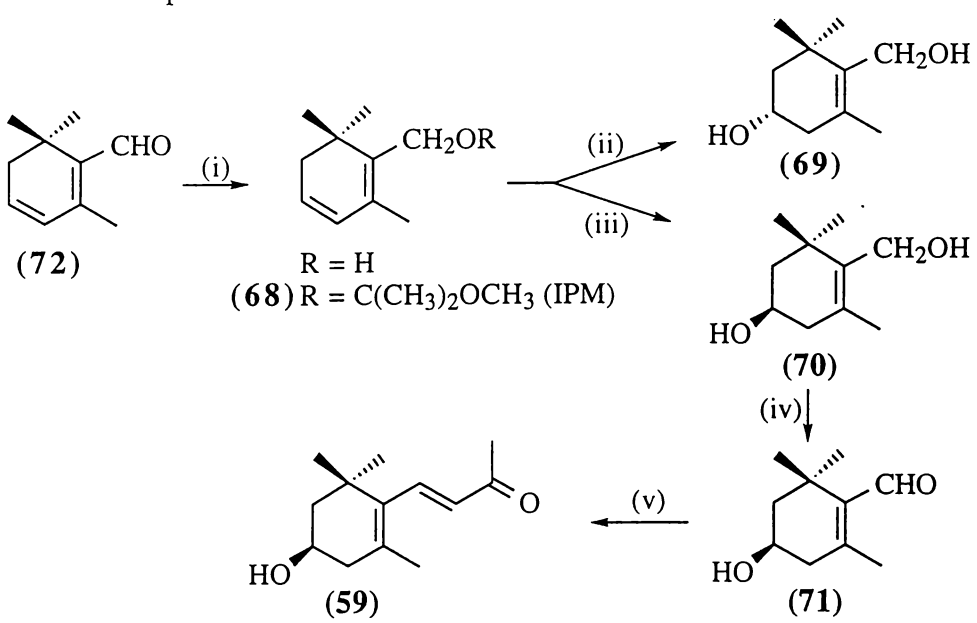
An alternative, more efficient strategy for synthesis of optically active C-9 carotenoid precursors was reported by Leuenberger *et al.* (1976) who introduced chirality biochemically (Scheme 2.4). Enantioselective fermentative reduction of the double bond of 6-oxo-isophorone (**9**) using baker's yeast gave the optically active diketone **65**. Subsequent diastereo- and regioselective reduction gave moderately good selectivity affording a 1:4 mixture of the epimeric hydroxy ketones **66** and **67**, respectively, which were easily separated. Chain lengthening was effected in a similar manner to that described by Mori (1974).



Reagents: i) Air/catalyst, ii) baker's yeast, 80%, iii) H_2/Raney Ni, 77%.

Scheme 2.4. Synthesis of C-9 optically active 3-hydroxy carotenoid precursors by biological methods.

The application of asymmetric hydroboration was another efficient method for preparation of the optically active 3-hydroxy-β end-group. Hydroboration of safranol isopropenylmethylether (**68**) with (-)- and (+)-$(IPC)_2BH$ gave the optically pure key intermediates **69** and **70** in yields of 27 and 30%, respectively, following crystallisation (Rüttimann and Mayer, 1980) (Scheme 2.5). Thus, the chiral hydroborating reagent achieved enantio- and regioselective functionalisation of the cyclohexadiene system. The enantioselectivity of the hydroboration reaction itself was not reported.

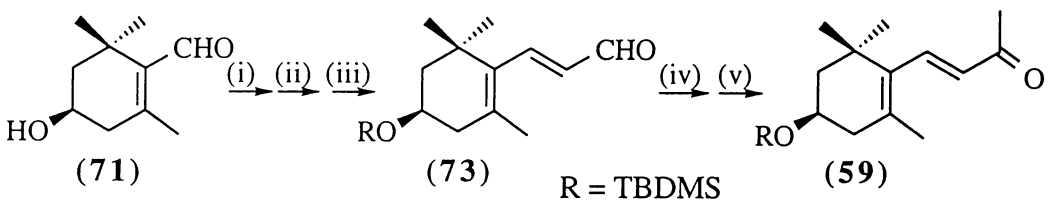


Reagents: i) DIBAL, $H_2CC(CH_3)OCH_3$, TsOH, 90%; ii) (-)-$(IPC)_2BH$, H_2O_2, NaOH, 27%; iii) (+)-$(IPC)_2BH$, H_2O_2, NaOH, 30%; iv) pyridinium chlorochromate, NaOAc, 53%; v) acetone, $NaOCH_3$, 73%.

Scheme 2.5. Synthesis of (*3R*)-3-hydroxy-β-ionone (**59**) from safranal (**72**) utilising asymmetric hydroboration.

Chain lengthening of diol **70** could be effected relatively easily and efficiently in two steps. Selective oxidation of the allylic hydroxy group with pyridinium chlorochromate afforded aldehyde **71** which underwent aldol condensation with acetone/$NaOCH_3$ to gave the corresponding α,β-unsaturated ketone **59** (Scheme 2.5).

Ok *et al.* (1988) modified the method of Rüttimann and Mayer (1980) to synthesise chiral precursors of retinal derivatives. The hydroboration step gave chemical yields of 52% and moderate optical yields of 75% *e.e.*, however chemical yields of the optically pure forms were not improved. A lengthy step-wise route to the corresponding ionones was reported. It entailed addition of acetonitrile to aldehyde **71**, subsequent reduction of the nitrile using DIBAL, and addition of methyl magnesium bromide to the resulting aldehyde **73** (Scheme 2.6). All steps were reported to occur in high yields.

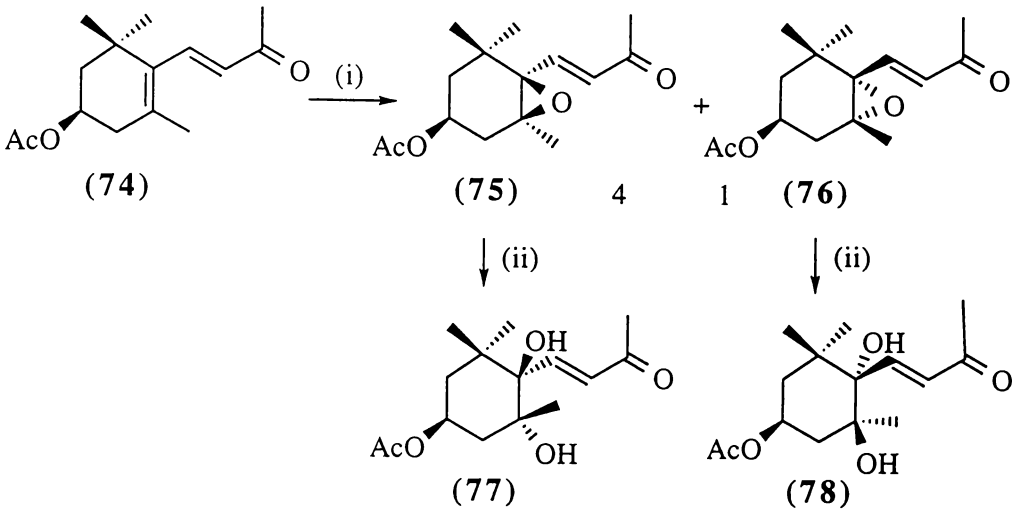


Reagents: i) $(EtO_2)_2P(O)CH_2CN$, NaH, 78%; ii) TBDMSCl, DMF, imidazole, 95%; iii) DIBAL, 70%; iv) CH_3MgBr, 92%; v) MnO_2, 98%.

Scheme 2.6. Chain lengthening of (*3R*)-3-hydroxy-β-cyclocitral (**71**).

The syntheses of several ionone and xanthophyll derivatives bearing the optically active 3,5,6-trihydroxy-5,6-dihydro-β end-group were subsequently reported by Buchecker *et al.* (1984). Epoxidation of (*3R*)-3-acetoxy-β-ionone **74** (Rüttimann and Mayer, 1980) with MCPBA gave the diastereoisomeric epoxides **75** and **76** in a ratio of 4:1, respectively (Scheme 2.7), consistent with that previously reported by Mori (1973). The moderate stereoselectivity of this reaction required separation of epoxides which was achieved by a combination of chromatography and fractional crystallisation.

Acid-catalysed hydrolysis of the epoxides using sulphuric acid in THF, led to the triols whose structures **77** and **78** were established by X-ray crystallographic analysis. The hydrolysis step occurred in a stereoselective manner, with exclusive inversion at C-5 (Scheme 2.7). Details of the synthesis of carotenoid epoxides, and their reactions were reviewed by Eugster (1985).



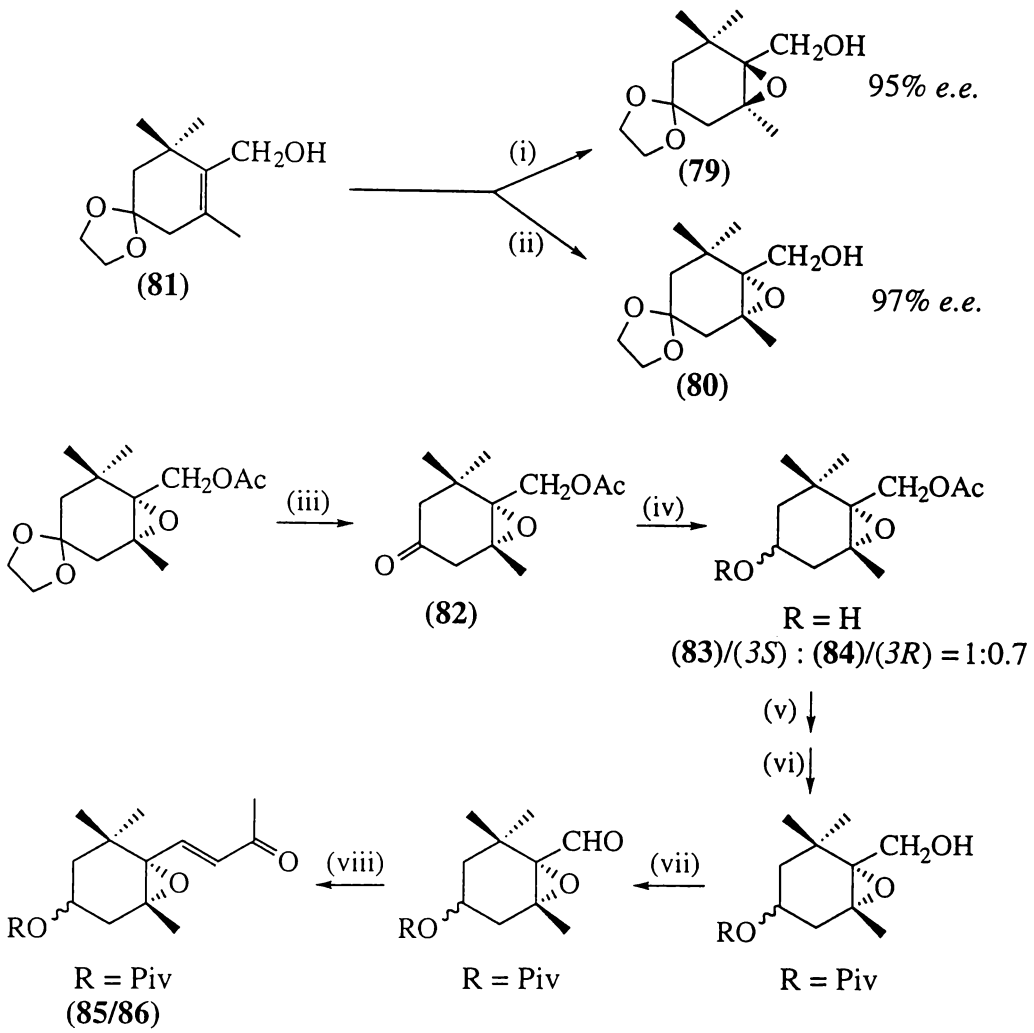
Reagents: (i) MCPBA, 80% (total yield); (ii) H_2SO_4, 60-70%.

Scheme 2.7. Stereoselective epoxidation and epoxide opening of (*3R*)-3-acetoxy-β-ionone (**74**).

Sharpless-Katsuki epoxidation (Katsuki and Sharpless, 1980; Gao *et al.*, 1987) and Sharpless dihydroxylation (Sharpless *et al.*, 1992) are important chemical reactions applied to asymmetric synthesis. The epoxidation reaction has been applied with success to the synthesis of xanthophylls with 3-hydroxy-5,6-epoxy-β end-groups such as violaxanthin (**31**), and related degraded carotenoids, dehydrovomifoliol (**1**) and ABA (**11/12**) (Acemoglu *et al.*, 1988).

The synthetic route used by Acemoglu *et al.* (1988) introduced the epoxide functionality as the initial step. This was in contrast to the previous reported synthesis of this end-group, which involved epoxidation of a precursor having a chiral hydroxyl group at C-3 (Buchecker *et al.*, 1984). The epoxides **79** and **80** were prepared by Sharpless-Katsuki oxidation (Gao *et al.*, 1987) of compound **81** (Scheme 2.8). Yields of 95 and 93%, respectively, and enantiomeric purities of 95 and 97% *e.e.*, respectively, were obtained. This was an unexpected result as hindered alkenes are not generally good substrates for asymmetric epoxidation using Sharpless-Katsuki conditions.

Although a stereoselective epoxidation was achieved, additional derivatisation and hydrolysis steps were required for separation of the epoxy alcohols. Reduction of the epoxy ketone **82** with $NaBH_4$ afforded the diastereoisomeric epoxy alcohols **83** and **84** in a ratio of 1:0.7, which were separated as their pivaloxy esters following conversion to the corresponding ionones **85/86** (Scheme 2.8).



Reagents: (i) *t*-BuOOH, Ti(*i*-PrO)$_4$, (+)-DET, 95% (95% *e.e.*); (ii) *t*-BuOOH, Ti(*i*-PrO)$_4$, (-)-DET, 93% (97% *e.e.*); (iii) $MgSO_4$, H_2O, montmorillonite ('clay 10'), 87%; (iv) $NaBH_4$, 88%; (v) pyridine, pivaloyl chloride, 90%; (vi) KOH/MeOH, 96%; (vii) $(COCl)_2$/DMSO, (*i*-Pr)$_2$EtN, 96%; (viii) (2-oxopropylidene)triphenylphosphorane, 52%.

Scheme 2.8. Synthesis of protected optically active 3-hydroxy-5,6-epoxy-5,6-dihydro-β-ionone derivatives **85/86** using Sharpless-Katsuki epoxidation.

Resolutions of non-enantioselective products have the disadvantage that the maximum theoretical yield of each diastereoisomer can be no greater than 50%. A resolution step was not problematic in the study of Acemoglu *et al.* (1988), as both diastereoisomers were required as precursors for diastereoisomeric violaxanthins.

2.2. Results and Discussion

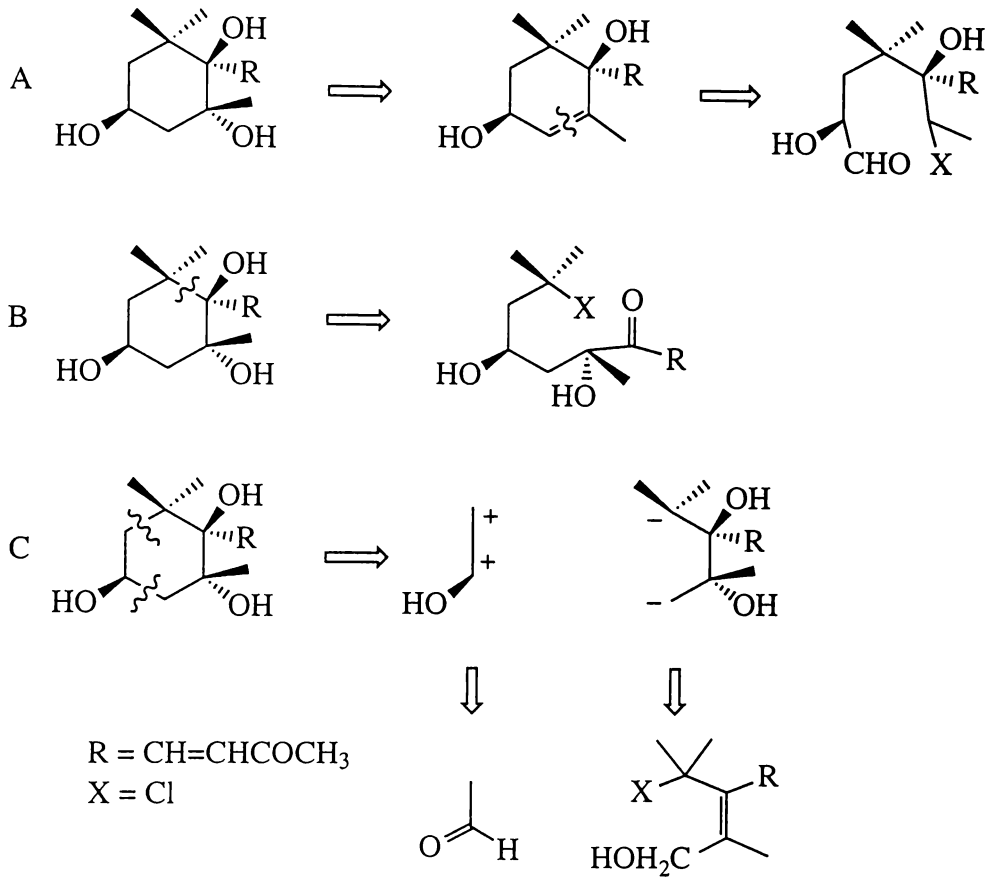
2.2.1. Synthesis of Triol 22 from Acyclic Materials

Ionone **22** had not been previously synthesised, although the monoacetyl-derivative **77** had been prepared by Buchecker *et al.* (1984) (Scheme 2.7), utilising the chiral precursor prepared by Rüttimann and Mayer (1980). In general, syntheses of ionone derivatives were *via* C-9 or C-10 cyclic precursors, with subsequent chain lengthening. In the present study, synthetic routes starting from acyclic starting materials and readily available ionones were evaluated.

Retrosynthetic analysis is a systematic approach to synthesis which transforms the structure of a synthetic target molecule to a sequence of progressively simpler structures, ultimately leading to a simple or commercially available starting material. The transformation of a molecule to a synthetic precursor is accomplished by the application of a transform, the exact reverse of a synthetic reaction (Corey and Cheng, 1989).

The target molecule of the present study was a six-membered cyclic ring with an alkyl side chain. The cyclic ring contained three stereocentres, one of which was a secondary hydroxyl group, the other two being adjacent tertiary hydroxyl groups. In construction of this molecule from acyclic starting materials, retrosynthetic analysis required consideration of both ring-disconnective and stereoselective transforms. Various retrosynthetic schemes devised for synthesis of **22** are depicted in Scheme 2.9.

A general principle of organic synthesis is that one should attempt to introduce stereocentres early on in the synthesis, with ring connections to be left as late as possible. However, retrosynthetic consideration of such an approach to **22** (Scheme 2.9) (even only as far as the first or second disconnection) showed that construction of the key stereochemical features in this manner would be somewhat inefficient. The main problem centred on the adjacent tertiary asymmetric carbons. Pathways A and B required independent construction of all three stereocentres and hence, ultimately three asymmetric inductions of high enantiomeric excess. However, pathway C allowed construction of the two adjacent tertiary centres (possibly by Sharpless chemistry), with the ring closure giving rise to the final secondary alcohol.



Scheme 2.9. Retrosynthetic analysis of 3,5,6-trihydroxy-5,6-dihydro-β-ionone (**22**).

The alternative, approach to synthesis of **22** was to start from a cyclic compound and introduce stereochemical centres. This was the approach taken by Rüttimann and Mayer (1980) and Buchecker *et al.* (1984) for syntheses of cyclic carotenoids, zeaxanthin (**30**) and violaxanthin (**31**). The advantages of this approach are that the initially introduced secondary chiral centre is produced early in the synthesis with excellent stereo-control, and that the stereoselective ring opening of the epoxide produced the required stereochemistry at the adjacent tertiary centres.

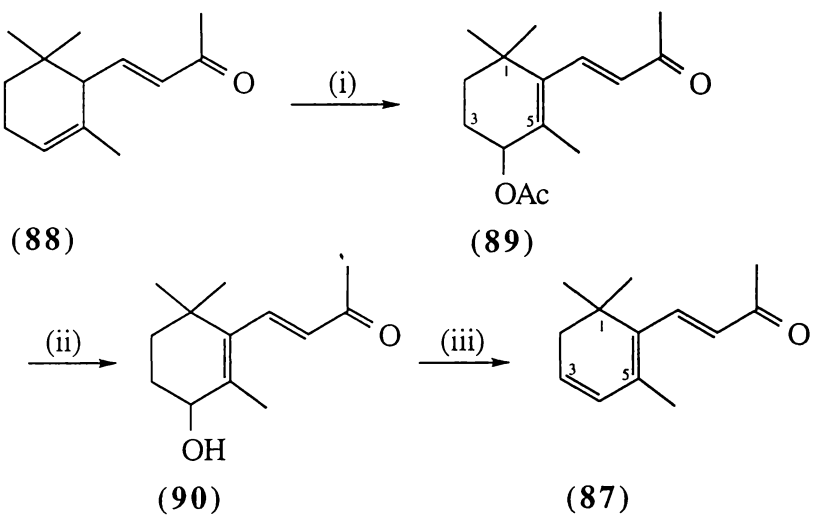
The logical extension of this approach was to develop a synthesis where the butenone side-chain was already incorporated in the starting material and to carry out similar transformations as those developed by the above authors. This was seen as a potentially more efficient approach to previously reported syntheses. Ionones were readily available and were chosen as starting materials for the synthesis. Previously, optically active α-ionone had been used for the synthesis of chiral ionone derivatives with 4-hydroxy- (Haag *et al.*, 1980), 4,5-epoxy- (Uebelhart *et al.*, 1986), 5,6-epoxy- (Acemoglu *et al.*, 1981), and 5,6-dihydroxy-

(Eschenmoser *et al.*, 1981) substitutions. However, it was apparent that ionones had not been used as starting materials for synthesis of optically active 3-hydroxy-, 3-hydroxy-5,6-epoxy- and 3,5,6-trihydroxy-5,6-dihydro-β-ionones.

2.2.2. Synthesis of Triol 22 from α- and β-Ionone

Preparation of 3,4-Dehydro-β-ionone (87)

The proposed synthetic route required preparation of 3,4-dehydro-β-ionone (**87**) using either α- or β-ionone as starting material (Scheme 2.10). Selenium dioxide (SeO_2) oxidation of α-ionone (**88**) using acetic anhydride as solvent (House, 1972a) afforded a monoacetoxy derivative in 52% yield following purification by flash chromatography. This product was identified as the more thermodynamically stable allylic rearrangement product, 4-acetoxy-β-ionone (**89**).



Reagents and Conditions: (i) SeO_2, Ac_2O, 50–60°C, 52%; (ii) KOH, MeOH, reflux; 88%; (iii) TsOH, toluene, 50–60°C, 62%.

Scheme 2.10. Synthesis of 3,4-dehydro-β-ionone (**87**) from α-ionone (**88**).

The use of acetic anhydride as solvent complicated work-up of the reaction mixture due to formation of emulsions, and required introduction of a deacetylation step to the synthesis. However, increased yields of the allylic oxidation product were obtained using this method. SeO_2 oxidation of both α-and β-ionone in ethanol were slow (in the order of days) and poor yields of the desired product were obtained. In comparison, Oritani and Yamashita (1973) reported the SeO_2 oxidation of (-)-α-ionone afforded (-)-6-hydroxy-α-ionone in 30% yield. A

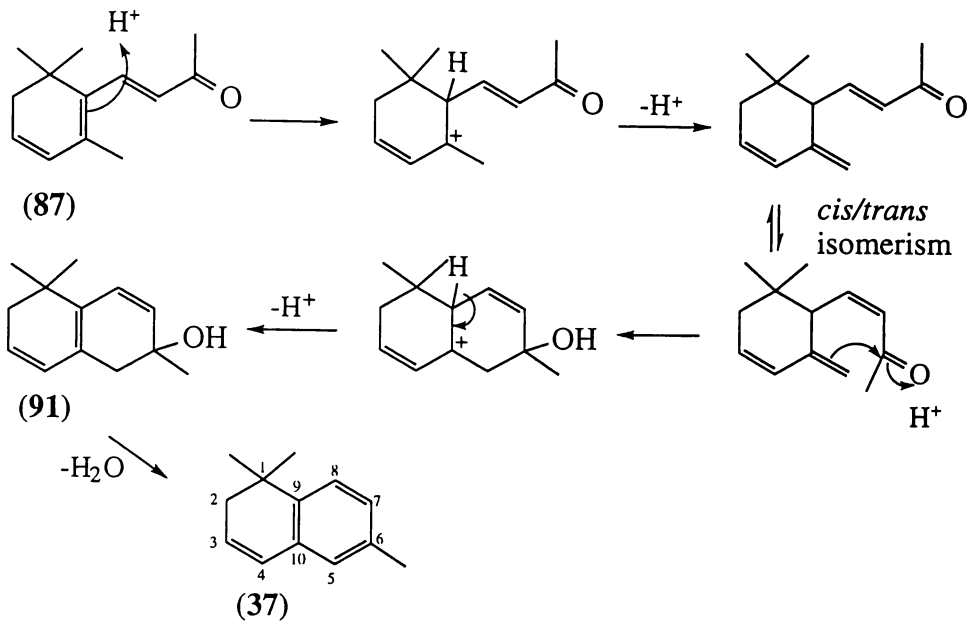
similar oxidation of methyl 2-*cis*-α-ionylideneacetate afforded the allylic rearrangement product, methyl 2-*cis*-4-hydroxy-β-ionylideneacetate in 32% yield, and the 3- and 6-hydroxy-α-ionylideneacetates as minor products (Oritani and Yamashita, 1974).

Hydroxy-β-ionone **90** was obtained in high yield by base hydrolysis of acetate **89**. Acid-catalysed dehydration of alcohol **90** was carried out on a small scale initially, using dry refluxing toluene and *p*-toluene sulphonic acid (*p*-TsOH) as dehydrating agent. TLC revealed the major product of the reaction was an extremely non-polar product, and GCMS suggested that the molecular weight of this compound was 172, and not 190 as expected for 3,4-dehydro-β-ionone (**87**). A computer search against the NBS Mass Spectrometry Database gave 1,1,6-trimethyl-1,2-dihydronaphthalene (**37**) (Scheme 2.11) as a compatible structure. Consequently, confirmation of this structure was sought by NMR.

The ^{1}H NMR spectrum showed a typical ABC pattern characteristic of a 1,2,4-trisubstituted aromatic system with resonances between δ 6.90 and δ 7.22. The methyl groups appeared at δ 1.29 (6H) and 2.33 (3H). A two proton doublet of doublets at δ 2.25 could be ascribed to the allyl protons which were coupled to the vinyl protons at δ 5.95 and 6.44. The NMR and MS data characteristic of this compound corresponded to that reported in the literature for 1,1,6-trimethyl-1,2-dihydronaphthalene (**37**) (Stevens *et al.*, 1975).

Under the reaction conditions, it was likely the desired dehydration product **87**, was formed, but underwent acid-catalysed rearrangement as outlined in Scheme 2.11. Evidence for this mechanism was the observation of a compound **91** of molecular mass 190 in the crude reaction mixture. The mass spectrum of that compound displayed a strong peak at *m/z* 172 consistent with loss of a molecule of water.

Naphthalene **37** has been identified as an aroma component from several natural sources including wine (Strauss *et al.*, 1986; Sefton *et al.*, 1989) and apricots (Bolzoni *et al.*, 1990). The formation of non-megastigmanes such as **37**, is attributed to acid-catalysed rearrangement of primary degraded carotenoids (Stevens *et al.*, 1975; Strauss *et al.*, 1986). Dehydro-β-ionone (**87**) has been proposed as a precursor of **37** in tobacco leaves (Wahlberg and Enzell, 1987). The results of the present study support this proposal. A two step synthesis of **37** from α-ionone (**88**) has been reported (Miginiac, 1990). That synthesis involved



(37)

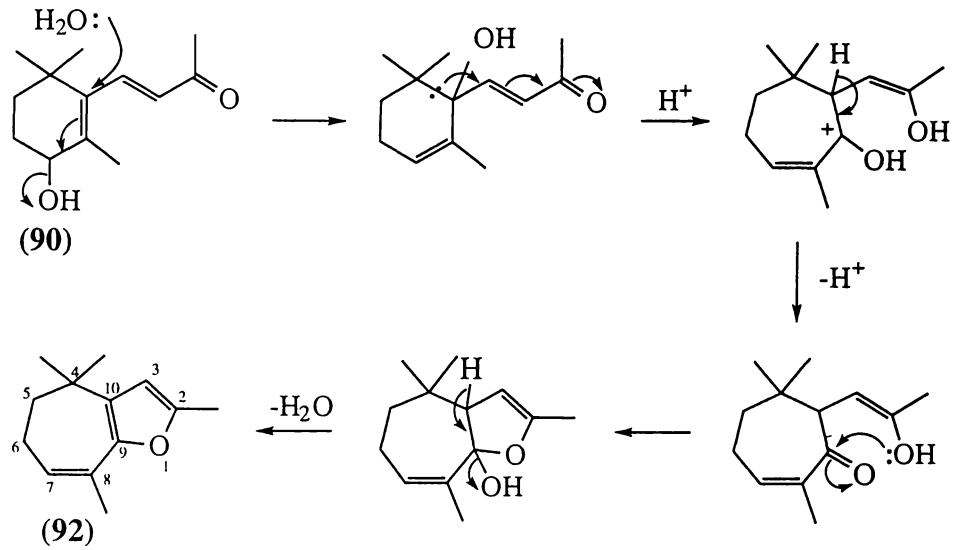
Scheme 2.11. Proposed mechanism for formation of 1,1,6-trimethyl-1,2-dihydronaphthalene (**37**) from 3,4-dehydro-β-ionone (**87**) in acidic conditions.

cyclisation of the butenone chain followed by bromination with *N*-bromosuccinimide (NBS), and dehydrobromination with *N,N*-diethylaniline.

^{1}H and ^{13}C NMR identifed the minor product of the dehydration reaction in the present study, as the desired dehydration product, 3,4-dehydro-β-ionone (**87**). Data for ^{1}H NMR were consistent with those reported by Findlay and MacKay (1971). The optimum yield (62%) of alkene **87** (Scheme 2.10) was obtained when the reaction temperature was maintained between 50 and 60°C. Above 60°C the yield of **87** was reduced by formation of several less polar products, the major components being naphthalene **37**, and a compound of unknown structure.

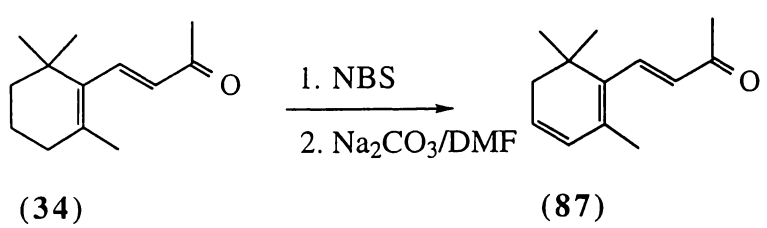
Mass spectral data of the unknown product suggested a molecular weight of 190; however it differed from that thought to be the naphthalene precursor **91** (Scheme 2.11). Mass spectral data were consistent with that found for furan **92** (Skorianetz and Ohloff, 1974) (Scheme 2.12). Reported ^{1}H and ^{13}C NMR, although recorded in deuterobenzene, were also consistent with those observed in the present study. A tentative assignment of the unknown product as furan **92** was made. Full structural elucidation using 2D COSY and long-range ^{13}C-^{1}H correlation experiments was not carried out to confirm that structure.

The formation of putative furan **92** from 4-hydroxy-β-ionone (**90**) in acidic conditions may occur *via* a 1,2-alkyl shift as depicted in Scheme 2.12. β-Ionone-5,6-epoxide undergoes similar acid-catalysed ring enlargement and contraction, affording isomeric cycloheptafuran and cyclopentane derivatives, respectively (Skorianetz and Ohloff, 1974).



Scheme 2.12. Proposed mechanism for formation of furan **92** from 4-hydroxy-β-ionone (**90**) in acidic conditions.

An alternative preparation of 3,4-dehydro-β-ionone (**87**) was a one-pot synthesis involving allylic bromination of β-ionone (**34**) with NBS, and subsequent dehydrobromination using base (Findlay and MacKay, 1971) (Figure 2.1). However, Miginiac (1990) noted that the reaction of α- or β-ionone with NBS followed by *N,N*-diethylaniline gave in low yield, a mixture of naphthalene **37** and alkene **87**, from which it was very difficult to isolate either product. Numerous supporting references were cited.



(**34**) (**87**)

Figure 2.1. One-pot synthesis of 3,4-dehydro-β-ionone (**87**) (Findlay and MacKay, 1971).

In the present study, the method of Findlay and MacKay (1971) was found to give good yields (70%) of the alkene, although several by-products with similar R_fs hindered efforts to purify the product chromatographically. ^1H NMR indicated one of the products was possibly the intermediate bromide (a resonance at δ 3.5), so the dehydrobromination step was lengthened. That adjustment did not improve either the yield or attempts to purify alkene **87**, and led to larger quantities of by-products. As had been reported by Miginiac (1990), one by-product of the reaction was naphthalene **37**, although this product was readily separated from alkene **87** by flash chromatography. In comparison to the method developed in the present study, the one-pot synthesis was a more efficient route to **87**, despite the requirement for several chromatographical separations for product purification.

Hydroboration of 3,4-Dehydro-β-ionone

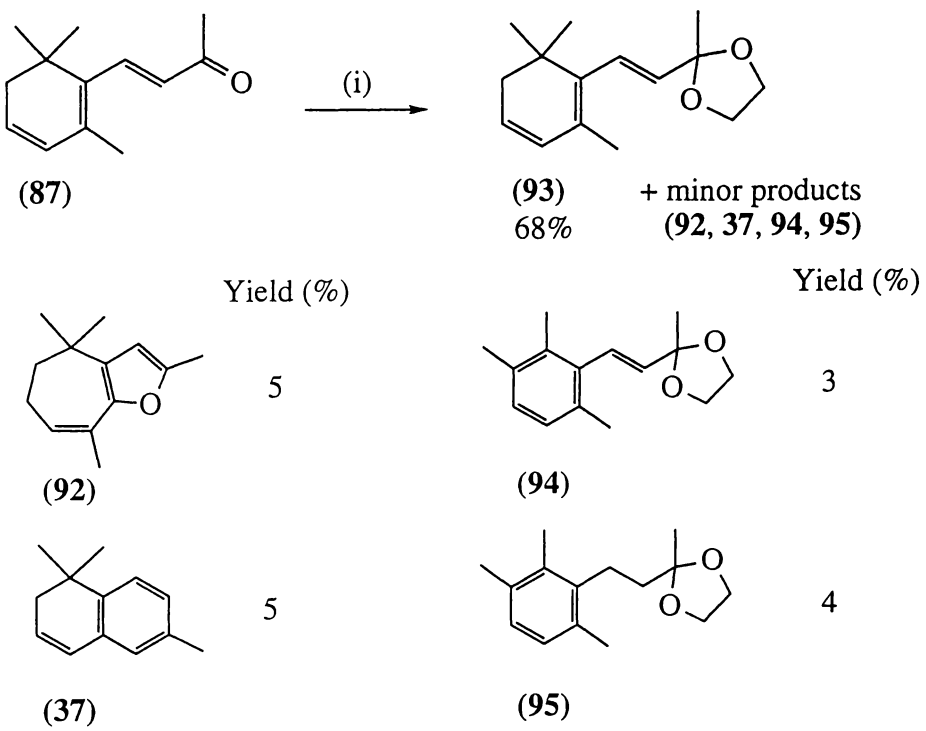
Protection of the ketone group of 3,4-dehydro-β-ionone (**87**) was necessary prior to hydroboration, owing to the reactivity of ketones, particularly unsaturated ketones, towards borane reagents (House, 1972b). The 1,3-dioxolane protective group was chosen, principally for its ease of preparation. The stability of this protective group under hydroboration reaction conditions was uncertain (Greene, 1981; Greene and Wuts, 1991), therefore this had to be assessed experimentally. Other protective groups reported to exhibit high stability under such reaction conditions included several *S,S*-acetals[1] (Greene, 1981).

Using standard acid-catalysed dioxolanation methods (*p*-TsOH catalyst, refluxing benzene), the 1,3-dioxolane of α,β-unsatuated ketone **87** was prepared in reasonable yield (68%). Utilisation of Dean-Starck apparatus allowed azeotropic distillation of the water generated from the reaction, which was subsequently removed from the solvent prior to recycling. Poor yields were observed when reagents and solvents were not dried prior to use. Progress of the reaction was monitored by TLC, which indicated quick conversion to the cyclic acetal (ketal) **93**, but following *ca* 75% conversion, a much slower reaction rate. Continued reaction at this stage resulted in the appearance of additional products (Scheme 2.13).

A non-polar fraction recovered from the reaction mixture consisted of previously identified acid-catalysed rearrangement products, naphthalene **37** and

[1]According to IUPAC rules (Rule C-331.1), the term aectal now applies to all 1,1-*bis*-ethers whether derived from aldehyde or ketone.

alleged furan **92**, both in *ca* 5% yield. Two additional products obtained in *ca* 3–4% yield were identified by 1- and 2D NMR and MS as the aromatic derivatives **94** and **95**. MS and NMR characteristics of the compounds obtained by removal of the protecting groups were consistent with those of 4-(2',3',6'-trimethylphenyl)-3-buten-2-one (**96**) and 4-(2',3',6'-trimethylphenyl)-butan-2-one (**97**), respectively, confirming those structures. Starting material was recovered in 10% yield. Acid-sensitive compounds may be dioxolanated using adipic acid as catalyst (Greene, 1981; Greene and Wuts, 1991); however this method of dioxolanation was unsuccessful when applied to α,β-unsaturated ketone **87**.

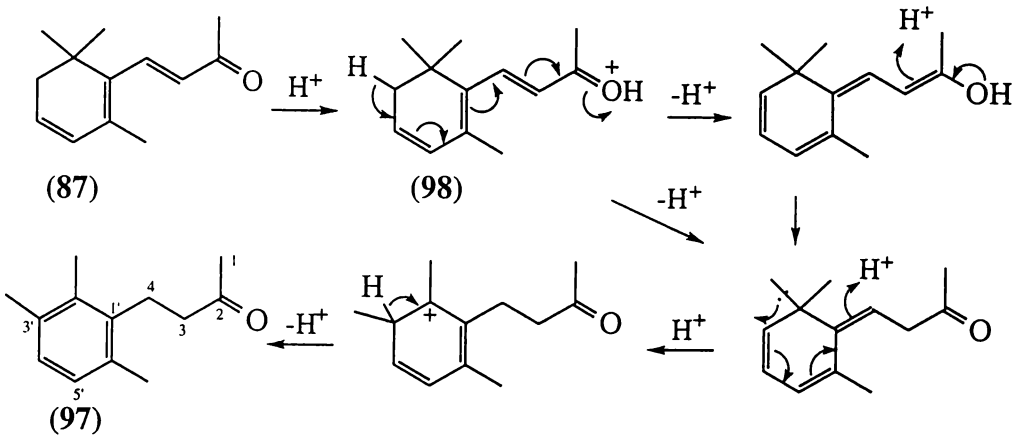


Reagents and Conditions: (i) $HOCH_2CH_2OH$, *p*-TsOH, benzene, reflux (Dean-Starck).

Scheme 2.13. Preparation of the 1,3-dioxolane of 3,4-dehydro-β-ionone (**87**). Various acid-catalysed rearrangement products were identified from the reaction mixture.

The formation of 4-(2',3',6'-trimethylphenyl)-3-butan-2-one (**97**) from alkene **87** may occur *via* protonation of the α,β-unsaturated ketone **98**, followed by [1,2] methyl migration and concomitant aromatisation (Scheme 2.14). Acetal formation occurs after this rearrangement. Similar aromatisations have been observed for ionones in acidic conditions (Stevens *et al.*, 1975; Strauss *et al.*, 1986). The mechanism by which alkene **87** is oxidised to the trimethylphenyl

derivative **96** is uncertain. The corresponding reduction reaction was not identified.



Scheme 2.14. Aromatisation of 3,4-dehydro-β-ionone (**87**) in acidic conditions.

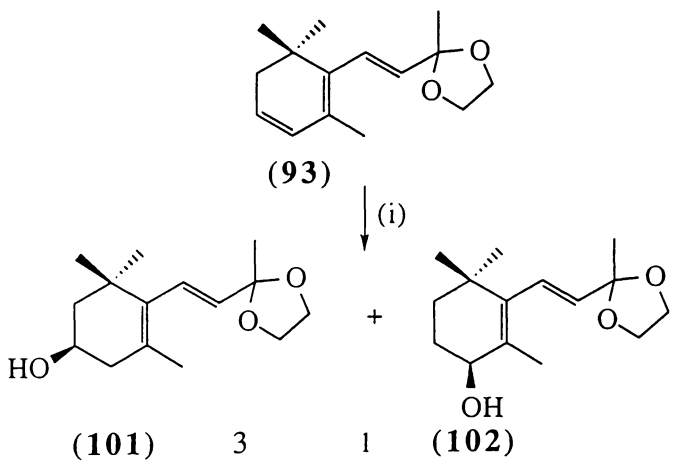
Rüttimann and Mayer (1980) demonstrated the utility of Brown's asymmetric hydroboration (Srebnik and Ramachandran, 1987) for the synthesis of enantiomeric 3-hydroxy-β-ionones from safranal (**72**) (Scheme 2.5). Reasonable asymmetric induction was achieved by reaction of chiral $(Ipc)_2BH$ (Brown *et al.* 1982a) with the diene, safranol IPM ether (**68**), affording optically active alcohols in approximately 90% *e.e.* and 30% chemical yield. The optically pure alcohols were obtained by fractional crystallisation. With slight modification, Ok *et al.* (1988) prepared the same chiral 3-hydroxylated precursors in lower optical yields of 75% *e.e.* and 50% chemical yield.

Attempts at hydroboration of triene **93** using $(Ipc)_2BH$ under the conditions optimised by Brown *et al.* (1982a), were unsuccessful in the present study. Following alkaline hydrolysis, pinanol (i.e unreacted $(Ipc)_2BH$), and starting material were recovered. Increasing the reaction temperature from -20°C to -10°C gave the same result, whilst at temperatures of 0°C a mixture of products was obtained, including starting material and the desired homoallylic alcohol in low yield. These reactions were monitored by GCMS analysis of hydrolysed aliquots. The molecular masses of the observed products, and NMR of the crude reaction mixture, suggested the 1,3-dioxolane group was intact.

The use of monoisopinocampheylborane ($IpcBH_2$) as chiral hydroborating reagent was also investigated. $IpcBH_2$ has wide application for hydroboration of alkenes that react slowly, or not all, with $(Ipc)_2BH$ (this is usually for steric

reasons). It is readily liberated from the crystalline $IpcBH_2$.TMEDA complex (Alpine-Boramine™) with boron trifluoride etherate ($BF_3.Et_2O$) (Brown *et al.*, 1978a, Brown *et al.*, 1982b). The procedure was carried out without filtration of the solid TMED.$2BF_3$ (removal is not crucial for further hydroboration (Brown *et al.*, 1978)). This reagent exhibited similarities in reactivity to that observed for $(Ipc)_2BH$. Consequently, asymmetric hydroboration was not pursued further.

Treatment of **93** with $BH_3.SMe_2$ resulted in the formation of isolable quantities of the required homoallylic alcohol **101**[1]. Reasonable regioselectivity for the terminal alkene of the triene system was obtained with a 3:1 mixture of **101** and **102** isolated in a yield of 36%, following flash chromatography (Scheme 2.15).



Reagents: (i) $BH_3.SMe_2$/3 M NaOH, 30% H_2O_2, 36%.

Scheme 2.15. Hydroboration of triene **93**.

The major hydroboration product was consistent with attack of the borane reagent at the least hindered position (Cragg, 1973). TLC indicated a mixture of products of varying polarities were also formed in the reaction, but attempts to obtain fractions in sufficient quantity and purity for identification were not successful. It was concluded the borane reagent may have been non-selective in its attack of the double bonds of triene **93**. In the hydroboration of conjugated dienes using borane, the unsaturated organoborane initially formed can undergo further reaction in preference to the less reactive diene, resulting in dihydroboration (Brown, 1972; Cragg, 1973). Yields of 30–45% of the monohydroboration product are common in such hydroxylation reactions.

[1]While one stereoisomer is depicted, the products are racemates.

The identity of a non-polar fraction (*ca* 10% yield) from the hydroboration reaction was subsequently elucidated. MS analysis indicated this fraction contained rearrangement products, naphthalene **37** and furan **92** in equal quantities, and trace amounts of a compound with molecular mass 170. Mass spectral data for this compound were consistent with 1,2,6-trimethylnaphthalene as its structure (NBS library). Formation of these compounds indicated that under hydroboration conditions, removal of the protecting group was occurring, allowing subsequent rearrangement, similar to that observed for 3,4-dehydro-β-ionone (**87**) in acidic (Schemes 2.11–2.14) and basic (Migianic, 1990) conditions.

Although the yield of monohydroboration product observed in the present study was in accord with that generally observed for cyclic conjugated dienes (Cragg, 1973), it was concluded that any future attempts to hydroborate 3,4-dehydro-β-ionone (**87**) might benefit from use of an alternative protecting group. In addition, regioselectivity may be increased by use of a larger attacking molecule, such as 9-borabicyclo[3.3.1]nonane (9-BBN) (Cragg, 1973; Brown *et al.*, 1978b). A bulky hydroborating reagent may also limit dihydroboration. Chen and Liu (1994) have subsequently employed the approach of Broom *et al.* (1992) for the synthesis of dehydroretinal isomers.

Separation of alcohols **101** and **102** by PLC proved difficult, although removal of the 1,3-dioxolane group facilitated this step. Deprotection was carried out at room temperature (RT) using oxalic acid and acetonitrile as solvent (Greene, 1981), affording the corresponding ketone derivatives (±)-**59** and **90** in quantitative yield.

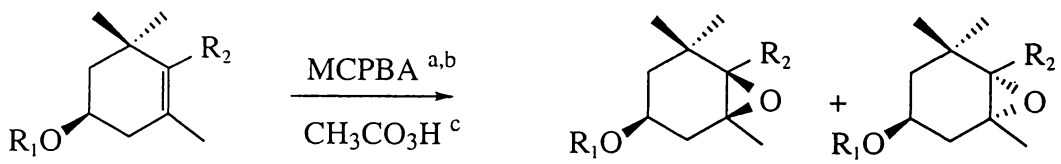
Epoxidation and Epoxide Opening

As asymmetric induction was not achieved, a resolution step was required (Section 2.2.3). Stereoselective epoxidation and epoxide opening steps were necessary in order to generate the required chirality at C-5 and C-6, following generation of the enantiomeric homoallylic alcohols (*S*)- and (*R*)-(**59**).

The use of epoxides as intermediates in the synthesis of stereochemically complex compounds is well established. Epoxidation is widely utilised in organic synthesis as the epoxide group is readily opened to produce a 1,2-functionality in a stereoselective manner. Moreover, in the context of asymmetric synthesis, epoxidation can create two adjacent chiral centres in a single reaction. Synthetic

applications of the epoxidation reaction have been reviewed (Rao, 1983; Finn and Sharpless, 1983; Hoveyda *et al.*, 1993).

The epoxidation reaction has been utilised in a number of carotenoid end-group syntheses. Buchecker *et al.* (1984) synthesised 3,5,6-trihydroxy carotenoid end-groups from epoxide derivatives of the chiral precursor, (*3R*)-acetoxy-β-ionone (**74**). Epoxidation using *m*-chloroperbenzoic acid (MCPBA) demonstrated moderate stereoselectivity, with the *syn* - and *anti*-epoxides formed in a 4:1 ratio. This was consistent with that previously reported by Mori (1973; 1974) for the same epoxidation reaction. In addition, Mori (1974) reported MCPBA epoxidation of the acetylenic compound (**103**) gave an epoxide ratio of 2:1. Kienzle *et al.* (1978) obtained the *syn*- and *anti* -epoxides in a ratio of 5:1 following peracetic acid (CH_3CO_3H) epoxidation of compound (**104**) (Scheme 2.16).



(**74**) R_1 = Ac, R_2 = CH=CHCOCH$_3$	4	1 [a]
(**103**) R_1 = Ac, R_2 = C≡CCH(CH$_3$)OAc	2	1 [b]
(**104**) R_1 = H, R_2 = CH=CHC(CH$_3$)=CHCOOCH$_3$	5	1 [c]

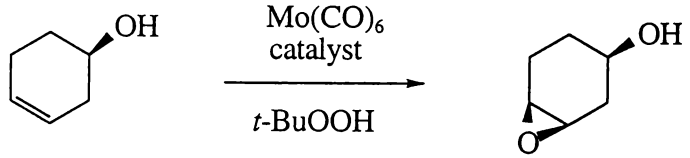
[a] Buchecker *et al.*, 1984; Mori, 1973; 1974. [b] Mori, 1974. [c] Kienzle *et al.*, 1978.

Scheme 2.16. Peracid epoxidation of ionone derivatives.

MCPBA epoxidation of homoallylic alcohol (±)-**59** afforded a 3:1 mixture of epoxides in moderate yield (Scheme 2.17). ^{1}H NMR signals of each epoxide were assigned by the 2D COSY experiment. Off-diagonal connectivities from the COSY spectrum, in conjunction with scalar coupling constants obtained from resolution-enhanced ^{1}H NMR spectrum enabled assignment of resonances of H-$2_{ax/eq}$ and H-$4_{ax/eq}$ from each diastereoisomer. Confirmation of the relative stereochemistry of epoxides was attempted using NOE-difference experiments; however the results of these experiments were inconclusive. The MM2*[1]-calculated internuclear distances of H-4_{eq}–5-Me for the *syn*-(±)-**23** and *anti*-(±)-**24** epoxides were 2.3 and 2.7 Å, respectively. It was assumed that the major product of the reaction was the *syn*-epoxide, in accord with previously reported epoxidations of ionone derivatives (Scheme 2.16).

[1] MM2* is the MacroModel implimentation of the MM2 force field (Allinger, 1977).

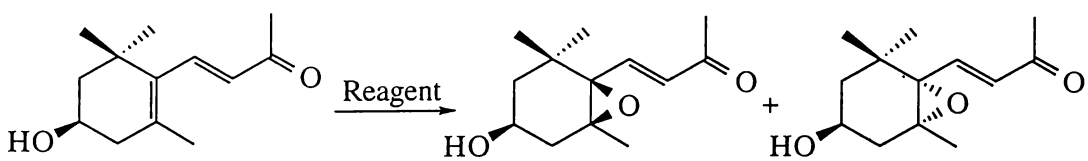
An alternative method of epoxidation was sought which would give *syn*-epoxide **23** stereoselectively. Sharpless and Michaelson (1973) reported high stereoselectivities for the transition metal-catalysed epoxidations of olefinic alcohols by *tert*-butyl hydroperoxide (*t*-BuOOH). Of significant interest, was the reaction of homoallylic alcohol **105** with *t*-BuOOH using molybdenum hexacarbonyl ($Mo(CO)_6$) as catalyst (Figure 2.2). The *syn*-epoxy alcohol **106** was obtained with an isomeric purity of 98%, in contrast to the 2:1 ratio of *syn:anti* epoxides obtained using perbenzoic acid.



(105) **(106)** 90% yield
98% *e.e.*

Figure 2.2. $Mo(CO)_6$-catalysed epoxidation of 3-cyclohexen-1-ol (**105**).

Epoxidation of homoallylic alcohol (±)-**59** with *t*-BuOOH and molybdenum catalyst ($Mo(CO)_6$/*t*-BuOOH) proceeded rapidly and afforded one major product in high yield. ^1H and ^{13}C NMR indicated that the epoxide product was isomerically pure (> 98%), and that it was the *syn*-epoxide (±)-**23** (Scheme 2.17).



(±)-(**59**) (±)-(**23**) (±)-(**24**)

Reagent	Stereoselectivity	
MCPBA	75%	25%
$Mo(CO)_6$/*t*-BuOOH	>98%	>2%

Scheme 2.17. Comparison of peracid and metal-catalysed epoxidation of homoallylic alcohol (±)-**59**.

The *syn*-directive effect of the hydroxyl group of allylic alcohols is well known in peracid and metal-catalysed epoxidation reactions. Henbest (1957) first established that peracid epoxidation of allylic alcohols occurred principally *cis* to the hydroxyl group, and that the rate of epoxidation of the alcohol was about ten

times that of the corresponding allylic acetate. The directive effect of cyclic olefins with allylic hydroxyl groups has been suggested to arise from hydrogen bonding between the hydroxyl and attacking peracid, with pseudo-equatorial functionalities directing the oxidation reaction more effectively (Hoveyda *et al.*, 1993) (Figure 2.3a). This has been rationalised on the basis of geometric and stereoelectronic factors (Hoveyda *et al.*, 1993).

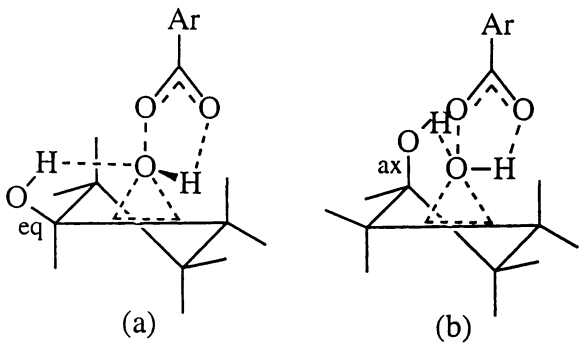
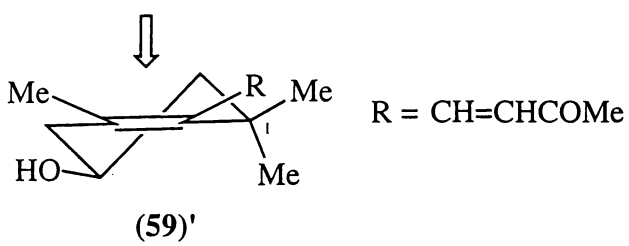


Figure 2.3. Transition structures for the peracid epoxidation of cyclic (a) allylic, and (b) homoallylic alcohols (Hoveyda *et al.*, 1993). *Syn*-directivity requires equatorial and axial hydroxyl groups, respectively.

Homoallylic hydroxyl groups have also been demonstrated to direct the course of peracid epoxidation reactions. In this instance, directivity is more efficient with pseudo-axially disposed hydroxyl group (Hoveyda *et al.*, 1993) (Figure 2.3b). The preferred generation of *cis*-epoxides in ionone derivatives has been rationalised on the basis of steric hinderence caused by the quasi-axial methyl group at C-1 (Mori, 1974). This can be observed in stereo-structure.**59'** (Figure 2.4). It is assumed the equatorial hydroxyl group in such compounds, is unfavourably disposed for directivity of the peracid.



(59)'

Figure 2.4. Stereo-structure of **59'** showing steric hinderence of the quasi-axial C-1 methyl group (Mori, 1974). The hydroxyl group is not in a position to alter the effect of the methyl group.

In contrast to peracid epoxidations, vanadium- and molybdenum-catalysed epoxidations of both allylic and homoallylic alcohols are considerably faster, and are essentially stereoselective (> 95:5) (Sharpless and Michaelson, 1973; Hoveyda, 1993). The stereoselectivity and rate acceleration of such epoxidations (including the well-known Sharpless reagent, *t*-BuOOH/Ti(O-*i*-Pr)$_4$/DET (Sharpless and Katsuki, 1980)) are explained by the mechanism which requires the equilibrium formation of a alkoxohydroperoxo-metal complex. Intramolecular transfer of the peroxide oxygen in close proximity to the double bond is assumed to be readily accomplished (Finn and Sharpless, 1983) (Figure 2.5). Detailed mechanistic pathways for these metal-catalysed epoxidations have not been elucidated to date (Hoveyda *et al.*, 1993).

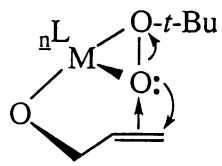


Figure 2.5. Proposed mechanism for metal carbonyl-catalysed epoxidation of allylic alcohols (Finn and Sharpless, 1983).

The stereoselectivities and rate acceleration of transition metal-catalysed epoxidations of various homoallylic alcohols (Hoveyda *et al.*, 1993), suggest the homoallylic hydroxyl is sufficiently near the double bond to direct the epoxidation, perhaps by a similar mechanism to that put forth for allylic alcohols. For the latter substrates, it has been demonstrated that a pseudo-axial alcohol is more favourable for metal-catalysed epoxidation reactions (*cf.* Figure 2.3a).

There are relatively few examples of metal-catalysed epoxidations of homoallylic alcohols. Those reported feature hydroxyl groups which are axially positioned, creating a more favourable geometry for directed epoxidation (Wender and Hubbs, 1980; Hauser *et al.*, 1988). Homoallylic alcohol (±)-**59** has an equatorial hydroxyl group (this was established by the large coupling constants of H-3, and NOE-difference experiments), however it may assume another half-chair conformation with a axial hydroxyl group. Some cyclohexane substrates react *via* a minor conformer, particularly if the rate of reaction of that conformer is relatively fast (Eliel *et al.*, 1994)

Treatment of the epoxy alcohol (±)-**23** with a catalytic amount of sulphuric acid in THF (Buchecker *et al.*, 1984) afforded a stereoselective opening of the

epoxide group. The trihydroxy product, racemic-**22**, was sparingly soluble in $CDCl_3$, therefore ^1H and ^{13}C NMR data were obtained using an elevated sample temperature (310 K). The relative configurations of the three hydroxyl groups were confirmed by NOE-difference experiments as ^1H NMR coupling constants did not allow stereochemical assignment at the tertiary centres (C-5 and C-6). Due to the poor solubility of (±)-**22** in $CDCl_3$, those experiments were carried out in $CDCl_3$:CD_3OD (1:1). All resonances showed sufficient dispersion for selective irradiation, including H-2_{ax}/H-2_{eq}, for which coupling constants could not be determined in $CDCl_3$. Through-space NOE connectivities were determined for all protons to confirm assignment of ^1H resonances using that solvent system (the 2D COSY experiment would have achieved the same result).

The NOE-difference experiments which established the relative stereochemistry of (±)-**22** are shown in Figure 2.6. Irradiation of H-3 (δ 3.84) resulted in enhancements in resonances from the adjacent protons, H-2_{eq} and H-4_{eq}, consistent with an axial position for H-3 (Figure 2.6(b)). Irradiation of 5-Me (δ 0.89) induced enhancements of similar intensities at δ 1.45 and 1.54, attributed to H-4_{ax} and H-4_{eq}, respectively, indicating an equatorial orientation for that methyl group (Figure 2.6(c)). Figure 5.6(d) shows enhancements in H-3, H-2_{eq} and H-7' following irradiation of 1-Me_{ax} (δ 1.05), and corroborates the relative stereochemistries depicted. Enhancements in resonances of the olefinic protons in Figure 5.6(c) and (d) were consistent with an equatorial butenone side-chain.

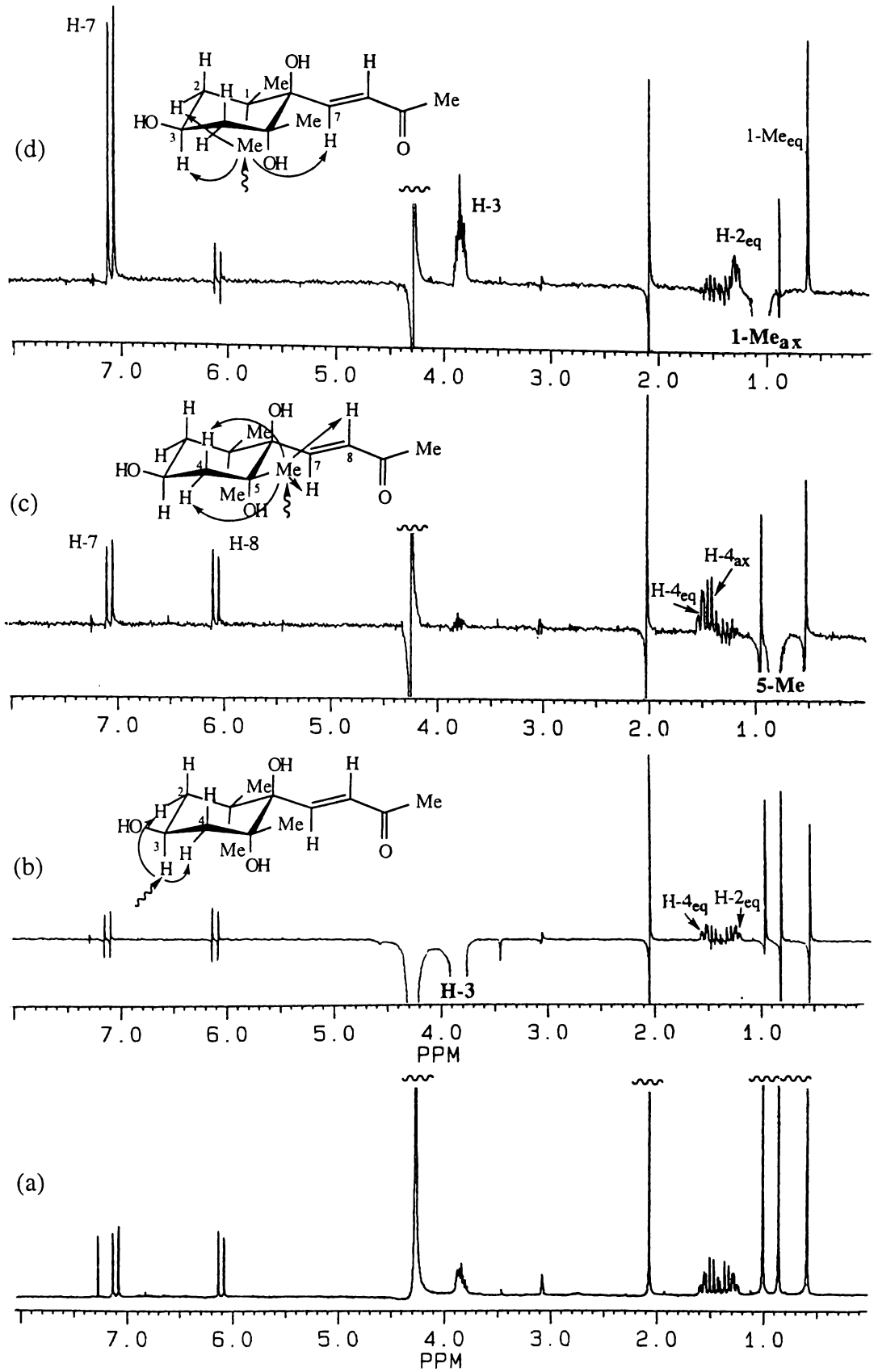
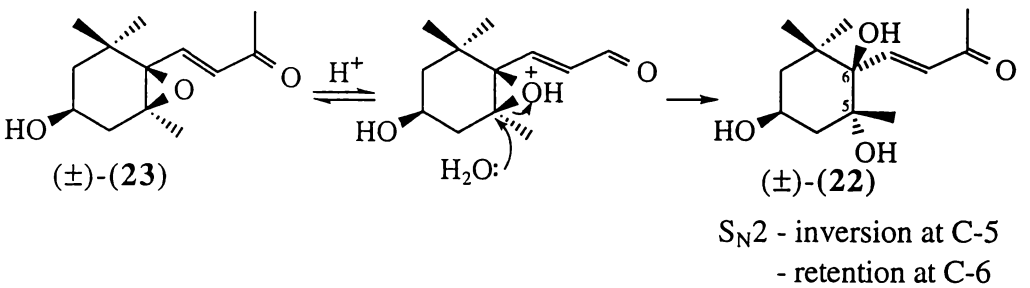


Figure 2.6. ^{1}H NMR spectrum (a) and NOE-difference spectra (b)-(d) showing the relative configurations of synthetic (±)-**22**. Spectra were acquired in 1:1 $CDCl_3$:CD_3OD.

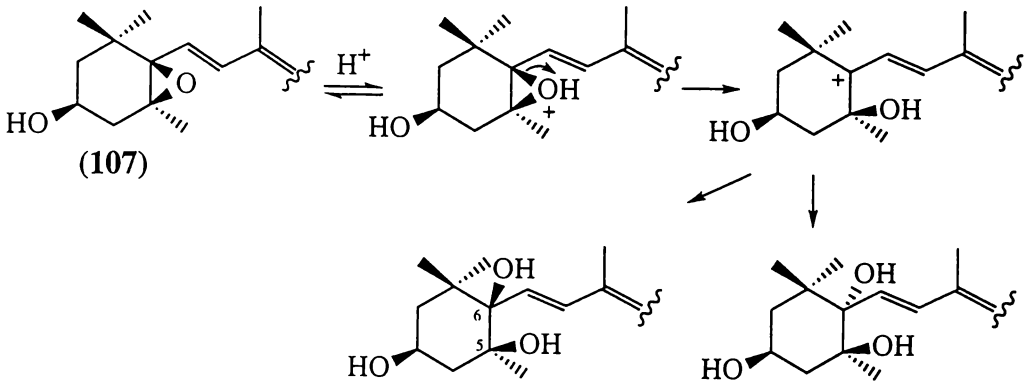
In accordance with acid-catalysed epoxide opening of 3-acetoxy-5,6-epoxy-β-ionones (Buchecker *et al.*, 1984), the reaction afforded the *trans*-1,2-diaxial product (Rao *et al.*, 1983; Eliel *et al.*, 1994), with exclusive inversion of configuration at C-5. That result was ascribed by Buchecker *et al.* (1984) as being consistent with the weaker C-5–O bond breaking. The high stereoselectivity observed for epoxide opening of (±)-**23** suggests it follows an S_N2 mechanism, with attack of the nucleophile occurring at the less hindered carbon atom (Scheme 2.18). Attack at C-6 is subject to greater steric hinderence due to the geminal methyl groups at C-1.



Scheme 2.18. S_N2 acid-catalysed stereoselective epoxide opening of racemic 3-hydroxy-5,6-epoxy-β-ionone (±)-(**23**) in THF.

Opening of epoxides in acid media is believed to proceed through a 'borderline S_N2' mechanism (i.e. there is considerable S_N1 character), and hence there is a preference for nucleophiles to attack at tertiary, benzylic or allylic carbons which are better suited to accomodate the δ+ charge in the transition state (Rao *et al.*, 1983). It appears that steric factors, rather than electronic factors, control the outcome of epoxide opening of (±)-**23**. The stereoselectivity of the epoxide opening was in accord with the Fürst-Plattner rule for diaxial epoxide opening occurring in cyclohexane rings (Eliel *et al.*, 1994).

By contrast, the mild acid-catalysed epoxide opening of carotenoid 5,6-epoxides (e.g. **107**) in THF:H_2O (2:1) has been demonstrated to proceed with retention of configuration at C-5, with either inversion or retention at C-6 (Buchecker *et al.*, 1984). The observation of two diastereoisomeric products under these conditions may be explained by formation of a stabilised (allylic) carbocation, resulting in S_N1 type ring opening (Scheme 2.19).



S_N1 - retention at C-5
- retention or inversion at C-6

Scheme 2.19. Acid-catalysed epoxide opening of synthetic carotenoid **107** in THF:H_2O proceeds with retention or inversion at C-6, consistent with a S_N1 ring opening (*cf.* Scheme 2.18).

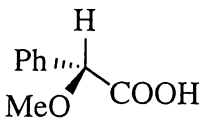
^1H and ^{13}C NMR, and MS spectral data of synthetic (±)-**22** were consistent with those previously reported for the natural product (Tan, 1989). The racemate exhibited a significantly higher melting point and different solubility properties.

2.2.3. Resolution of Secondary Alcohols

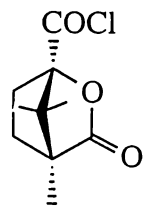
As chirality was not introduced at C-3, a resolution step was necessary. By current asymmetric synthetic standards such resolutions are undesirable. The enantiomer possessing the unwanted absolute configuration is discarded following the resolution step, a practice limiting the maximum yield of usable product to 50%. However, as both enantiomers of the triol **22** were required, a resolution step would provide the enantiomeric homoallylic alcohols required to prepare both enantiomers of **22**, *via* stereoselective epoxidation and epoxide opening as outlined previously.

The basis of a chemical separation of a racemic mixture is the reaction of the racemate with a chiral agent to give a pair of diastereoisomers. Those diastereoisomers have different chemical and physical properties and can usually be separated by conventional means such as distillation, chromatography or crystallisation. This approach neccessitates regeneration of the enantiomers by removal of the chiral auxillary.

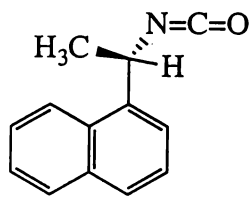
Trost *et al.* (1986) reported derivatisation with α-methoxyphenylacetic acid (MPA) (**108**) allowed easy chromatographic resolution of secondary alcohols using preparative HPLC. Other resolving agents employed in conjunction with HPLC include (-)-camphanoyl chloride (**109**) (Haag *et al.*, 1980) and α-(1-naphthyl)ethyl isocyanate (**110**) (Rüttimann *et al.*, 1983). HPLC was not available when this work was carried out. GC enantiomer separation was not pursued, as the chiral stationary phases were considered too expensive for use in the present study. König *et al.* (1982) reported that isopropyl urethane derivatives of chiral aliphatic, aromatic and monoterpene alcohols (formed from **111**) were separated using an XE60-*S*-valine-*S*-α-phenylethylamide-coated stationary phase.



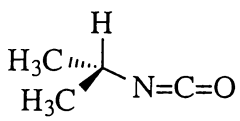
(**108**)



(**109**)



(**110**)



(**111**)

Biological Methods

Enzymes are catalysts which have demonstrated utility and potential in synthetic organic chemistry largely due to their specificity. Enzymes are generally selective in terms of the types of reaction catalysed and with respect to the structures and stereochemistries of substrates and products. The ability of enzymes to discriminate between the enantiomers of racemic substrates is well documented. Enzyme specificity is now increasingly exploited for the purpose of asymmetric synthesis, as many enzyme reactions can be carried out in non-aqueous media with high degrees of enantioselectivity. The general utility of enzymes in synthesis, and in particular, asymmetric synthesis, has been extensively reviewed (Whitesides and Wong, 1985; Chen and Sih, 1989; Santaniello *et al.*, 1992).

Hydolytic enzymes, such as lipases, have been used extensively as catalysts in enantioselective syntheses (Chen and Sih, 1989). Lipases have the ability to assume a variety of conformations to accomodate substrates of varying sizes and

stereochemical complexities. Moreover, their inherent affinity for hydrophobic environments distinguishes them from other hydrolytic enzymes, and enables them to catalyse reactions in non-aqueous media with high enantioselectivity. Lipases are useful candidates for practical transformations because they are commercially available, relatively inexpensive and require no cofactors for their action.

Lipases catalyse acyl transfer reactions such as asymmetric enzymatic hydrolysis and ester synthesis. In an aqueous environment the hydrolytic reaction mode is strongly favoured (Figure 2.7; equation 1). In organic solvents, lipases are particularly stable, and catalyse esterification and transesterification reactions in which acyl groups from suitable donors are transferred to a wide variety of acceptors other than water (e.g. alcohols) (Figure 2.7; equations 2/3). Enantioselectivities of lipase-catalysed esterification and transesterifications in organic media are often higher than that of the corresponding hydrolytic reactions in water. Moreover, ester synthesis is a highly attractive alternative to hydrolysis as it is applicable to substrates insoluble or unstable in aqueous media. The high enantioselectivity of enzymatic kinetic resolutions arises from the large difference in the reaction rates of the two competing enantiomers.

$$\text{Acyl-OR} + H_2O \underset{\text{esterification}}{\overset{\text{hydrolysis}}{\rightleftharpoons}} \text{Acyl-OH} + \text{ROH} \qquad (1)$$

$$\text{Acyl-OR'} + \text{enzyme} \rightleftharpoons [\text{Acyl-enzyme}] + \text{R'OH} \qquad (2)$$

$$[\text{Acyl-enzyme}] + \text{R''OH} \rightleftharpoons \text{Acyl-OR''} + \text{enzyme} \qquad (3)$$

Figure 2.7. Lipase-catalysed hydrolysis, esterification and transesterification.

Enzymatic hydrolysis or synthesis of esters has provided a simple route to a large number of enantiomerically pure acyclic secondary alcohols (Santaniello *et al.*, 1992). Few examples exist of enzymatic resolutions of cyclohexanols. The cyclohexane ring constitutes a central structural moiety in a large number of natural, biologically active products, and most of these compounds are chiral. A general method for synthesis of enantiomerically pure cyclohexanols would be beneficial.

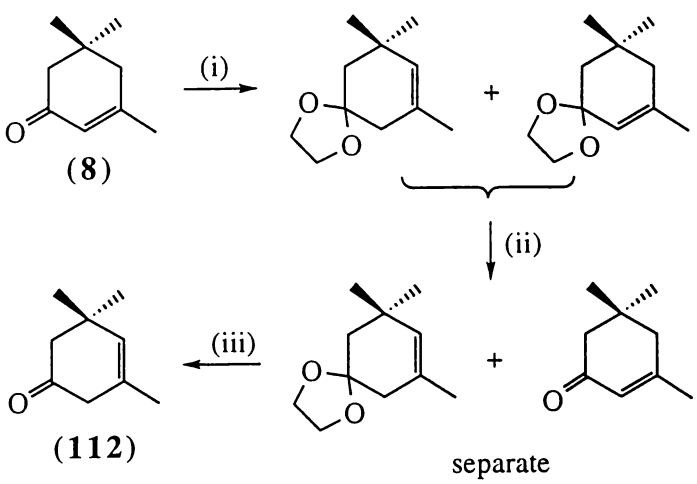
Some work has been directed towards the enzymatic resolution of 2-substituted cyclohexanols for which a satisfactory technique has been established

(Langrand *et al.*, 1985; Hönig and Seufer-Wasserthal, 1990). However, no studies have investigated cyclohexanols where the stereodifferentiating groups are remote from the centre of chirality (i.e. cyclohexanols without vicinal substituents).

Preparation of a Model Compound

Investigation of the optical resolution of 3,5,5-trimethylcyclohexenols, such as homoallylic alcohol (±)-**59**, was facilitated by synthesis of a model compound. Criteria for selection of a model included structural and substitutional similarity and availability by simple synthesis or commercial source. Compound **112** (β-phorone) was selected as a model for the preliminary experiments. Its synthesis from readily available isophorone (**8**) required deconjugation of the α,β-enone functionality, for which several methods have been reported. Those methods lead to mixtures of both enones (Babler *et al.*, 1978), or can give inconsistent results (Meinwald and Hendry, 1971).

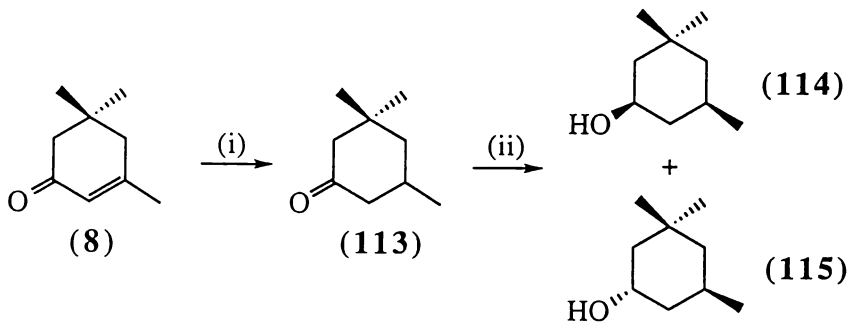
Pure β,γ-enones, including that of **8**, have been obtained from the corresponding α,β-enones *via* dioxolanation followed by hydrolysis (Babler *et al.*, 1978) (Scheme 2.20). Ketals of **8** were prepared (Babler *et al.*, 1978; Constantino *et al.*, 1986); however their hydrolysis was problematic, leading to a mixture of products and low yields of β-γ-enone **112**. Given the poor yields of that procedure, and the possibility of isomerisation of β-phorone **112** upon storage (Meinwald and Hendry, 1971), and in subsequent steps, an alternative model was sought.



Reagents: (i) $HOCH_2CH_2OH$, TsOH; (ii) $(COOH)_2$, MeCN; (iii) AcOH.

Scheme 2.20. Attempted synthesis of β-phorone (**112**) from isophorone (**8**).

Model compounds **114** and **115** were prepared from **8** by a simple synthetic route (Scheme 2.21). Reduction of ketone **113** gave a mixture of diastereoisomeric alcohols in a ratio of *ca* 1:2. The alcohols were readily separated by flash chromatography. Spin-spin coupling information in the ^1H NMR spectrum indicated that the major diastereoisomer was the *anti*-alcohol **115**, consistent with that reported for $NaBH_4$ reduction of isophorone (**8**) (House, 1972c).



Reagents: (i) H_2/Pd, 0.66 atm; (ii) $NaBH_4$.

Scheme 2.21. Synthesis of *cis*- and *trans*-3,3,5-trimethylcyclohexanol (**114**)/(**115**).

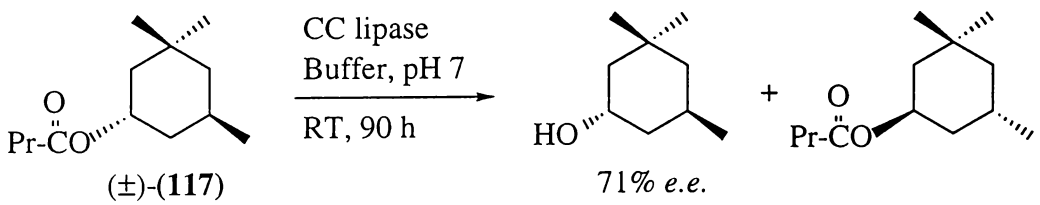
Enzymatic Hydrolysis

Enzymatic hydrolysis was assessed as a potential method for differentiation of enantiomers. There are approximately 20 different commercially available lipases (Chen and Sih, 1989). Yeast (*Candida cylindradea,* CC) and porcine pancreatic (PPL) lipases in particular, are used extensively as they have broad substrate specificity (Kirchner, 1985). These enzymes were used in the present study and were obtained in crude form without further purification. Unless stated, *anti*-alcohol **115** was used in enzymatic resolutions.

Some studies have demonstrated significant preference for hydrolysis of butanoates compared to acetates or longer chain esters by most lipases (Hönig *et al.*, 1989; Hönig and Seufer-Wasserthal, 1990). Thus, acetate **116** and butanoate **117** derivatives of alcohol **115** were prepared. Both enzymes showed poor activity for hydrolysis of those derivatives in a biphasic reaction medium (1:1 $CHCl_3$:H_2O). GCMS showed that transformations were extremely slow, with detectable quantities of alcohol observed only after five days.

The most successful lipase-mediated hydrolysis reactions have been carried out at neutral pH with use of phosphate buffers (pH 6.5-7.0). As lipases are active at water-organic interfaces (Chen and Sih, 1989), solubility of the organic substrate in water is not necessary. A number of 2-substituted cyclohexanols have been enantioselectively hydrolysed by CC and PPL in phosphate buffer in times of 1–60 h (Hönig and Seufer-Wasserthal, 1990). Enantiomeric excesses for hydrolysed alcohols were moderate to good (83–98% *e.e.*).

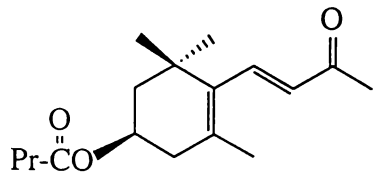
In the present study, CC and PPL-mediated hydrolyses of acetate **116** in phosphate buffer were extremely slow at both room temperature and 35°C, with no detectable reaction in 48 h. In contrast, CC-mediated hydrolysis of butanoate **117** (Figure 2.8) was significantly faster; GCMS analysis indicated approximately 50% conversion after 90 h. The enantiomeric purity of the alcohol product was deduced by the ^{1}H NMR spectra of the diastereoisotopic (*S*)-α-methoxyphenylacetic acid (MPA) derivatives (Dale and Mosher, 1973; Trost *et al.*, 1986) (Chapter Three), which indicated an enantiomeric purity of 71% *e.e.* Theoretical calculations (Sih *et al.*, 1982) have shown optimum chemical and optical yields for the faster-hydrolysed enantiomer are expected near 40% conversion. Thus, it was possible that 'over-hydrolysis' may have contributed to the low enantioselectivity.



(±)-(**117**) 71% *e.e.*

Figure 2.8. CC-mediated hydrolysis of butanoate (**117**).

The enantiodifferentiation observed for butanoate **117**, indicated investigation of the CC-catalysed hydrolysis of racemic homoallylic alcohol (±)-**59** was warranted. The corresponding butanoate **118** hydrolysed at a similar rate to that observed for the model compound **117**. However, analysis of the optical purity of resultant alcohol indicated no enantioselectivity. The method of esterification with MPA was examined for possible racemisation using optically active menthol as substrate; no racemisation was detected.



(118)

Lipase-catalysed Transesterification

A transesterification was evaluated as an alternative route to optically active homoallylic alcohol **59**. A wide range of cyclic and acyclic optically pure alcohols are obtained by lipase-catalysed esterification or transesterification of the corresponding racemic alcohols in an organic solvent. These enzymes retain a high degree of activity in organic systems (Langrand *et al.*, 1986; Kirchner *et al.*, 1985).

The transesterification reaction has proven to be more effective than the conventional esterification procedure (Santaniello *et al.*, 1992). Acylation of the alcohol may be performed with an ester like ethyl acetate acting as solvent and acylating agent. However, transesterification reactions are generally slower and afford poorer enantioselectivities compared to hydrolyses (Wang *et al.*, 1988). Acyl donors with good leaving groups such as trichloroethyl or trifluoro esters (Kirchner *et al.*, 1985) or anhydrides (Bianchi *et al.*, 1988) have been used to shift the equilibrium.

The best experimental procedure however, appears to be the irreversible transesterification which can be achieved using vinyl carboxylates as acylating agents (Wang *et al.*, 1988). In this case, the reverse reaction is prevented as the vinyl alcohol product irreversibly tautomerises to acetaldehyde (Figure 2.9). The high reactivity of enol esters in lipase transesterifications is well established (e.g. Wang and Wong, 1988; Burgess and Jennings, 1991).

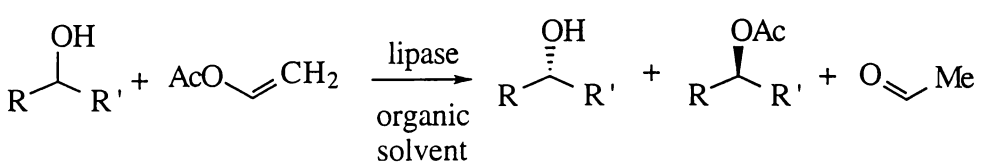


Figure 2.9. Transesterification of racemic alcohols using vinyl acetate as acylating agent.

Solvents may have significant effects on the reaction kinetics and stability of the enzyme (Chen and Sih, 1989). Apolar solvents, such as hexane, toluene, benzene and cyclohexane, are suitable for reactions in which dry enzyme powders are used. Moderate to highly polar sovlents may reduce the enzymes catalytic efficiency (due to denaturing) but this can often be overcome by immobilising the enzyme on hydrophilic supports. Lipases have shown vigorous activity in solvents such as chloroform, diethyl ether, and THF. Another consideration in choice of solvent is the solubility of the substrate and product.

CC and PPL-catalysed acylations of both *syn*-**114** and *anti*-**115** alcohols were carried out with a stoichiometric amount of acetic anhydride in THF, with enzymes added either directly to the solution or immobolised on celite (Bianchi *et al.*, 1988). The mixture was vigorously shaken, and aliquots were periodically withdrawn for GCMS analysis. Both enzymes showed low activity towards the alcohols with *ca* 20% conversion achieved in 48 h. Moreover, in control experiments (same conditions without enzyme), some acylation was observed over the periods of time required for the enzyme-mediated reaction. That suggested the presence of acid, possibly due to partial hydrolysis of acetic anhydride.

Subsequent reactions were carried out in near-anhydrous conditions (N_2 atmosphere), using freshly distilled reagents. Under those conditions, no acylation was observed in the control experiments. In the enzyme-mediated reaction, no enantioselectivity was observed for the acetate product following deacetylation. The possibility that racemisation was occurring in the deacetylation step was discounted as the remaining substrate alcohol exhibited no enantiomeric enrichment. Both enzymes showed low activity for transesterification of alcohol **115** using vinyl acetate as acylating agent (Wang *et al.*, 1988; Burgess and Jennings, 1991), with 10% conversion attained in 120 h. No enantioselectivity was observed. Poor enantioselectivities have also been reported for similar CC- and PSL (*Pseudomonas sp.*)-catalysed transesterifications of 3-methylcyclohex-2-enol (Wang *et al.*, 1988).

A satisfactory biocatalytic resolution of homoallylic alcohols was not found using two lipases generally regarded as being non-substrate specific. A more extensive study would require a screening process of a larger number of commercially available lipases. Conceptually, such a study would incorporate identification of a suitable enzyme, followed by optimisation of reaction conditions. The lack of success in the present study might be attributed to focusing

on the latter, namely optimisation, rather than identification of an enzyme displaying good activity towards the substrate.

Asymmetric Reduction

The reduction of ketones by fermenting baker's yeast (*Saccharomyces cervisiae*) has long been known and much of that work has been reviewed (Csuk and Glänzer, 1991; Santaniello *et al.*, 1992). Baker's yeast is able to enantioselectively reduce variously substituted carbonyl compounds to the corresponding hydroxy compounds. The stereochemical outcome of such reductions depends on the presence of dehydrogenases which generally follow Prelog's rule. Only a few examples for the reduction of cycloalkanones bearing no functionalities have been described and in those cases, the enantioselectivity was not determined. In the present study, baker's yeast showed no activity towards the ketone model **113**.

2.3. Conclusions

The present study sought to develop an efficient route to optically active 3-hydroxy- and 3,5,6-trihydroxy-5,6-dihydro-β-ionones, using readily available ionones as starting materials. A one-pot synthesis afforded an efficient route to key precursor 3,4-dehydro-β-ionone (**87**) from β-ionone. Enantio- and regioselective hydroboration of a cyclic diene has been reported (Rüttimann and Mayer, 1980), but was not successful in the present study for 3,4-dehydro-β-ionone-9-(1,3-dioxolane) (**93**). The presence of a butenone side chain appears to modify the reactivity of the terminal cyclic double bond towards the hydroborating reagent. Hydroboration of the triene **93** was achieved with $BH_3.SMe_2$, with reasonable regioselectivity.

Stereoselective molybdenum-catalysed epoxidation was an efficient means of *syn*-epoxidation of 3-hydroxy-β-ionone. Epoxide opening, as previously reported for 5,6-epoxy-ionones, afforded (±)-**22**. Synthesis of both enantiomers of **22** required a resolution step following hydroboration. A satisfactory method of biocatalytic resolution for homoallylic alcohols was not developed. An alternative method for determination of the absolute configuration of **22** was therefore investigated (Chapter Three).

The most efficient route to optically active 3,5,6-trihydroxy-β-ionone (**22**) may be *via* the optically active C-10 precursor prepared by asymmetric hydroboration of safranal, as described by Rüttimann and Mayer (1980). Safranal is not commercially available. Chain lengthening is effected efficiently in two steps. Finally, stereoselective metal-catalysed epoxidation and epoxide opening of optically active 3-hydroxy-β-ionone, would provide the required relative stereochemistry at C-5 and C-6.

2.4. Experimental

2.4.1. Spectroscopic Techniques

All NMR spectra were recorded in $CDCl_3$ on a Bruker AC300 at a probe temperature of 300 K (unless stated otherwise) using standard Bruker pulse programs. 300.13 MHz ^1H NMR spectra were processed with an exponential multiplier of 0.1 Hz and transformed using 32 K data points resulting in *J* values accurate to 0.28 Hz. NOE-difference experiments were carried out using NOEMULT. NOE-difference experiments involving multiplets were carried out with sequential irradiation of each line in the multiplet (Kinns and Sanders, 1984). Homonuclear shift correlation was established with the 2D absolute-value DQFCOSY experiment. 1 K data points were collected in the F_2 dimension with 256 increments, zero filled to 512 in the F_1 dimension. Data sets were transformed using unshifted sinebell window functions, and symmetrised after transformation.

75.47 MHz ^1H-decoupled ^{13}C spectra were transformed using 64 K data points. Multiplicity assignments were achieved with the DEPT pulse sequence ($\tau = 1/2J$ = 3.45 ms and θ = 135°) optimised for an average $^1J_{CH}$ of 145 Hz. Heteronuclear shift correlation spectra were established by 2D absolute-value ^{13}C-^1H chemical shift correlation (XHCORR). Long-range ^{13}C-^1H connectivities were established by changing the Δ_1 and Δ_2 delays in the XHCORR experiment to 35 and 25 ms, respectively. The δ 7.26 resonance of residual $CHCl_3$, and the center line of the $^{13}CDCl_3$ triplet (δ 77.06) were used as internal references for the ^1H and ^{13}C spectra.

Unless stated, all products were analysed by 2D ^1H-^1H COSY, ^1H-^{13}C correlation and long-range ^1H-^{13}C correlation experiments. Where a definitive assignment of resonances or couplings was not possible, the interchangeable

assignments are given. The assignment of ^{1}H and ^{13}C NMR spectra using these techniques is discussed in Chapters Three and Four.

Mass spectra were obtained on a Varian MAT CH5 mass spectrometer coupled with a Varian 2700 gas chromatograph *via* an open-split interface. An OV-1 FSOT column, 25 m x 0.32 mm (diameter), was used with helium as carrier gas and the temperature programmed from 40 to 280°C at 8°C/min (no initial hold, 10 min final hold). The mass spectra were recorded in electron ionisation (EI) mode at 70 eV, ion source temperature 200°C. The scan repetition rate was 4 s over a mass range of 35 to 480 amu. High resolution GCMS were obtained on the Kratos instrument of the Ruakura Agricultural Research Centre.

Infrared spectra were obtained on a Pye Unicam SP3-200S spectrophotometer as nujol mulls between KBr discs. Ultraviolet spectra were obtained on a Cary 1 UV/VIS spectrophotometer.

2.4.2. Analytical Techniques

TLC was carried out on silica coated aluminium sheets. Flash chromatography was carried out under the conditions described by Still *et al.* (1978). PLC was carried out on 2 mm silica gel (Merck Kieselgel) coated glass plates. Compounds were visualised by UV light (254 or 365 nm) or treatment with methanolic H_2SO_4.

2.4.3. Synthetic Methods

All reagents and solvents were purified according to the methods of Perrin and Armarego (1988).

*4-Acetoxy-β-ionone (4-(3-acetoxy-2,6,6-trimethylcyclohex-1-yl)-3-buten-2-one) (**89**).* To a solution of α-ionone (**88**) (8.2 g, 43 mmol) in acetic anhydride (60ml) at 80°C was added SeO_2 (1.6 g, 14 mmol). Following the addition of a further two portions of SeO_2 (2 x 1.6 g) at 1 h intervals, the reaction was left stirring at 90-100°C for 4 h. The mixture was filtered into water (300 ml) and extracted with diethyl ether (Et_2O) (3 x 80 ml). The combined ethereal extracts were washed with water (3 x 100 ml), saturated $NaHCO_3$, (2 x 150 ml), water

(150 ml) and dried over $MgSO_4$. Removal of the solvent under reduced pressure yielded a brown oil (11.1 g) which was purified by flash chromatography (7:3 petroleum ether (40-60°C):diethylether (PE:Et_2O)) twice to give compound **(89)** (Haag *et al.*, 1980) (5.6 g, 52%) as a pale yellow oil. ^1H NMR (300 MHz, $CDCl_3$) δ: 1.04 (3H, s, 1-Me), 1.08 (3H, s, 1-Me), 1.45 (1H, ddd, $J_{2eq,2ax}$ = 13.1, $J_{2eq,3}$ = 7.4, $J_{2eq,3}$ = 3.0 Hz, H-2_{eq}), 1.61 (1H, ddd, $J_{2ax,2eq}$ = 13.1, $J_{2ax,3}$ = 10.7, $J_{2ax,3}$ = 3.1 Hz, H-2_{ax}), 1.69 (3H, s, 5-Me), 1.75 (1H, m, H-3_{ax} or H-3_{eq}), 1.91(1H, m, H-3_{eq} or H-3_{ax}), 2.07 (3H, s, OAc), 2.30 (3H, s, H-10), 5.22 (1H, t, $J_{4,3ax}$ = $J_{4,3eq}$ = 4.8 Hz, H-4), 6.12 (1H, d, $J_{8,7}$ = 16.4 Hz, H-8), 7.17 (1H, dt, $J_{7,8}$ = 16.4, ^5J = 1.1 Hz[a], H-7). ^{13}C NMR (75.5 MHz, $CDCl_3$) δ: 18.2 (q, 5-Me), 21.2 (q, OCO<u>C</u>H_3), 25.0 (t, C-3), 27.2 (q, 1-Me), 27.4 (q, C-10), 28.6 (q, 1-Me), 34.4 (s, C-1), 34.7 (t, C-2), 71.8 (d, C-4), 129.9 (s, C-5), 133.3 (d, C-8), 141.6 (s, C-6), 142.0 (d, C-7), 170.8 (s, O<u>C</u>OCH_3), 198.0 (s, C-9). IR: υ 1737 (C=O acetate), 1673 cm^{-1} (α,β-unsaturated C=O). UV (EtOH): λ_{max} 232 (log ε 3.6). EIMS: *m/z* (rel. int.) 235 ($M^{+\cdot}$-Me, 9), 190 (26), 175 (21), 147 (13), 123 (28), 109 (16), 91 (12), 43 (101).

[a] A five-bond coupling of H-7 to H-4 and/or 5-Me in carotenoid β end-groups is well documented (Englert, 1996). This proton is generally reported as broad doublet; however in some cases, high-field NMR resolves the five-bond coupling.

4-Hydroxy-β-ionone (4-(3-hydroxy-2,6,6-trimethyl-1-cyclohexenyl)-3-buten-2-one) (90). To a solution of acetate **89** (5.4 g, 22 mmol) in methanol (80 ml) was added an aqueous solution of NaOH (1 g, 2.2 mmol; 5 ml). The reaction mixture was refluxed for 15 min, cooled then poured into water (300 ml). The product was extracted with ether (3 x 80 ml) and the extracts washed with 5% H_2SO_4 (100 ml), saturated $NaHCO_3$ (100 ml) and water (2 x 100 ml). The ether layer was dried over $MgSO_4$ and the solvent removed under reduced pressure affording a brown oil (4.1 g). Purification by flash chromatography using 3:7 EtOAc:PE gave alcohol **90** (Haag *et al.*, 1980) (3.95 g, 88 %) as a pale yellow oil. ^1H NMR (300 MHz, $CDCl_3$) δ: 0.97 (3H, s, 1-Me), 1.00 (3H, s, 1-Me), 1.39 (1H, ddd, $J_{2eq,2ax}$ = 13.3, $J_{2eq,3}$ = 7.8, $J_{2eq,3}$ = 3.2 Hz, H-2_{eq}), 1.60 (1H, ddd, $J_{2ax,2eq}$ = 13.2, $J_{2ax,3ax}$ = 10.6, $J_{2ax,3eq}$ = 3.0 Hz, H-2_{ax}), 1.66 (1H, m, H-3_{ax} or H-3_{eq}), 1.78 (3H, s, 5-Me), 1.84 (1H, m, H-3_{eq} or H-3_{ax}), 2.24 (3H, s, H-10), 2.46 (s(br.), OH), 3.94 (1H, t, $J_{4,3ax}$ = $J_{4,3eq}$ = 4.9 Hz, H-4), 6.05 (1H, d, $J_{8,7}$ = 16.5 Hz, H-8), 7.13 (1H, dquintet, $J_{7,8}$ = 16.5, ^5J = 1.1 Hz, H-7). ^{13}C NMR (75.5 MHz, $CDCl_3$) δ: 18.4 (q, 5-Me), 27.3 (q, 1-Me), 27.6 (q, C-10), 28.5 (t, C-3), 28.8 (q, 1-Me), 34.6 (s, C-1), 34.7 (t, C-2), 69.8 (d, C-4), 133.0 (d, C-8),

134.1 (s, C-5), 139.3 (s, C-6), 142.8 (d, C-7), 198.5 (s, C-9). IR: υ 3416 (OH), 1673 cm^{-1} (α,β-unsaturated C=O). UV (EtOH): λ_{max} 221 (log ε 3.6). EIMS: *m/z* (rel. int.) 208 ($M^{+\cdot}$, 18), 193 ($M^{+\cdot}$-Me, 10), 175 ($M^{+\cdot}$-Me-H_2O, 13), 165 (12), 151 (12), 137 (16), 123 (23), 109 (62), 91 (17), 77 (13), 55 (15), 43 (101).

3,4-Dehydro-β-ionone (4-(2,6,6-trimethyl-1,3-cyclohexadiene)-3-buten-2-one) (87). Compound **90** (3.95 g, 19 mmol) was dissolved in toluene (80 ml) and the resulting solution was heated to 50°C. *p*-TsOH (1.2 g, 6.3 mmol) was then added maintaining the temperature at 50-60°C with constant stirring. After 4 h TLC indicated that no starting material remained. The reaction mixture was cooled, washed with water (100 ml), saturated $NaHCO_3$ (2 x 100 ml), water (2 x 100 ml), then dried over $MgSO_4$. The solvent was removed under vacuum to give a brown oil (3.8 g) which was purified by flash chromatography using 3:7 Et_2O:PE to yield i) *alkene 87* (Findlay and MacKay, 1971) (2.2 g, 62%) (R_f = 0.6) as a bright yellow oil. ^1H NMR (300 MHz, $CDCl_3$) δ: 1.04 (6H, s, 2 x 1-Me), 1.89 (3H, s, 5-Me), 2.11 (2H, d, $J_{2,3}$ = 2.2 Hz, 2 x H-2), 2.30 (3H, s, H-10), 5.87 (2H, m, H-3 and H-4), 6.20 (1H, d, $J_{8,7}$ = 16.0 Hz, H-8), 7.26 (1H, d(br.), $J_{7,8}$ = 16.0 Hz, H-7). ^{13}C NMR (75.5 MHz, $CDCl_3$) δ: 20.4 (q, 5-Me), 26.6 (q, 2 x 1-Me), 27.4 (q, C-10), 34.1 (s, C-1), 40.1 (t, C-2), 128.3 (d, C-3), 129.7 (d, C-4), 130.4 (d, C-8), 132.8 (s, C-5), 136.0 (s, C-6), 141.8 (d, C-7), 198.5 (s, C-9). IR: υ 1672 and 1603 cm^{-1} (α,β-unsaturated C=O). UV (EtOH): λ_{max} 240 (log ε 3.8). EIMS: *m/z* (rel. int.) 190 ($M^{+\cdot}$, 10), 175 ($M^{+\cdot}$-Me, 42), 157 (8), 147 (13), 131 (14), 115 (13), 105 (12), 91 (15), 77 (9), 43 (101); ii) *1,1,6-trimethyl-1,2-dihydronaphthalene (37)* (Stevens *et al.*, 1975; Miginiac, 1990) (R_f = 0.9) (0.4 g, 11%) as a pale yellow oil. ^1H NMR (300 MHz, $CDCl_3$) δ: 1.29 (6H, s; 2 x 1-Me), 2.26 (2H, dd, $J_{2,3}$ = 4.6, $J_{2,4}$ = 1.8 Hz, 2 x H-2), 2.33 (3H, s, 6-Me), 5.95 (1H, dt, $J_{3,4}$ = 9.5, $J_{3,2ax}$ = $J_{3,2eq}$ = 4.6 Hz, H-3), 6.44 (1H, dt, $J_{4,3}$ = 9.5, $J_{4,2ax}$ = $J_{4,2eq}$ = 1.8 Hz, H-4), 6.90 (1H, s(br.), H-5), 7.03 (1H, dq, $J_{7,8}$ = 7.8, $J_{7,6\text{-Me}}$ = 1.0 Hz, H-7), 7.22 (1H, d, $J_{8,7}$ = 7.8 Hz, H-8). ^{13}C NMR (75.5 MHz, $CDCl_3$) δ: 20.9 (q, 6-Me), 28.5 (q, 2 x 1-Me), 33.2 (s, C-1), 39.1 (t, C-2), 123.7 (d), 127.2 (d), 127.3 (d), 127.6 (d), 128.1 (d), 133.3 (s), 135.4 (s), 141.2 (s). EIMS: *m/z* (rel. int.) 172 ($M^{+\cdot}$, 26), 157 ($M^{+\cdot}$-Me, 101), 142 (48), 128 (4), 115 (9), 91 (2), 77 (3), 63 (1), 44 (3); iii) *2,4,4,8-tetramethyl-5,6-dihydro-4H-cyclohepta-[b]-furan (92)* (Skorianetz and Ohloff, 1974) (0.3 g, 8%) (R_f = 0.85) as a colourless oil. ^1H NMR (300 MHz, $CDCl_3$) δ: 1.34 (6H, s, 2 x 4-Me), 1.71 (2H, m, 2 x H-5), 1.98 (3H, dt, $J_{8\text{-Me},7}$ = $J_{8\text{-Me},6ax}$ = $J_{8\text{-Me},6eq}$ =1.4 Hz, 8-Me), 2.28 (3H, d, $J_{2\text{-Me},3}$ = 1.2 Hz, 2-Me), 2.30 (2H, m, 2 x H-6), 5.65 (1H, tq, $J_{7,6ax}$ = $J_{7,6eq}$ = 5.9, $J_{7,8\text{-Me}}$= 1.4 Hz, H-7), 5.94 (1H, q, $J_{3,2\text{-Me}}$ = 1.2 Hz, H-3). ^{13}C NMR

(75.5 MHz, $CDCl_3$) δ: 13.4 (q, 8-Me), 23.2 (q, 2-Me), 24.8 (t, C-5), 29.5 (q, 2 x 4-Me), 37.4 (s, C-4), 39.3 (t, C-6), 105.8 (d, C-3), 118.8 (s, C-10), 126.1 (d, C-7), 128.1 (s, C-8), 148.6 (s, C-2 or C-9), 157.0 (s, C-9 or C-2). EIMS: *m/z* (rel. int.) 190 ($M^{+\cdot}$, 40), 175 ($M^{+\cdot}$-Me, 100) 157 (12), 147 (12), 133 (18), 131 (13), 119 (10), 105 (10), 91 (14), 77 (9), 43 (52), 41 (7).

One pot synthesis of 3,4-dehydro-β-ionone (87) (Findlay and MacKay, 1971). A solution of β-ionone (3.8 g, 19 mmol) and *N*-bromosuccinimide (4.6 g, 26 mmol) in dry CCl_4 (200 ml, redistilled over P_2O_5) was refluxed for 40 min whilst irradiated with a 100 W lamp. After cooling to room temperature, the succinimide was filtered off, anhydrous Na_2CO_3 (4.8 g, 45 mmol) and DMF (50 ml, freshly distilled) were added and the bulk of CCl_4 was distilled off (*ca* 100 ml). The reaction mixture was cooled to room temperature and then washed with 5% HCl solution (2 x 100 ml), water (100 ml), saturated $NaHCO_3$ solution (100 ml), saturated saline solution (100 ml) and dried over $MgSO_4$. Removal of the solvent afforded a brown oil (3.7 g) which was chromatographed on silica gel (flash chromatography) giving **87** (2.5 g, 70%) and *1,1,6-trimethyl-1,2-dihydronaphthalene (37)* (0.2 g, 6%).

3,4-Dehydro-β-ionone-9-(ethyleneacetal) (4-(2,6,6-trimethyl-1,3-cyclohexadiene)-3-buten-2-(1,3-dioxolane)) (93). A mixture of ethylene glycol (3.5 g, 50 mmol), *p*-TsOH (0.1 g) in benzene (150 ml) was distilled using Dean Starck apparatus to remove residual water. A solution of alkene **87** (2.0 g, 10 mmol) in dry benzene (10 ml) was then added and the mixture was refluxed for 8 hr. Following cooling, the reaction mixture was washed with water (2 x 200 ml), saturated $NaHCO_3$ (100 ml), water (100 ml), then dried over $MgSO_4$. Removal of the solvent by vacuum distillation yielded a brown oil (2.0 g) which was chromatographed using 1:9 Et_2O:PE to give compound **93** (R_f = 0.4) (1.59 g, 68%) as an orange oil. ^1H NMR (300 MHz, $CDCl_3$) δ: 0.98 (6H, s, 2 x 1-Me), 1.52 (3H, s, H-10), 1.79 (3H, s, 5-Me), 2.05 (2H, dd, $J_{2,3}$ = 4.3, $J_{2,4}$ = 1.6 Hz, 2 x H-2), 3.95 (4H, m, -O($C\underline{H}_2$)$_2$O-), 5.47 (1H, d, $J_{8,7}$ = 16.1, H-8), 5.70 (1H, dt, $J_{3,4}$ = 9.5, $J_{3,2ax}$ = $J_{3,2eq}$ = 4.3 Hz, H-3), 5.80 (1H, dt, $J_{4,3}$ = 9.5, $J_{4,2eq}$ = $J_{4,2ax}$ = 1.6 Hz, H-4), 6.21 (1H, d(br.), $J_{7,8}$ = 16.1 Hz, H-7). ^{13}C NMR (75.5 MHz, $CDCl_3$) δ: 19.8 (q, 5-Me), 25.3 (q, C-10), 26.5 (q, 2 x 1-Me), 33.6 (s, C-1), 39.6 (t, C-2), 64.5 (t, -O($\underline{C}H_2$)$_2$O-), 107.7 (s, C-9), 124.9 (d, C-3), 126.2 (s, C-5), 127.0 (d, C-7), 129.4 (d, C-4), 133.2 (d, C-8), 37.1 (s, C-6). UV (EtOH): λ_{max} 279 (log ε 3.9) and 230 (log ε 4.1). EIMS: *m/z* (rel. int.) 234 ($M^{+\cdot}$, 39), 219 ($M^{+\cdot}$-Me, 76), 147 (29), 119 (22), 87 (101), 73 (31). Found *m/z*

234.1629, $C_{15}H_{22}O_2$ requires *m/z* 234.1619. ii) *3,4-dehydro-β-ionone* (200 mg, 10.0%). iii)*1,1,6-trimethyl-1,2-dihydronaphthalene (37)* (100 mg, 5%). iv) *2,4,4,8-tetramethyl-5,6-dihydro-4H-cyclohepta-[b]-furan (92)* (100 mg, 5%). v) *4-(2',3',6'-trimethylphenyl)-3-buten-2-(1,3-dioxolane)* (69 mg, 3%) (R_f = 0.35). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.59 (3H, s, H-1), 2.19 (3H, s, Ar-Me), 2.23 (3H, s, Ar-Me), 2.24 (3H, s, Ar-Me), 4.01 (4H, m, -O(C$\underline{H}_2$)$_2$O-), 5.57 (1H, d, $J_{3,4}$ = 16.3 Hz, H-3), 6.70 (1H, d, $J_{4,3}$ = 16.3 Hz, H-4), 6.95 (2H, d, J = 3.1 Hz, H-4' and H-5'). ^{13}C NMR (75.5 MHz, $CDCl_3$) δ: 16.8 (q, Ar-Me), 20.4 (q, Ar-Me), 20.8 (q, Ar-Me), 25.2 (q, C-1), 64.6 (t, -O($\underline{C}$H$_2$)$_2$O-), 107.6 (s, C-2), 127.1 (d, C-4' or C-5'), 128.3 (d, C-5' or C-4'), 128.6 (d, C-4), 133.2 (s, C-3' or C-6'), 134.2 (s, C-6' or C-3'), 134.9 (d, C-3), 136.5 (s, C-2'), 139.5 (s, C-1'). EIMS: *m/z* (rel. int.) 232 ($M^{+\cdot}$, 13), 217 ($M^{+\cdot}$-Me, 100), 187 (2), 173 (12), 145 (15), 115 (9), 87 (18), 43 (26). The compound obtained by removal of the 1,3-dioxolane group gave ^{1}H NMR and MS data consistent with that reported for 4-(2',3',6'-trimethylphenyl)-3-buten-2-one (**96**) in the NBS library; EIMS: *m/z* (rel. int.) 188 ($M^{+\cdot}$, 7), 173 ($M^{+\cdot}$-Me, 100), 158 ($M^{+\cdot}$-Me-Me, 5), 145($M^{+\cdot}$-Me-CO, 5), 129 (19), 15 (12), 91 (5), 77 (4), 44 (11), 43 (9). vi) *4-(2',3',6'-trimethylphenyl)-butan-2-(1,3-dioxolane) (95)* (94 mg, 4%) (R_f = 0.30). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.43 (3H, s, H-1), 1.79 (2H, m, 2 x H-3), 2.24 (3H, s, Ar-Me), 2.26 (3H, s, Ar-Me), 2.32 (3H, s, Ar-Me), 2.77 (2H, m, 2 x H-4), 4.02 (4H, m, -O(C$\underline{H}_2$)$_2$O-), 6.92 (2H, s, H-4' and H-5'). ^{13}C NMR (75.5 MHz, $CDCl_3$) δ: 15.1 (q, 2'-Me), 19.8 (q, 3'-Me or 6'-Me), 20.7 (q, 6'-Me or 3'-Me), 23.7 (q, C-1), 24.3 (t, C-4), 38.2 (t, C-3), 64.8 (t, -O($\underline{C}$H$_2$)$_2$O-), 109.8 (s, C-2), 127.4 (d, C-4' and C-5'), 133.5 (s, C-6'), 134.5 (s, C-2' and C-3'), 138.5 (s, C-1'). EIMS: *m/z* (rel. int.) 234 ($M^{+\cdot}$, 3), 219 ($M^{+\cdot}$-Me, 2), 189 (1), 172 (27), 157 (12), 133 (16), 91 (7), 87 (101), 43 (26). The parent compound obtained by removal of the 1,3-dioxolane group gave identical ^{1}H NMR and MS data to that reported for 4-(2',3',6'-trimethylphenyl)-2-butanone (**97**) (Stevens *et al.*, 1975), and was compatible with the mass spectrum for that compound found in the NBS data base. EIMS: *m/z* (rel. int.) 190 ($M^{+\cdot}$, 12), 172 (66), 157 (73), 147 (18), 133 (71), 132 (101), 117 (33), 105 (15), 91 (22), 77 (7), 43 (18).

3-Hydroxy-β-ionone-9-(ethyeneacetal) (4-(4-hydroxy-2,6,6-trimethyl-1,3-cyclohexadiene)-3-buten-2-(1,3-dioxolane)) (101). A 50 ml flask[1] equipped with a septum inlet, magnetic stirrer and N_2-inlet was charged with $BH_3.SMe_2$[2] (5.2 ml,

[1] Reaction flasks and other glass equipment were dried in an oven (100°C, 12 h) and cooled in a desiccator over P_2O_5.

[2] Experimental techniques used in handling air-sensitive materials, as described by the manufacturer (Aldrich Chemical Co.), were followed.

9.4 mmol) then cooled to 0°C. A solution of alkene **93** (1.0 g, 4.3 mmol; dried over P_2O_5) in dry THF (2 ml) was added dropwise and the mixture was stirred at 0°C for 2 h. The reaction mixture was methanolysed (0.2 ml) followed by treatment with 3 M NaOH (3.2 ml, 9.4 mmol) and careful addition of 30% H_2O_2 (3 ml, 29 mmol), then stirred at 50°C for 1 h to ensure complete hydrolysis. The reaction mixture was cooled and extracted into ether (3 x 40 ml). The combined ethereal extracts were washed with water (2 x 50 ml) and saturated NaCl solution (50 ml) and dried over $MgSO_4$. The ether was removed at reduced pressure and the oil obtained purified by flash chromatography using 4:6 Et_2O:PE to give the alcohols **101** and **102** (390 mg, 36%) as a pale yellow oil. Further purification by PLC using 2:8 Et_2O:PE afforded alcohol **101** (250 mg, 23%) as a colourless oil. ^1H NMR (300 MHz, $CDCl_3$) δ: 1.02 (3H, s, 1-Me), 1.05 (3H, s, 1-Me), 1.44 (1H, t, $J_{2ax,2eq} = J_{2ax,3} = 12.0$ Hz, H-2_{ax}), 1.51 (3H, s, H-10), 1.67 (3H, s, 5-Me), 1.75 (1H, ddd, $J_{2eq,2ax} = 12.0$, $J_{2eq,3} = 3.6$, $J_{2eq,4eq} = 1.6$ Hz, H-2_{eq}), 1.98 (1H, ddq, $J_{4ax,4eq} = 14.7$, $J_{4ax,3} = 9.6$, $^4J = 1.2$ Hz, H-4_{ax}), 2.34 (1H, ddq, $J_{4eq,4ax} = 14.7$, $J_{4eq,3} = 5.6$, $J_{4eq,2eq} = 1.6$ Hz, H-4_{eq}), 3.92 (5H, m, H-3 and -O(C$\underline{H}_2$)$_2$O-), 5.35 (1H, d, $J_{8,7} = 16.1$ Hz, H-8), 6.15 (1H, ddt, $J_{7,8} = 16.1$, $^5J = 2.5$, $^5J = 1.2$ Hz[a], H-7). ^{13}C NMR (75.5 MHz, $CDCl_3$) δ: 21.1 (q, 5-Me), 25.2 (q, C-10), 28.4 (q, 1-Me), 30.1 (q, 1-Me), 36.7 (s, C-1), 42.1 (t, C-4), 48.1 (t, C-2), 64.5 (t, -O(C$\underline{H}_2$)$_2$O-), 64.9 (d, C-3), 107.5 (s, C-9), 125.8 (s, C-5), 127.4 (d, C-7), 134.4 (d, C-8), 138.2 (s, C-6). IR: υ 3422 cm^{-1} (br.) (OH). UV (EtOH): λ_{max} 220 (log ε 3.5). EIMS: *m/z* (rel. int.) 252 ($M^{+\cdot}$, 14), 237 ($M^{+\cdot}$-Me, 100), 219 ($M^{+\cdot}$-Me-H_2O, 7), 193 (5), 175 (8), 147 (10), 133 (8), 119 (7), 105 (12), 91 (18), 87 (44), 73 (11), 43 (50). Found *m/z* 252.1713, $C_{15}H_{24}O_3$ requires *m/z* 252.1752.

[a] Couplings to H-4_{eq}, H-4_{ax} and 5-Me were indicated by 2D COSY.

3-Hydroxy-β-ionone (4-(4-hydroxy-2,6,6-trimethylcyclohex-1-yl)-3-buten-2-one) (±)-(59). A solution of alcohol **101** (100 mg, 0.4 mmol) and oxalic acid (500 mg, 0.4 mmol) in acetonitrile (10 ml) was stirred at room temperature for 4 h after which no starting material could be detected by TLC. The solution was diluted with water (20 ml) and extracted with ether (2 x 20 ml). The ethereal extracts were washed with water (30 ml), saturated $NaHCO_3$ (20 ml), water (30 ml) and finally with saturated NaCl (20 ml). Following drying over $MgSO_4$, the solvent was removed yielding a pale yellow oil (100 mg). PLC using 1:1 Et_2O:PE gave (±)-**59** (Rüttimann and Mayer, 1980) (82 mg, 98%) as a colourless oil. ^1H NMR (300 MHz, $CDCl_3$) δ: 1.10 (3H, s, 1-Me), 1.12 (3H, s, 1-Me), 1.51 (1H, t, $J_{2ax,2eq} = J_{2ax,3} = 12.0$ Hz, H-2_{ax}), 1.78 (3H, s, 5-Me), 1.80 (1H, ddd, $J_{2eq,2ax} = 12.0$, $J_{2eq,3} = 3.7$, $J_{2eq,4eq} = 1.8$ Hz, H-2_{eq}), 2.11 (1H, ddd, $J_{4ax,4eq} = 14.6$,

$J_{4ax,3}$ = 9.5, ^4J = 1.8 Hz, H-4_{ax}), 2.31 (3H, s, H-10), 2.43 (1H, ddd, $J_{4eq,4ax}$ = 14.6, $J_{4eq,3}$ = 4.2, $J_{4eq,2eq}$ = 1.8 Hz, H-4_{eq}), 4.01 (1H, m, H-3), 6.12 (1H, d, $J_{8,7}$ = 16.1 Hz, H-8), 7.21 (1H, ddt, $J_{7,8}$ = 16.1, ^5J = 2.5, ^5J = 1.2 Hz, H-7). ^{13}C NMR (75.5 MHz, $CDCl_3$) δ: 21.6 (q, 5-Me), 27.3 (q, C-10), 28.6 (q, 1-Me), 30.1 (q, 1-Me), 36.9 (s, C-1), 42.8 (t, C-4), 48.4 (t, C-2), 64.5 (d, C-3), 132.39 (s, C-5), 132.4 (d, C-8), 135.6 (s, C-6), 142.3 (d, C-7), 198.5 (s, C-9). EIMS: *m/z* (rel. int.) 208 ($M^{+\cdot}$, 18), 193 ($M^{+\cdot}$-Me, 101), 175 ($M^{+\cdot}$-Me-H_2O, 18), 149 (8), 147 (10), 133 (10), 131 (12), 121 (10), 109 (13), 105 (14), 91 (13), 79 (8), 77 (9), 43 (52).

3-Hydroxy-5,6-epoxy-5,6-dihydro-β-ionone (4-(1,2-epoxy-4-hydroxy-2,6,6-trimethylcyclohexyl)-3-buten-2-one (±)-(23)/(24). A solution of (±)-**59** (150 mg, 0.72 mmol) in $CHCl_3$ (10 ml) was cooled to 0°C. A solution of MCPBA (180 mg, 0.86 mmol) in $CHCl_3$ (2 ml) was added dropwise and the reaction mixture was stirred at 0°C for 4 h. A further portion of MCPBA (35 mg, 0.20 mmol) in $CHCl_3$ (1 ml) was added and the reaction mixture maintained at RT for a further 20 h. The reaction mixture was diluted with ether (20 ml), washed with saturated $NaHCO_3$ (30 ml), water (2 x 30 ml), saturated NaCl (20 ml) and dried over $MgSO_4$. Following removal of the solvent under vacuum, the resulting oil (150 mg) was purified by PLC using 1:1 then 7:3 Et_2O:PE as developing solvent, affording a colourless oil (95 mg, 59%) containing a 3:1 mixture of *syn*- and *anti*-epoxides, and alcohol (±)-**59** (35 mg, 22%). NMR data was taken from the mixture: *syn*-Epoxide (±)-**23**: ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.00 (3H, s, 1-Me_{eq}), 1.17 (3H, s, 5-Me), 1.21 (3H, s, 1-Me_{ax}), 1.38 (1H, ddd, $J_{2eq,2ax}$ = 12.3, $J_{2eq,3}$ = 5.8, $J_{2eq,4eq}$ =1.2 Hz, H-2_{eq}), 1.56 (1H, t, $J_{2ax,2eq}$ = $J_{2ax,3}$ = 12.3 Hz, H-2_{ax}), 1.87 (1H, dd, $J_{4ax,4eq}$ = 14.8, $J_{4ax,3}$ = 8.7 Hz, H-4_{ax}), 2.20 (1H, ddd, $J_{4eq,4ax}$ = 14.8, $J_{4eq,3}$ = 6.4, $J_{4eq,2eq}$ = 1.2 Hz, H-4_{eq}), 2.23 (3H, s, H-10), 3.89 (1H, m, H-3), 6.24 (1H, d, $J_{8,7}$ = 16.0 Hz, H-8), 6.95 (1H, d, $J_{7,8}$ = 16.0 Hz, H-7). ^{13}C NMR (75.5 MHz, $CDCl_3$) δ: 21.0 (q, 5-Me), 25.6 (q, 1-Me_{ax}), 26.8 (q, C-10), 28.4 (q, 1-Me_{eq}), 34.8 (s, C-1), 38.9 (t, C-4), 43.4 (t, C-2), 63.5 (d, C-3), 65.6 (s, C-5), 70.5 (s, C-6), 133.1 (d, C-7), 141.2 (d, C-8), 197.4 (s, C-9). IR: υ 3417 (br.) (OH), 1672 cm^{-1} (α,β-unsaturated C=O). UV (EtOH): λ_{max} 233 (log ε 4.0). EIMS: *m/z* (rel. int.) 224 ($M^{+\cdot}$, 12), 206 (6), 191 (17), 175 (12), 173 (12), 166 (12), 163 (18), 149 (17), 123 (101), 109 (20), 95 (29), 91 (31), 79 (26), 67 (18), 55 (22), 43 (75). Found *m/z* 224.1405, $C_{13}H_{20}O_3$ requires *m/z* 224.1412. *anti*-Epoxide (±)-(**24**): ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.98 (3H, s, 1-Me_{eq}), 1.19 (3H, s, 5-Me), 1.21 (3H, s, 1-Me_{ax}), 1.28 (1H, t, $J_{2ax,2eq}$ = $J_{2ax,3}$ = 12.0 Hz, H-2_{ax}), 1.62 (1H, ddd, $J_{2eq,2ax}$ = 12.0, $J_{2eq,3}$ = 6.1, $J_{2eq,4eq}$ = 1.4 Hz,

H-2_{eq}), 1.64 (1H, dd, $J_{4ax,4eq}$ = 14.3, $J_{4ax,3}$ = 8.7 Hz, H-4_{ax}), 2.23 (3H, s, H-10), 2.42 (1H, ddd, $J_{4eq,4ax}$ = 14.3, $J_{4eq,3}$ = 5.9, $J_{4eq,2eq}$ = 1.4 Hz, H-4_{eq}), 3.89 (1H, m, H-3), 6.22 (1H, d, $J_{8,7}$ = 16.0 Hz, H-8), 7.02 (1H, d, $J_{7,8}$ = 16.0 Hz, H-7). ^{13}C NMR (75.5 MHz, $CDCl_3$) δ: 19.8 (q, 5-Me), 24.9 (q, 1-Me_{ax}), 26.8 (q, C-10), 29.3 (q, 1-Me_{eq}), 35.1 (s, C-1), 40.5 (t, C-4), 46.6 (t, C-2), 63.8 (d, C-3), 67.3 (s, C-5), 69.5 (s, C-6), 132.6 (d, C-7), 142.5 (d, C-8), 197.5 (s, C-9).

$Mo(CO)_6$/t-BuOOH epoxidation. To a solution of alcohol (±)-**59** (100 mg, 0.48 mmol) and $Mo(CO)_6$ (1.7 mg, $4.8x10^{-3}$ mmol) in benzene (5 ml) at reflux temperature was added *t*-BuOOH (70%) (0.07 ml, 0.53 mmol) dropwise. TLC showed no starting material was present after 30 min. After cooling, the reaction mixture was diluted with benzene (10 ml) and washed with aqueous sodium metabisulphite solution (2M, 20 ml) and water (20 ml). Removal of the solvent gave a white residue which was purified by PLC using 6:4 Et_2O:PE as solvent to give epoxide (±)-**23** (80 mg, 74%) as the major product.

3,5,6-Trihydroxy-5,6-dihydro-β-ionone (4-(1,2,4-trihydroxy-2,6,6-trimethylcyclohexyl)-3-buten-2-one) (±)-(22). To a solution of epoxide (±)-**23** (30 mg, 0.13 mmol) in THF (2 ml) at RT was added H_2SO_4 (30%, 0.05 ml, 0.28 mmol) dropwise. After 5 h a further portion of H_2SO_4 (0.05 ml) was added and the reaction mixture was stirred for a further 12 h. The reaction mixture was extracted with EtOAc (3 x 20 ml), washed with $NaHCO_3$ solution and dried over $MgSO_4$. Removal of the solvent under reduced pressure gave a colourless oil (30 mg) which was purified by PLC using 7:3 Et_2O:PE as solvent to give triol (±)-**22** as a white solid. Recrystallisation from toluene afforded white crystals (22 mg, 75%), m.p. 151-152°C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.85 (3H, s, 1-Me_{eq}), 1.15 (3H, s, 5-Me), 1.26 (3H, s, 1-Me_{ax}), 1.60 (2H, d, $J_{2ax/eq,3}$ = 8.4 Hz, H-2_{ax} and H-2_{eq}), 1.77 (1H, dd, $J_{4ax,4eq}$ = 13.0, $J_{4ax,3}$ = 11.3 Hz, H-4_{ax}), 1.89 (1H, ddd, $J_{4eq,4ax}$ = 13.0, $J_{4eq,3}$ = 4.5, $J_{4eq,2eq}$ = 1.0 Hz, H-2_{eq}), 2.31 (3H, s, H-10), 4.16 (1H, m, H-3), 6.37 (1H, d, $J_{8,7}$ = 16.0 Hz, H-8), 7.28 (1H, d, $J_{7,8}$= 16.0 Hz, H-7). ^{1}H NMR (300 MHz, 1:1 $CDCl_3$:CD_3OD) δ: 0.58 (3H, s, 1-Me_{eq}), 0.84 (3H, s, 5-Me), 0.99 (3H, s, 1-Me_{ax}), 1.27 (1H, ddd, $J_{2eq,2ax}$ = 12.3, $J_{2eq,3}$ = 4.7, $J_{2eq,4eq}$ = 1.9 Hz, H-2_{eq}), 1.30 (1H, t, $J_{2ax,2eq}$ = $J_{2ax,3}$ = 12.3 Hz, H-2_{ax}), 1.45 (1H, dd, $J_{4ax,4eq}$ = 13.0, $J_{4ax,3}$ = 11.3 Hz, H-4_{ax}), 1.54 (1H, ddd, $J_{4eq,4ax}$ = 13.0, $J_{4eq,3}$ = 4.6, $J_{4eq,2eq}$ = 1.9 Hz, H-4_{eq}), 2.06 (3H, s, H-10), 3.83 (1H, m, H-3), 6.10 (1H, d, $J_{8,7}$ = 16.3 Hz, H-8), 7.10 (1H, d, $J_{7,8}$ = 16.3 Hz, H-7). ^{13}C NMR (75.5 MHz, $CDCl_3$) δ: 25.6 (q, 1-Me_{ax}), 26.7 (q, 1-Me_{eq}), 27.4 (q, 5-Me), 27.8 (q, C-10), 40.3 (s, C-1), 45.4 (t, C-4), 45.6 (t, C-2), 64.3 (d, C-3), 77.1 (s,

C-5), 78.8 (s, C-6), 131.2 (d, C-7), 148.8 (d, C-8), 198.2 (s, C-9). EIMS: *m/z* (rel. int.) 224 ($M^{+\cdot}$-H_2O, 6), 141 (9), 140 (8), 125 (43), 124 (10), 123 (17), 109 (8), 99 (7), 97 (23), 95 (6), 83 (9), 71 (13), 69 (8), 55 (17), 43 (100). Found *m/z* 224.1379, $C_{13}H_{20}O_3$ ($M^{+\cdot}$-H_20) requires *m/z* 224.1412.

3,3,5-Trimethylcyclohexanone (113). A mixture of isophorone (**8**) (10.0 g, 72 mmmol) and Pd/C (5% Pd, 0.2 g) in ethanol (100 ml) was hydrogenated at 0.66 atm H_2 in a parr hydrogenator for 15 h. The reaction mixture was filtered from the Pd/C, dried with molecular sieves and the ethanol removed under vacuum. Ketone **113** (Cossy and BouzBouz, 1995) was obtained as a colourless liquid (9.9 g, 98%), and GCMS showed it was pure enough for use in subsequent reactions without further purification. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.84 (3H, s, 3-Me_{ax}), 0.97 (3H, d, $J_{5\text{-}Me,5}$ = 6.3 Hz, 5-Me), 1.01 (3H, s, 3-Me_{eq}), 1.25 (1H, t, $J_{4ax,4eq} = J_{4ax,5}$ = 12.7 Hz, H-4_{ax}), 1.53 (1H, ddt, $J_{4eq,4ax}$ = 12.7, $J_{4eq,5}$ = 4.1, $J_{4eq,2eq} = J_{4eq,6eq}$ = 1.9 Hz, H-4_{eq}), 1.84 (1H, td, $J_{6ax,6eq} = J_{6ax,5}$ = 13.0, J = 0.9 Hz, H-6_{ax}), 1.95 (1H, m, H-5), 2.10 (1H, dt, $J_{2eq,2ax}$ = 13.1, $J_{2eq,4eq} = J_{2eq,6eq}$ = 1.9 Hz, H-2_{eq}), 2.11 (1H, d, $J_{2ax,2eq}$ = 13.1, H-2_{ax}), 2.21 (1H, ddt, $J_{6eq,6ax}$ = 13.0, $J_{6eq,5}$ = 3.9, $J_{6eq,2eq} = J_{6eq,4eq}$ = 1.9 Hz, H-6_{eq}). ^{13}C NMR (75.5 MHz, $CDCl_3$) δ: 22.5 (q, 5-Me), 25.8 (q, 3-Me_{ax}), 29.7 (d, C-5), 32.1 (q, 3-Me_{eq}), 35.7 (s, C-3), 47.3 (t, C-4), 49.3 (t, C-6), 54.2 (t, C-2), 211.9 (s, C-1). EIMS: *m/z* (rel. int.) 140 ($M^{+\cdot}$, 16), 125 ($M^{+\cdot}$-Me, 12), 107 (30), 97 (7), 83 (100), 69 (53), 56 (43), 55 (51), 41 (61).

3,3,5-Trimethylcyclohexanol (114)/(115). To a solution of ketone **113** (7.5 g, 53 mmol) in EtOH (80 ml) was added $NaBH_4$ (2.4 g, 64 mmol). The mixture was stirred at RT for 15 h and then poured into saturated NH_4Cl solution (100 ml). Once effervesence had subsided, the mixture was extracted with CH_2Cl_2 (3 x 50 ml), washed with NH_4Cl solution and water (2 x 80 ml). The organic phase was dried over anhydrous $MgSO_4$ and the solvent removed under reduced pressure to give a mixture of two alcohols (6.0 g, *syn*-**114**:*anti*-**115** = 2:3) as a pale yellow oil. Flash chromatography using 2:3 Et_2O:PE as solvent yielded *syn*-alcohol **114** (R_f = 0.3) (1.4 g, 19%) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.70 (1H, t, $J_{4ax,4eq} = J_{4ax,5}$ = 12.5 Hz, H-4_{ax}), 0.74 (1H, t, $J_{6ax,6eq} = J_{6ax,1}$ = 11.7 Hz, H-6_{ax}) 0.85 (3H, s, 3-Me_{eq}), 0.86 (3H, d, $J_{5\text{-}Me,5}$ = 7.0 Hz, 5-Me), 0.89 (3H, s, 3-Me_{ax}), 0.94 (1H, t, $J_{2ax,2eq} = J_{2ax,1}$ = 12.1 Hz, H-2_{ax}), 1.26 (1H, ddt, $J_{4eq,4ax}$ = 12.5, $J_{4eq,5}$ = 3.2, $J_{4eq,2eq} = J_{4eq,6eq}$ = 1.7 Hz, H-4_{eq}), 1.60 (1H, m, H-5), 1.63 (1H, ddt, $J_{2eq,2ax}$ = 12.1, $J_{2eq,1}$ = 4.1, $J_{2eq,4eq} = J_{2eq,6eq}$ = 1.7 Hz, H-2_{eq}), 1.91 (1H, m, H-6_{eq}), 2.17 (1H, s(br.), OH), 3.71 (1H, m, H-1). ^{13}C NMR (75.5 MHz, $CDCl_3$) δ: 22.3 (q, 5-Me), 25.7 (q, 3-Me_{ax}), 27.2 (d,

C-5), 32.2 (s, C-3), 33.1 (q, 3-Me_{eq}), 44.6 (t, C-6), 47.6 (t, C-4), 48.1 (t, C-2), 67.7 (d, C-1). EIMS: *m/z* (rel. int.) 142 ($M^{+\cdot}$, 5), 124 ($M^{+\cdot}$-H_2O, 9), 109 (100), 83 (24), 71 (23), 67 (24), 55 (28), 43 (28), 41 (44). *Anti*-alcohol (**115**) (Cossy and BouzBouz, 1995) (R_f = 0.4) (3.0 g, 40%) as a pale pink solid. ^1H NMR (300 MHz, $CDCl_3$) δ: 0.81 (1H, t, $J_{4ax,4eq} = J_{4ax,5}$ = 12.5 Hz, H-4_{ax}), 0.87 (3H, s, 3-Me_{eq}), 0.89 (3H, d, J = 6.4 Hz, 5-Me), 1.07 (1H, ddd, $J_{6ax,6eq}$ = 13.9, $J_{6ax,5}$ = 12.0, $J_{6ax,1}$ = 3.2 Hz, H-6_{ax}), 1.09 (3H, s, 3-Me_{ax}), 1.27 (1H, dd, $J_{2ax,2eq}$ = 14.3, $J_{2ax,1}$ = 3.2 Hz, H-2_{ax}), 1.42 (1H, ddt, $J_{4eq,4ax}$ = 12.5, $J_{4eq,5}$ = 3.2, $J_{4eq,2eq} = J_{4eq,6eq}$ = 2.1 Hz, H-4_{eq}), 1.53 (1H, ddd, $J_{2eq,2ax}$ = 14.3, $J_{2eq,1}$ = 5.0, $J_{2eq,4eq} = J_{2eq,6eq}$ = 2.1 Hz, H-2_{eq}), 1.73 (1H, ddt, $J_{6eq,6ax}$ = 13.9, $J_{6eq,1}$ = 5.5, $J_{6eq,2eq} = J_{6eq,4eq}$ = 2.1 Hz, H-6_{eq}), 1.96 (1H, m, H-5), 4.14 (1H, m, H-1). ^{13}C NMR (75.5 MHz, $CDCl_3$) δ: 22.6 (q, 5-Me), 22.8 (d, C-5), 28.2 (q, 3-Me_{ax}), 30.7 (s, C-3), 34.1 (q, 3-Me_{eq}), 41.6 (t, C-6), 44.8 (t, C-2), 48.5 (t, C-4), 68.3 (d, C-1).

Typical procedure for esterification. The compound was dissolved in excess pyridine/acetic anhydride 1:1 and stirred at RT for 12 h. The reaction mixture was then poured into water (150 ml), extracted with Et_2O (2 x 100 ml), washed twice with 5% H_2SO_4, water, saturated $NaHCO_3$, and saturated NaCl (50 ml each). The organic layer was dried over anhydrous $MgSO_4$ and the solvent removed under reduced pressure.

*1-Acetoxy-3,5,5-trimethylcyclohexane (**116**).* Alcohol **115** (800 mg, 5.6 mmol) was acetylated using the standard procedure affording acetate **116** (860 mg, 83%) as a colourless oil. ^1H NMR (300 MHz, $CDCl_3$) δ: 0.79 (1H, t, $J_{4ax,4eq} = J_{4ax,5}$ = 12.7 Hz, H4_{ax}), 0.84 (3H, s, 3-Me), 0.85 (3H, d, $J_{5\text{-}Me\text{-}5}$ = 5.6 Hz, 5-Me), 0.98 (3H, s, 3-Me), 0.98 (1H, ddd, $J_{6ax,6eq}$ = 14.6, $J_{6ax,5}$ = 12.1, $J_{6ax,1}$ = 3.2 Hz, H-6_{ax}), 1.20 (1H, dd, $J_{2ax,2eq}$ = 14.8, $J_{2ax,1}$ = 3.7, H-2_{ax}), 1.39 (1H, ddt, $J_{4eq,4ax}$ = 12.7, $J_{4eq,5}$ = 5.5, $J_{4eq,2eq} = J_{4eq,6eq}$ = 2.2 Hz, H-4_{eq}), 1.64 (1H, dq, $J_{2eq,2ax}$ = 14.8, $J_{2eq,1} = J_{2eq,4eq}$ = 2.4 Hz, H-2_{eq}), 1.76 (1H, m, H-6_{eq}), 1.84 (1H, m, H-5), 1.97 (3H, s, OAc), 5.04 (1H, m, H-1). ^{13}C NMR (75.5 MHz, $CDCl_3$) δ: 21.5 (q, OAc), 22.4 (q, 5-Me or 3-Me), 23.2 (d, C-5), 25.8 (q, 3-Me), 30.6 (s, C-3), 33.9 (q, 3-Me or 5-Me), 38.4 (t, C-6), 41.2 (t, C-2), 48.1 (t, C-4), 71.0 (d, C-1), 170.4 (s, OAc). EIMS: *m/z* (rel. int.) 141 ($M^{+\cdot}$-CH_3CO, 5), 124 (29), 109 (79), 95 (13), 82 (21), 68 (29), 67 (29), 55 (18), 43 (100).

*1-Butoxy-3,3,5-trimethylcyclohexane (**117**).* To a solution of alcohol **115** (200 mg, 1.4 mmol) in CH_2Cl_2 (5 ml) was added a mixture of pyridine and butanoic anhydride (2 ml, 1:1) and DMAP (20 mg, 0.1 mmol). The reaction mixture was stirred at RT for 5 h then poured into water (70 ml). The aqueous

phase was extracted with Et_2O (2 x 20 ml), and the ethereal phase washed with 5% H_2SO_4, water, saturated $NaHCO_3$ (2 x 20 ml) and dried over anhydrous $MgSO_4$. Removal of the ether under reduced pressure yielded a pale yellow liquid which was purified by flash chromatography using 1:9 Et_2O:PE to give butanoate **117** (264 mg, 89%) as a colourless liquid. ^1H NMR (300 MHz, $CDCl_3$) δ: 0.82 (1H, t, $J_{4ax,4eq} = J_{4ax,5} = 12.7$ Hz, H-4_{ax}), 0.86 (3H, d, $J_{5\text{-}Me,5} = 6.5$ Hz, 5-Me), 0.87 (3H, s, 3-Me_{eq}), 0.93 (3H, t, $^3J = 7.5$ Hz, C$\underline{H}_3CH_2CH_2$-), 0.99 (1H, ddd, $J_{6ax,6eq} = 13.2$, $J_{6ax,5} = 12.0$, $J_{6ax,1} = 3.2$ Hz, H-6_{ax}), 1.00 (3H, s, 3-Me_{ax}), 1.24 (1H, dd, $J_{2ax,2eq} = 14.8$, $J_{2ax,1} = 3.6$ Hz, H-2_{ax}), 1.42 (1H, ddt, $J_{4eq,4ax} = 12.7$, $J_{4eq,5} = 5.3$, $J_{4eq,2eq} = J_{4eq,6eq} = 2.4$ Hz, H-4_{eq}), 1.63 (2H, q, $^3J = 6.2$ Hz, $CH_3C\underline{H}_2CH_2$-), 1.65 (1H, ddt, $J_{2eq,2ax} = 14.8$, $J_{2eq,1} = 3.2$, $J_{2eq,4eq} = J_{2eq,6eq} = 2.4$ Hz, H-2_{eq}), 1.78 (1H, m, H-6_{eq}), 1.85 (1H, m, H-5), 2.25 (2H, tq, $^3J = 7.4$, $^4J = 0.8$ Hz, $CH_3CH_2C\underline{H}_2$-), 5.09 (1H, dt, $^3J = 6.3$, $^3J = 3.2$ Hz, H-1). ^{13}C NMR (75.5 MHz, $CDCl_3$) δ: 13.7 (q, $\underline{C}H_3CH_2CH_2$-), 18.5 (t, $CH_3\underline{C}H_2CH_2$-), 22.5 (q, 5-Me or 3-Me_{ax}), 23.4 (d, C-5), 27.3 (q, 3-Me_{eq}), 30.6 (s, C-3), 33.9 (q, 3-Me_{ax} or 5-Me), 36.9 (t, $CH_3CH_2\underline{C}H_2$-), 38.5 (t, C-6), 41.4 (t, C-2), 48.1 (t, C-4), 70.8 (d, C-1), 173.1 (s, Pr$\underline{C}$=O). EIMS: *m/z* (rel. int.) 197 ($M^{+\cdot}$-Me, 5%), 141 (8), 124 (42), 109 (101), 95 (24), 82 (29), 71 (26), 69 (32), 68 (100), 55 (18), 43 (53).

*3-Butoxy-β-ionone (4-(4-butoxy-2,6,6-trimethylcyclohex-1-yl)but-3-en-2-one) (**118**).* Butanonate **118** was prepared from alcohol **59** according to the method of Hönig and Seufer-Wasserthal (1989): A solution of alcohol **59** (60 mg, 0.29 mmol) in CH_2Cl_2 (5 ml), butanoic anhydride (0.05 ml, 0.32 mmol), pyridine (0.05 ml, 0.62 mmol) and DMAP (0.001 g) was stirred at RT until conversion was complete (16 h). The reaction mixture was poured in water and extracted with ether (2 x 15 ml). The ethereal layer was washed with 5% H_2SO_4, water, saturated $NaHCO_3$ (2 x 30 ml), water (30 ml) and then dried over $NaSO_4$. The solvent was evaporated under reduced pressure to give a yellow oil (75 mg) which was contaminated with butyric acid (^1H NMR). The oil was dissolved in ether (20 ml) and washed with $NaHCO_3$ solution and after evaporation of the solvent, was purified by PLC using Et_2O:PE to give a pale yellow oil (40 mg, 50%). ^1H NMR (90 MHz, $CDCl_3$) δ: 0.96 (3H, t, 8.1 Hz, C$\underline{H}_3CH_2CH_2$-), 1.11 (3H, s, 1-Me_{eq}), 1.16 (3H, s, 1-Me_{ax}), 1.58-2.10 (3H, H-2_{ax}, H-2_{eq}, and H-4_{ax}[a]), 1.66 (2H, m, $CH_3C\underline{H}_2CH_2$-), 1.76 (3H, s, 5-Me), 2.29 (2H, t, $J = 7.5$ Hz, $CH_3CH_2C\underline{H}_2$-), 2.29 (3H, s, H-10), 2.41 (1H, d, $J_{4eq,4ax} = 13.0$, $J_{4eq,3} = 3.2$ Hz, H-4_{eq}), 5.05 (1H, m, H-3), 6.10 (1H, d, $J_{8,7} = 16.1$ Hz, H-8), 7.21 (1H, d, $J_{7,8} = 16.1$ Hz, H-7). EIMS: *m/z* (rel. int.) 217 (2), 121 (100), 105 (5), 91 (12), 77 (15), 55 (11).

[a] These resonances were not resolved from signals of the butanoate moiety in the 90 MHz spectrum.

Enzymes. Porcine pancreatic lipase (13 units/mg) and lipase from *Candida cylindracea* (500 units/mg) were purchased from Sigma Chemical Co. and were used without purification. Adsorption of enzymes on celite was carried out as described by Bianchi *et al.* (1988).

*Lipase-catalysed hydrolysis of acetate **116** and butanoates **117** and **118**.* To a solution of lipase (CC or PPL, 50 mg) in phosphate buffer (0.1 M, pH = 7.0, 30 ml) was added acetate **116** or butanoate **117** (150 mg). While vigorous stirring was maintained, the pH was kept constant at pH = 6.5 by dropwise addition of 1 M NaOH. Aliquots (0.5 ml) of reaction mixture were monitored by GCMS following filtration through cotton wool and extraction into Et_2O. When approximately 40% conversion was attained the reaction mixture was extracted with Et_2O (3 x 20 ml) and the solvent removed by vacuum yielding a colourless oil (60 mg). Flash chromatography using 1:9 then 2:9 Et_2O:PE yielded i) unreacted butanoate (35 mg, 23%) and ii) hydrolysed alcohol **115'** (15 mg, 10%).

Determination of enantiomeric excess. Alcohol **115'** and (-)-menthol were esterified with (*S*)-MPA using the procedure outlined in the Experimental Section of Chapter Three. Enantiomeric excess was determined by integration of the MPA methoxyl resonances. ^1H NMR indicated an *e.e.* of 71% for **115'** (methoxyl resonances at δ 3.40 and 3.42).

*Attempted lipase-catalysed acylation of alcohol **115** with anhydride* (Bianchi *et al.*, 1988). To a stirred solution of alcohol **115** (900mg, 6.33 mmol) and acetic anhydride (970mg, 9.50 mmol) in THF (15 ml) was added lipase (400 mg) or lipase adsorped on celite (900 mg). The reaction mixture was stirred at RT and then 35°C. Periodically, aliquots (0.1 ml) were withdrawn, filtered through cotton wool and analysed by GCMS.

*Attempted lipase-catalysed acylation of alcohol **115** with vinyl acetate* (Wang *et al.* 1988). Alcohol **115** (200 mg, 1.41 mmol), vinyl acetate (0.52 ml, 5.64 mmol) and ground 4Å molecular seives (210 mg) were dissolved in hexane (15 ml). Following addition of the enzyme, the suspension was stirred at 35°C and monitored by GCMS.

Alkaline hydrolysis of esters. The ester was dissolved in a 1 M solution of NaOH in EtOH (10 ml) and the solution stirred at 35°C and followed by GCMS. The solution was concentrated under vacuum, and the residue poured into water (50 ml), extracted with EtOAc (2 x 30 ml), washed with 5% H_2SO_4 (20 ml) and then water (20 ml). The solvent was removed and the resulting oil chromatographed by PLC. The enantiomeric excess was of the alcohol was determined as outlined above.

Chapter Three

Determination of the Absolute Stereochemistry of 3,5,6-Trihydroxy-5,6-dihydro-β-ionone

3.1. Introduction

The dominant component in the diethyl ether extractives of New Zealand native thyme honey was confirmed as *r*-3,*t*-5,*t*-6-trihydroxy-5,6-dihydro-β-ionone **22** by X-ray crystallography. Although triol **22** crystallised in an optically active space group, its absolute stereochemistry could not be determined from the data. Attempts to develop a facile enantioselective synthesis of both enantiomers of triol **22** were unproductive, although a simple racemic synthesis was achieved (Chapter Two). To complete the characterisation of **22**, analytical methods for determination of absolute stereochemistry were investigated.

3.1.1. Methods of Absolute Configuration Assignment

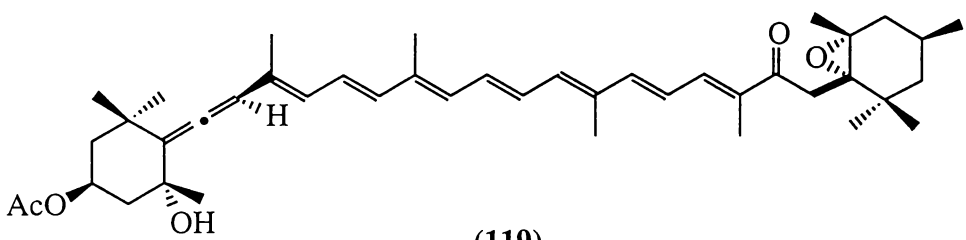
A number of techniques have been described for determination of absolute configuration. Currently, X-ray diffraction is the only reliable method. All other methods depend, to a greater or lesser extent, on the correlation of a compound of unknown configuration with one of established configuration. This correlation may be through chemical or physical properties.

The absolute configurations of optically active carotenoid end-groups are generally determined by chemical, chiroptical and X-ray methods. These, and other methods which are relevant to determination of the absolute stereochemistry of β-ionone derivatives, are outlined here. The use of NMR for assignment of absolute configuration is also reviewed

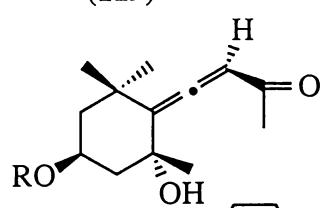
X-ray Diffraction

Determination of absolute configuration by anomalous X-ray diffraction is preferred, as it can distinguish directly between enantiomers (Eliel *et al.*, 1994 and references cited). Anomalous X-ray scattering usually depends on the presence of a heavy atom that can absorb, as well as diffract X-rays. The pattern obtained from this technique depends on the chirality of the molecule, and allows the unequivocal assignment of absolute configuration. Standard X-ray diffraction may be used in an indirect way to determine a chiral centre in a molecule which also possesses a group of known chirality. This may be achieved by derivatisation of an appropriate group in the molecule by a chiral reagent of known configuration.

In general, X-ray diffraction has not been successfully employed for carotenoids due to difficulties in obtaining suitable crystals (Weedon and Moss, 1995). However, the absolute configuration of fucoxanthin **(119)** was determined unequivocally by X-ray crystallographic analysis of the *p*-bromobenzoate derivative **120** (DeVille *et al.*, 1969).



(119)



(120) R = Br—C$_6$H$_4$—CO-

(121) R = H

Chemical Methods

Assignment of the absolute configuration of allenic end-group **121** (grasshopper ketone) enabled assignment of the absolute configurations of other carotenoids with β end-groups. The absolute stereochemistries of the 3-hydroxy-β end-groups (A and B) of zeaxanthin **(30)** and diatoxanthin **(122)**, 3-hydroxy-5,6-epoxy-5,6-dihydro-β end-group (C) of violaxanthin **(31)**, and the allenic end-

group (D) of neoxanthin (**32**) (Figure 3.1), and others, were established by chemical correlations with this end-group (Bartlett *et al.*, 1969; Cholnoky *et al.*, 1969; Hlubucek *et al.*, 1974). The absolute configuration of the 5,6-epoxy group remained to be established.

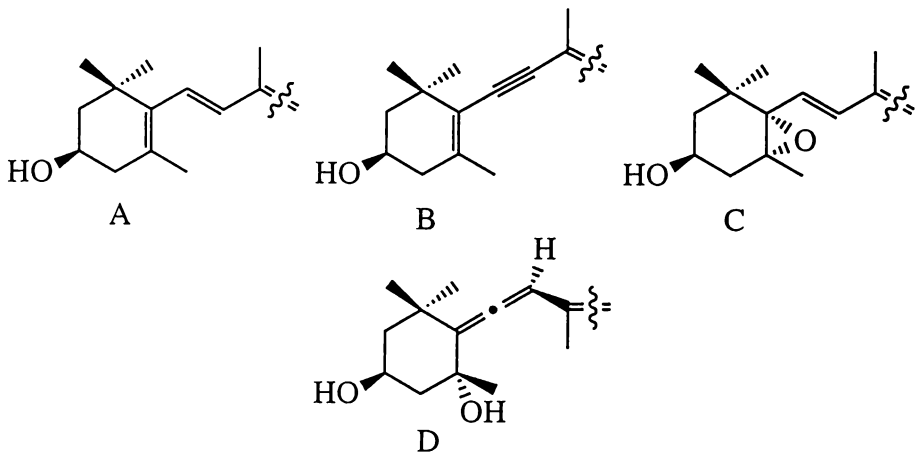
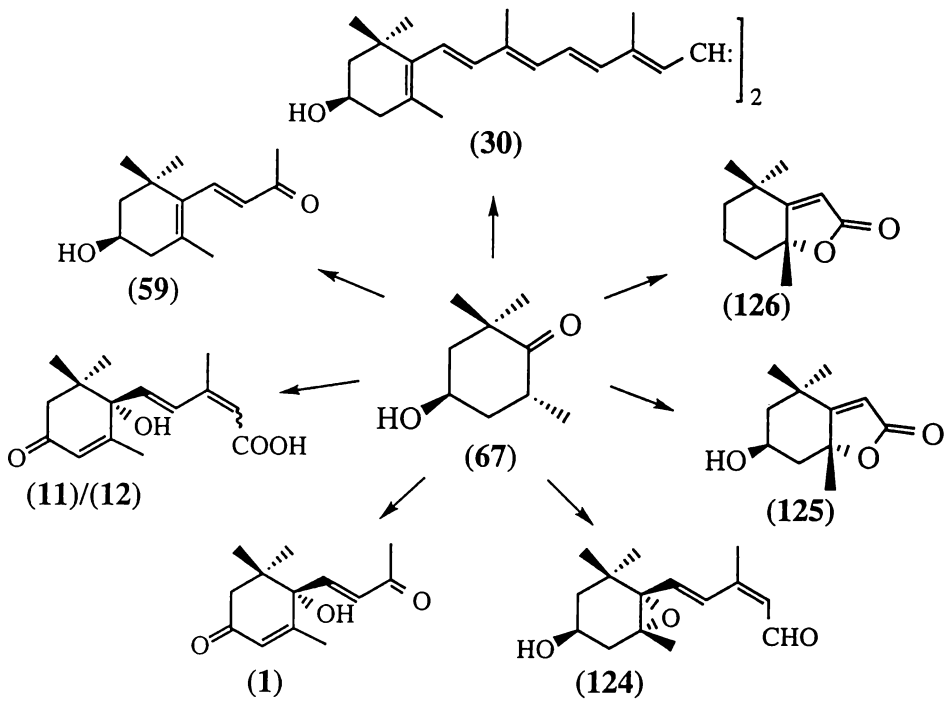


Figure 3.1. The absolute configurations of 3-hydroxylated-β end-groups determined by chemical correlation with grasshopper ketone (**121**).

Chemical methods are correlative, relying on the chemical transformation of a compound of unknown configuration, into a compound of known configuration. This correlation must take place through a series of steps which do not affect the bonds to the asymmetric carbon. Generally, chemical methods are reliable, but may be tedious, and of limited applicability. The studies of Bartlett *et al.* (1969) and Hlubucek *et al.* (1974) however, demonstrated that the structures and substitutional patterns of many carotenoid end-groups were conducive to correlation of chiral centres by chemical means. The same principle applies to degraded carotenoids (*nor*-isoprenoids). Recently, the absolute configuration of a novel 3,6-dihydroxy-5,6-dihydro-β-ionol was determined by chemical correlation with (*3R*)-3-hydroxy-β-ionone (**59**) (Pérez *et al.*, 1996).

Absolute stereochemistry may also be established by synthetic methods. This approach requires that the absolute stereochemistry of a synthetic precursor be determined. The chirality of a key carotenoid end-group precursor **67** (Scheme 2.4) was predicted by optical rotatory dispersion (ORD) (*vide infra*), and subsequently confirmed by transformation to grasshopper ketone (**121**) (Mori, 1974). Thus, the absolute structures of 3-hydroxy-β-ionone (**59**) (Mori, 1974), zeaxanthin (**30**) (Mayer 1979), ABA isomers (**11**)/(**12**) (Mori, 1974; Kienzle *et al.*, 1978), dehydrovomifoliol (**1**) (Mori, 1974), xanthoxin (**124**), loliolide

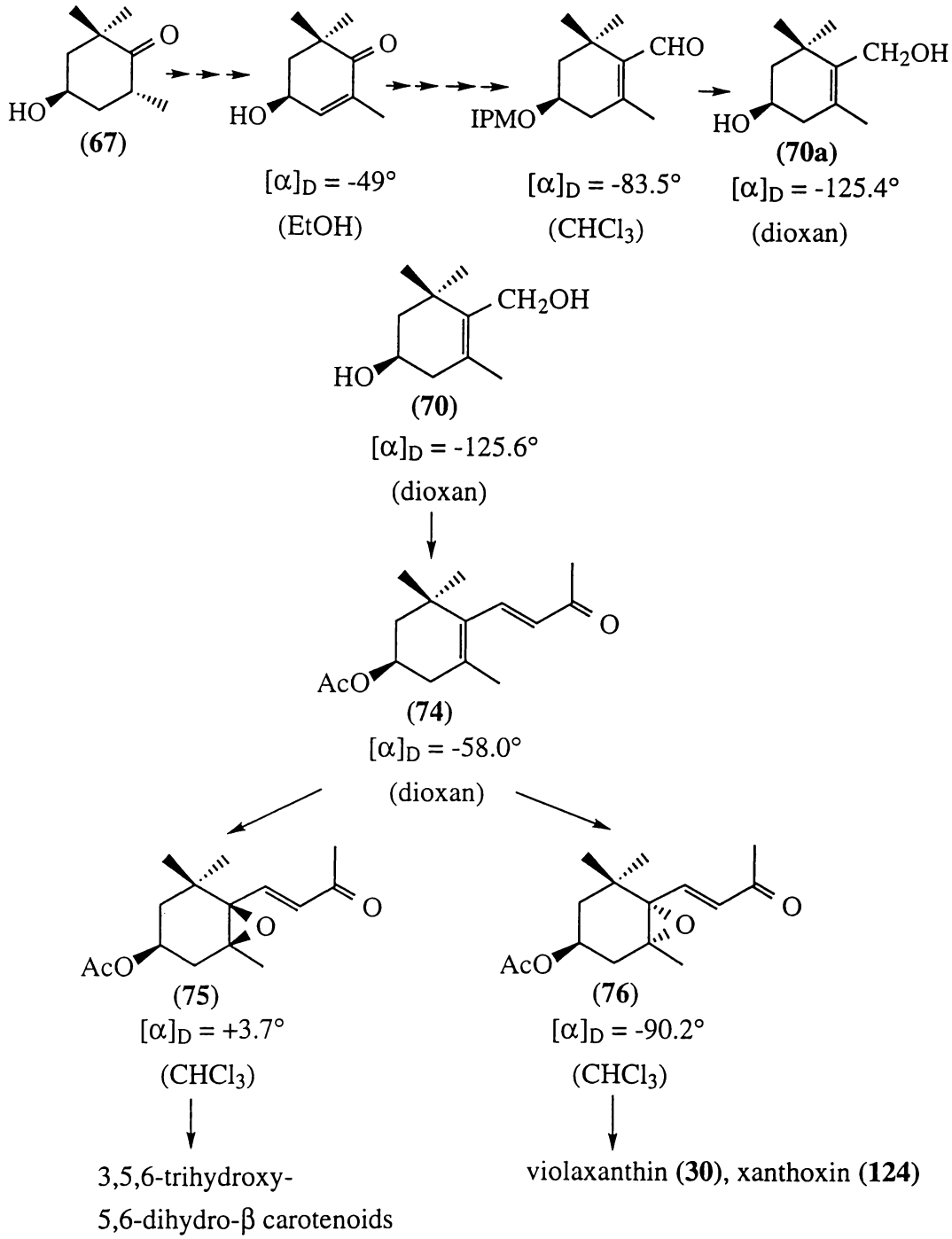
(**125**), and dihydroactinidiolide (**126**) (Kienzle *et al.*, 1978), were established by synthesis from chiral precursor **67** (Scheme 3.1).



Scheme 3.1. Synthesis of the optically active 3-hydroxy-β end-group (**59**) and structurally related compounds from chiral intermediate **67**.

The synthesis of (*3R*)-3-hydroxy-β-ionone (**59**) and zeaxanthin (**30**) by an alternative route (Mayer and Rüttimann, 1980), required that the absolute configuration and optical purity of C-10 intermediate, (*3R*)-3-hydroxy-β-cyclogeraniol (**70**), be established (Scheme 3.2). This was achieved by chemical and chiroptical correlation with compound **70a** prepared from C-9 chiral precursor **67** (Mayer, 1979). The absolute configurations of synthetic 3-acetoxy-5,6-epoxy-(**75**)/(**76**) and 3,5,6-trihydroxy-5,6-dihydro-β diastereoisomeric end-groups were subsequently correlated with (*3R*)-3-acetoxy-β-ionone (**74**) (Buchecker *et al.*, 1984) (Scheme 3.2).

Absolute configuration may also be determined by a stereoselective reaction of 'known' stereochemical course This approach relies on a configurational correlation between a chiral auxillary or reagent, and a newly created chiral centre produced in the reaction. Prelog's, Cram's and Sharpless' epoxidation rules are used in this manner. These, and other configuration correlations used in synthesis, are discussed by Eliel *et al.* (1994). Correlations of this type may be equivocal, particularly when the stereochemical outcome of the reaction is uncertain.



Scheme 3.2. The absolute configuration of C-10 synthetic intermediate **70** was determined by chemical correlation with **67**. The configurations of (*3R*)-3-acetoxy-β-ionone (**74**), 3-acetoxy-5,6-epoxy-β-ionones (**75**)/(**76**) and 3,5,6-trihydroxy-β-ionones, and the corresponding carotenoids, were established by synthesis from **70**.

Chiroptical Methods

Chemical methods are usually employed with chiroptical methods such as polarimetry, optical rotatory dispersion (ORD) and circular dichroism (CD). These methods distinguish chiral molecules from their mirror images by their interaction with chiral (polarised) light. ORD and CD were rapidly adopted in carotenoid research in the 1960s. Today, most studies of chiral carotenoids report CD spectra, or at least CD maxima. ORD was superseded by CD due to the simplicity of CD spectra.

Application of polarimetry, or optical rotation, is used only if the sign and magnitude of the specific rotation (α) of a compound of unknown configuration can be compared with that of the same compound of established configuration (Scheme 3.2). The more sophisicated chiroptical techniques such as ORD or CD afford spectra which exhibit peaks or troughs (positive and negative maxima) in the absorption region of the compound, known as Cotton effects. Enantiomers have oppositely signed mirror image Cotton effects (due to asymmetric electronic perturbations induced within the chromophoric group). The advantage of ORD and CD is that it is legitimate to compare two chemically different species, provided their structure is similar in the vicinity of the chromophore.

Determination of absolute configuration by ORD or CD is based mainly on empirical correlations (octant, quadrant, sector and helicity rules) with spectra of similar molecules with established stereochemistry. This approach was used by Mori (1974) and Leuenberger *et al.* (1976) for prediction of the absolute configurations of cyclohexanone intermediates (e.g **67**) used for carotenoid end-group syntheses.

The main features of the CD spectra of β-ionone derivatives are explained on the basis of a twisted diene model (i.e helicity rules) (Noack and Thomson, 1979). Two different conformations are proposed for a β-ionone ring in which the C-6–C-7 bond is twisted (steric interactions prevent the ring double bond and C-7–C-8 from being coplanar). Substitution at C-2 or C-3 in the ring causes one of these conformations to predominate, thereby imparting a resulting chirality to the overall system of conjugated bonds (i.e. an intinsically chiral molecule exists). Figure 3.2 shows one of the configurations of (*3R*)-3-hydroxy-β end-group (Noack and Thomson, 1979). The configuration in which C-2 and C-8 are on

different sides of the C-(1,6,5,4) plane, gives rise to a CD spectrum of opposite sign.

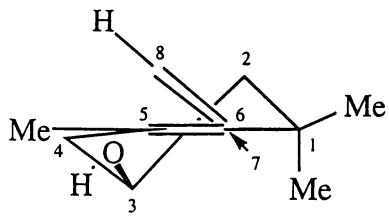


Figure 3.2. Carotenoid with (*3R*)-3-hydroxy-β end-group viewed along the C-6–C-7 bond (some H atoms omitted for clarity).

The CD spectrum of a 5,6-epoxidised-β end-group gives information about the absolute configuration of the epoxy group, irrespective of whether there is a hydroxyl group at C-3 (Buchecker and Noack, 1995). No reliable correlation between CD and the chirality of a specific centre has been found for saturated cyclic end-groups with two or three hydroxy groups, such as 5,6-diols and 3,5,6-triols (Buchecker and Noack, 1995). For these carotenoids, the chiral pertubation of the planar chromophore stems from chiral centres which do not induce a chiral twist of the conjugated system (i.e. an asymmetrically perturbed chromophore exists), and empirical rules are given. A number of rules have been proposed for the classification and interpretation of CD spectra of carotenoids. These rules, and the CD of carotenoids are discussed by Buchecker and Noack (1995). Theoretical aspects of chiroptical methods are reviewed by Eliel (1962), Crabbé (1972) and Eliel *et al.* (1994).

Chromatographic Methods

Identification of absolute configuration by GC or HPLC may be by resolution of diastereoisomeric derivatives on an achiral stationary phase, or by direct resolution using a chiral stationary phase. These methods require comparison of the order of peak emergence between the compound of unknown chirality, and a reference compound of known chirality. The major disadvantage of this approach is the requirement for a suitable reference compound. Further restrictions are requirements of compound resolvability, and for CG methods, compound volatility and thermal stability.

Determination of enantiomeric purity and absolute configuration by GC (Schurig, 1983) and HPLC (Pirkle and Finn, 1983) have been reviewed. Advances in these chromatographic methods are also discussed in detail by Eliel *et al.* (1994). Absolute configurational assignment by these methods is relatively uncommon, unless suitable reference compounds are available. HPLC has been used to assign the absolute configurations of carotenoids (Britton, 1989), whilst chiral capillary GC has been used to determine the absolute configurations of some ionone derivatives (Grayson, 1994; 1997).

The stereospecific analysis of natural products has progressed significantly with the recent introduction of multidimensional GC (MDGC) instrumentation. The absolute configurations of 3-hydroxy- and 3-hydroxy-5,6-epoxy-ionols have been determined by MDGC-MS and CD using reference compounds (Neugebauer *et al.*, 1995). The absolute stereochemistries of various other degraded carotenoids (e.g. vitispiranes, theaspiranes) have been analysed by MDGC (Winterhalter, 1996).

NMR Methods

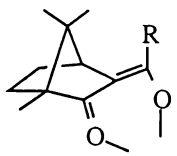
Determination of absolute configuration by NMR was in the early stages of development when carotenoid chirality was first determined by chiroptical and chemical correlations. Subsequently, the NMR method has undergone many improvements, becoming the most widely used and accurate method for determining enantiomeric excess and absolute configuration of organic compounds.

Enantiomers cannot be distinguished in an achiral medium, as the resonances of enantiotopic nuclei are isochronous. Diastereoisomers however, may be distinguished because the resonances (of certain diastereotopic nuclei) are anisochronous. Determination of enantiomeric purity or absolute configuration using NMR therefore requires the use of a chiral auxillary that converts enantiomers to diastereoisomers. Three types of chiral auxillary have been used for this purpose. These are the chiral lanthanide shift reagents, chiral solvating agents, and chiral derivatising agents.

Chiral shift reagents (CSR) have the capacity to effect different induced shifts for protons in different stereochemical environments due to the formation of diastereoisomeric complexes. Camphor-based complexes of lanthanides are

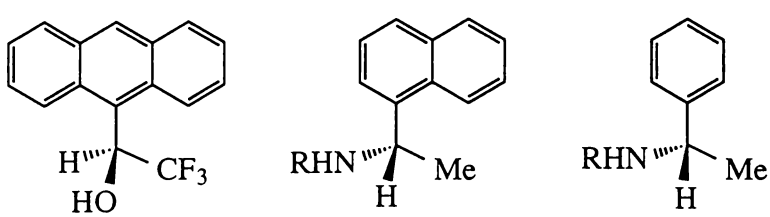
commonly employed (Table 3.1). Shifts in ^1H NMR resonances reflect the distance of each type of proton from the donor atom (e.g. an O atom) of the substrate (e.g. alcohol).

Table 3.1. Some common chiral shift reagents.

Structure of L in EuL_3		Abbreviation
	R = ^{t}Bu	$(Eu(pvc)_3)$
	R = CF_3	$(Eu(tfc)_3)$
	R = C_3F_7	$(Eu(hfc)_3)$

The major application of CSRs was to provide a direct measure of enantiomeric purity (Parker, 1991). It is generally accepted that many factors are potentially responsible for enantiomeric shift differences (such as stoichiometry of the complex, conformation of organic donor), and that any assignments of absolute configuration from CSR data would be ambiguous (Fraser, 1983; Eliel *et al.*, 1994).

Chiral solvating agents (CSAs), such as the aryltrifluromethylalcohols (e.g. **127**) and 1-arylethylamines (e.g. **128** and **129**) (Figure 3.3) form diastereoisomeric solvation complexes with solute enantiomers *via* rapidly reversible equilibria. Chemical shift anisochrony is dependent on the relative position of magnetically anisotropic groups (e.g. phenyl, carbonyl) with respect to substituents in the diastereoisomeric complex, in addition to the diastereoisomeric complexation constants.



(127) **(128)** **(129)**

R = H, Me

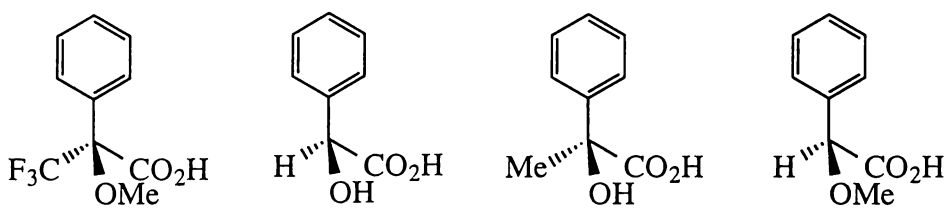
Figure 3.3. Some common chiral solvating agents.

CSAs generally induce small chemical shift differences and as a result have received less use for % *e.e.* determinations than CSRs. Various solvation models have been developed to allow determination of absolute stereochemistry from the

observed differential shifts of diastereoisomeric solvation complexes (Pirkle and Hoover, 1982; Eliel *et al.*, 1994). In general, CSAs have been more successful in this area than CSRs. CSAs have been reviewed by several authors (Pirkle and Hoover, 1982; Weisman, 1983; Parker, 1991; Eliel *et al.*, 1994).

In contrast to CSRs and CSAs, which form diastereoisomeric complexes that are in fast exchange on the NMR time scale, derivatisation yields discrete diastereoisomers for which the chemical shift non-equivalence is considerably greater. Although a derivatisation step is necessary, chiral derivativsing agents (CDAs) have largely superseded CSRs and CSAs for assessment of enantiomeric purity and absolute configuration.

NMR non-equivalence of diastereoisomeric esters was initially investigated for determination of stereochemical purity. A series of α-substituted phenylacetic acids **130–133** were assessed as chiral derivatising agents for NMR anaylsis (Dale and Mosher, 1968) (Figure 3.4). Some of these reagents were susceptible to racemisation due to their α-hydrogen, especially on reaction with hindered alcohols. α-Methoxy-α-trifluoromethylphenylacetic acid (MTPA) (**130**) had several advantages in that it did not racemise under the most severe reaction conditions, and the diastereoisomeric groups showed significant chemical shift differences (Dale *et al.*, 1969). The volatility and solubility of MTPA derivatives also allowed analysis by chromatographic methods. The MTPA derivatives of alcohols and amines were readily prepared by reaction with excess MTPA chloride with an acylation catalyst such as pyridine.



(130) **(131)** **(132)** **(133)**

Figure 3.4. MTPA (**130**), mandelic (**131**), atrolactic (**132**) and *O*-methylmandelic (**133**) acids assessed for determination of the absolute configuration of alcohols and amines (Dale and Mosher, 1973).

Subsequently, NMR configurational correlation schemes were devised which permitted assignment of the absolute configuration of alcohols and amines in MTPA and related mandelate derivatives (Dale and Mosher, 1973) (Figure 3.5).

These models rationalised the observed non-equivalence of diastereotopic NMR signals. When that study was published, NMR experiments were carried out on 60-100 MHz instruments and complete assignment of protons in complex organic molecules was almost impossible. For this reason, assignment of stereochemistry was usually made on the chemical shift differences of the two β-substituents (L_2, L_3). This method however, was not applicable when the ^1H NMR signals of the respective diastereoisomers were not sufficiently separated, or when crucial signals overlapped other resonances.

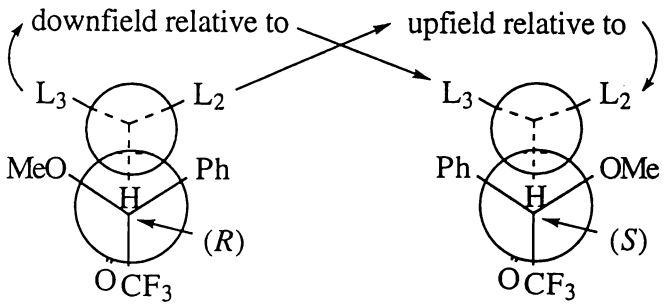


Figure 3.5. Configurational correlation model for the (*S*)- and (*R*)-MTPA derivatives (Dale and Mosher, 1973).

In such cases, ^{19}F NMR analysis of the MTPA moiety provided an alternative method (Sullivan *et al.*, 1973). ^{19}F NMR chemical shift differences were generally greater than those of the corresponding proton signals and the ^{19}F signals were usually found in an unobscured region of the spectrum. That method required that one know which of the diastereoisomers had a greater conformer population having the CF_3 group in the deshielding plane of the carbonyl. Steric repulsion between the phenyl group of the MTPA moiety and the β-substituents was essential to bring about the chemical shift difference of the CF_3 (^{19}F) or OMe (^1H) groups.

As this method was dependent on subtle differences in through-space steric and electronic effects between β-substituents and the Ph or OMe groups, it could be unreliable, particularly when applied to compounds other than those types already investigated (this was alluded to in the argument put forth by Mosher's group (Sullivan *et al.*, 1973)). Indeed, a number of recent investigators have demonstrated discrepancies in results obtained *via* this method (Kusumi *et al.*, 1988; Ohtani *et al.*, 1991; Rieser *et al.*, 1992.). Moreover, Kusumi *et al.* (1988) demonstrated that some compounds can adopt a conformation in which the β-

substituents are placed far from the ester moiety and other substituents (on other positions) may have a greater interaction with this group.

The use of achiral lanthanide shift reagents and accompanying empirical models extended the usefulness of MTPA esters in stereochemical studies (Yamaguchi *et al.*, 1976; Yamaguchi and Yasuhara, 1977; Yasahura and Yamaguchi, 1977). Those reagents, such as $Eu(fod)_3$ ('fod' is 6,6,7,7,8,8,8-heptafluoro-2,2-dimethyl-3,5-octane-dione), resulted in enhanced separation of signals from the OMe group from the MTPA moiety. Although these experiments proved a useful alternative, they could be tedious, and ambiguous results were obtained (Yamaguchi *et al.*, 1976). In addition, these reagents were less useful at high fields due to increased line broadening.

The above methods of stereochemical determination allowed only a single-point comparison. A more reliable method employing ^1H NMR, based on a number of clearly identifiable, assigned signals from diastereoisomeric esters was demonstrated by Ohtani *et al.* (1991). High-field FT NMR techniques such as COSY, HOHAHA and NOE-difference permitted the identification of a number of like sets of protons within L_2 and L_3 (Figure 3.5). This reinforced the assignment made by a comparison of the chemical shifts of only a single set. Incorporation of a large number of data points into the NMR configurational model provides a self-examination mechanism for the method (Kusumi *et al.*, 1991a; Ohtani *et al.*, 1991). Another advantage of high-field (300-500 MHz) ^1H NMR analysis was the improved dispersion, which allowed confident assignment of small chemical shift differences (e.g Pehk *et al.*, 1993).

The high-field FT NMR application of Mosher's methodology has been applied to a number of structurally diverse and complex compounds (Adamczeski *et al.*, 1990; Hundt *et al.*, 1990; Kusumi *et al.*, 1988, 1991a, 1992; Ohtani *et al.*, 1991; Rodríguez and Riguera, 1992; Rieser *et al.*, 1992; Pehk *et al.*, 1993; Latypov *et al.*, 1995a, 1996). Generally, the method has been limited to those with a secondary alcohol moiety (Figure 3.6), although it has been extended to primary amines such as amino acids (Kusumi *et al.*, 1991b and cited references; Latypov *et al.*, 1995b) and in one case, a tertiary alcohol (Izumi *et al.*, 1994). Kusumi *et al.* (1992) demonstrated that the method could be extended to non-hydroxyl compounds possessing a functional group able to be converted to a secondary alcohol. Instances in which the absolute configurations are not readily accessible from this analysis are attributable to unfavourable steric interactions

(e.g. 1,3-diaxial interactions) between the alcohol and acid moieties (Kusumi *et al.*, 1988, 1991a; Ohtani *et al.*, 1991).

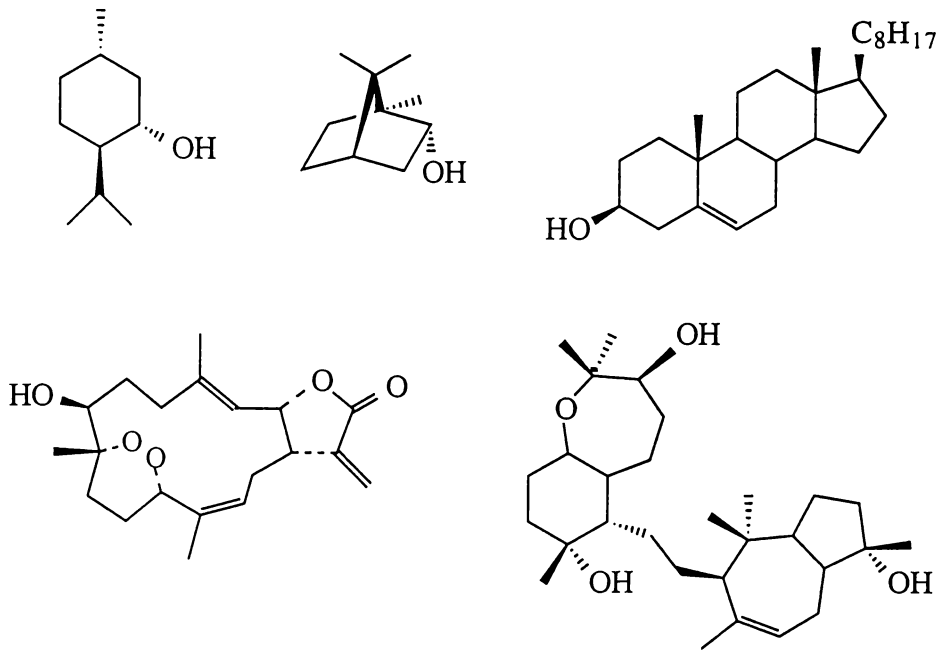


Figure 3.6. Examples of secondary alcohols of established absolute configuration for which the extended Mosher method (using MTPA) has been successfully applied (Ohtani *et al.*, 1991).

Most stereochemical studies employed MTPA as derivatising reagent because the quaternary character of the chiral centre suppressed racemisation. With confirmation of the NMR configurational correlation model for α-methoxyphenylacetate (MPA) (**133**) esters (Trost *et al.*, 1986) (Figure 3.7), and improvements in synthetic methodology for forming esters under non-racemising conditions, an increasing number of stereochemical determinations using MPA esters have been reported (e.g. Adamczeski *et al.*, 1990; Rodríguez and Riguera, 1992; Zhao *et al.*, 1996). Those studies used the high-field ^{1}H NMR method of Ohtani *et al.* (1991) and the conformational model for MPA esters (Trost *et al.*, 1986). Both MTPA and MPA are widely used chiral anisotropic reagents as they are commercially available. Recently, it has been demonstrated that MPA is superior to MTPA, as it leads to higher values of chemical shift difference (Δδ) for diastereoisomeric derivatives (Pehk *et al.*, 1993; Seco *et al.*, 1994; Latypov *et al.*, 1996).

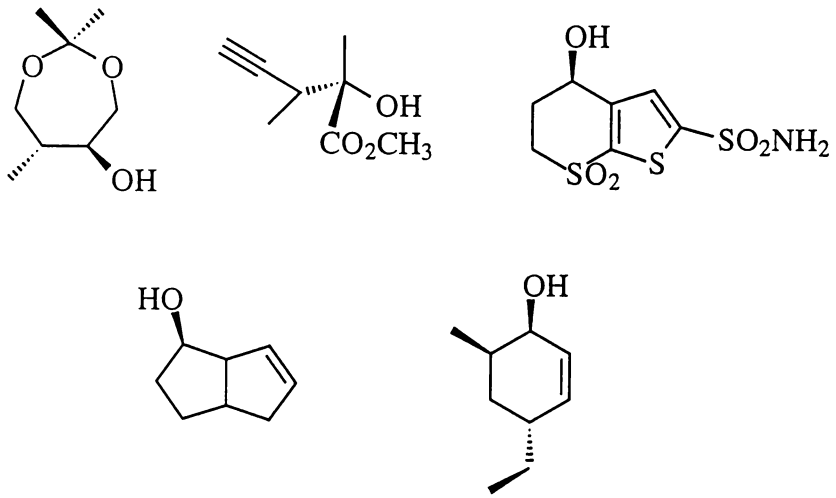
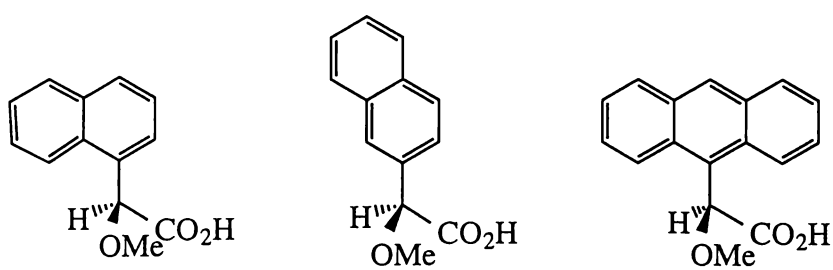


Figure 3.7. Compounds of known configuration used to devise the NMR configurational model for MPA esters (Trost *et al.*, 1986)

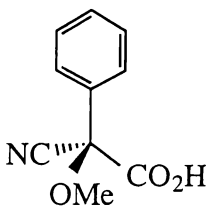
More recently, focus has turned to development of new chiral derivatising reagents for determining the absolute configurations and enantiomeric purities of optically active compounds. These include Trost-type compounds such as 1-naphthylmethoxyacetic acid (**134**), 2-naphthylmethoxyacetic acid (**135**) and 9-anthranylmethoxyacetic acid (**136**) (Seco *et al.*, 1994; Kusumi *et al.*, 1994; Latypov *et al.*, 1995a). The latter reagent is most superior, providing shifts 2-3 times larger than MTPA and longer-range anisotropic effects. Arylmethoxyacetic acids (AMAA) are particularly useful for determining the absolute configurations of secondary alcohols involved in long-chain compounds (Kusumi *et al.*, 1994). Moreover, the $\Delta\delta$ values are so large that only one enantiomer of the reagents may be needed (Kusumi *et al.*, 1994).



(134) **(135)** **(136)**

Reagents with enhanced reactivity, such as α-cyano-α-fluorophenylacetic acid (**137**), are being studied for use on hindered alcohols (Takeuchi *et al.*, 1993). Chiral anisotropic reagents which do not exhibit carbon-centrality, for example the axially chiral binaphthyl derivatives **138** and **139** (Fukushi *et al.*, 1994a; 1994b), have also shown potential for assignment of configuration. This method requires

relatively complicated configurational correlation schemes, but is applicable to sterically hindered alcohols. Configurational correlation schemes have been devised for alcoholic tetra-*O*-benzoylglucosides (**140**) (Trujillo *et al.*, 1994). The anisotropic effect and glycosylation-induced ^{1}H NMR shifts combine to afford larger $\Delta\delta$ values than those of MTPA or MPA esters, thus a single derivatisation may be sufficient. At present, reagents **137–140** are at the developmental stage and are not commercially available.



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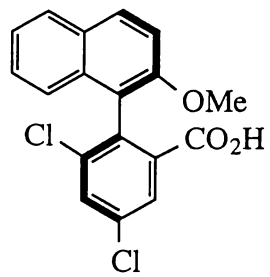

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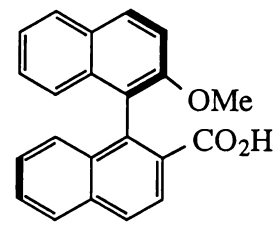

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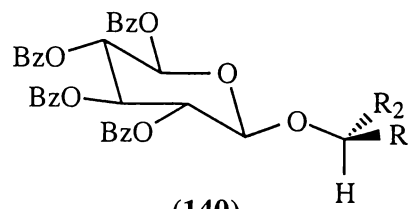


(140)

3.2. Results and Discussion

An indirect approach to determination of the absolute stereochemistry of 3,5,6-trihydroxy-5,6-dihydro-β-ionone (**22**) was to develop an enantioselective synthesis whereby the absolute configuration of an optically active precursor could be established. This could be achieved by comparison of the optical rotation or CD spectrum of chiral 3-hydroxy-β-ionone (**59**), data which were reported by several investigators (e.g. Rüttimann and Mayer, 1980). An enantiomeric synthesis of ionone **22** was attempted in the present study and was unsuccessful (Chapter Two).

Several other methods were unsuitable for assignment of the absolute stereochemistry of triol **22**. Chemical and chiroptical correlation of **22** with chiral 3-hydroxy-β-ionone (**59**) was not feasible due to the small quantity of sample available. Chromatographic methods, which required a reference compound of established absolute configuration, were not applicable.

The absolute configuration of **22** could be established directly by comparison of its CD data with that of the previously synthesised enantiomeric 3-acetoxy-5,6-dihydroxy-5,6-dihydro-β-ionones (**75**)/(**76**) (Scheme 3.2). This comparison was valid provided the C-3 hydroxy and acetoxy groups in these compounds stabilised the same conformation of the cyclohexane ring. It has been demonstrated that the influence of substitution on the sign (chirality) of CD spectra of carotenoid β end-groups is dependent on the position of substitution, rather than the nature of the substituent (Buchecker and Noack, 1995).

As the instrumentation required for CD studies was not available, the NMR method using chiral derivatives was chosen as a means of determining the absolute stereochemistry of triol **22**. This method has been used successfully to determine the absolute configurations of a number of secondary alcohols, many similar in structure to **22**. In addition, providing suitable crystals of a chiral derivative could be obtained, X-ray crystallographic analysis would afford an unequivocal determination of absolute stereochemistry.

3.2.1. Isolation of Triol 22

The isolation of triol **22** from unifloral thyme honey has been reported by Tan (1989). A small scale extraction of thyme honey with diethyl ether, followed by GC analysis of the extractives, revealed the presence of a dominant peak, the mass spectrum of which was consistent with that reported for **22** (Tan, 1989). A bulk extraction of thyme honey (600 g) afforded a mixture of extractives which were chromatographed by PLC to give **22** (10 mg). ^{1}H and ^{13}C NMR indicated that **22** was a single diastereoisomer.

3.2.2. Preparation of MPA Esters of Triol 22

Several methods have been reported for preparation of MPA esters without racemisation of the acid:- (a) dicyclohexylcarbodiimide (DCC) and dimethylaminopyridine (DMAP) (Hassner and Alexanian, 1978), (b) DCC, pyridine, and 2-hydroxybenzotriazole (Trost *et al.*, 1986), (c) dimethylformamide (DMF) and oxalyl chloride (Trost *et al.*, 1986). Trost *et al.* (1986) found the first method was successful for esterification of most alcohols, although it led to

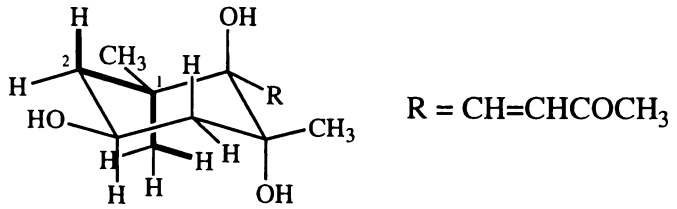
substantial racemisation when applied to hindered alcohols, where the rate of esterification was slow.

Esterification of triol **22** with *(S)*- and *(R)*-MPA (**133**) was carried out *via* DCC coupling using DMAP (method (a)). Each reaction was complete within *ca* 2 h. ^{1}H NMR spectra of the ester products (**22a/22b**) exhibited resonances indicative of the 3,5,6-trihydroxy-5,6-dihydro-β-ionone ring system of **22**, in addition to signals at δ 3.41, 4.73, and 7.34–7.45, attributed to an MPA moiety. Acylation was characterised by a large downfield shift in the H-3 resonance (δ 4.16 in **22**, δ 5.29 in **22a/22b**). The observation of one product from each reaction mixture, giving rise to one set of ^{1}H and ^{13}C NMR signals indicated that no racemisation had occurred. Moreover, these data indicated that **22** was optically active.

3.2.3. Characterisation of MPA Esters 22a and 22b

Unambiguous assignment of the proton resonances from both (*S*)-**22a** and (*R*)-(**22b**) MPA esters was required in order to determine the absolute stereochemistry of triol **22** from the NMR correlation model (Dale and Mosher, 1973; Trost *et al.*, 1986; Ohtani *et al.*, 1991). The 300 MHz ^{1}H NMR of (*S*)-MPA ester **22a** showed resonances attributed to four methyl groups, and three poorly resolved proton resonances in the region δ 1.72–1.82. Another resonance at δ 1.63 was partially obscured by the hydroxyl resonances. Improved spectral dispersion was obtained at 400 MHz. Assignment of proton resonances was made by ^{1}H-^{1}H and ^{1}H-^{13}C correlation spectroscopy, and NOE-difference experiments.

The COSY-45 spectrum (Bax and Freeman, 1981a) (Figure 3.8) identified the two geminal methyl signals *via* their mutual ^4J coupling. The downfield geminal methyl group (δ 1.31) also exhibited a cross-correlation peak (cross-peak) to the proton at δ 1.75, identifying this proton as H-2_{ax}. This coupling, attributed to a W-type interaction, is typical of that between a methyl group and proton which are 1,2-diaxial (Tan, 1989).



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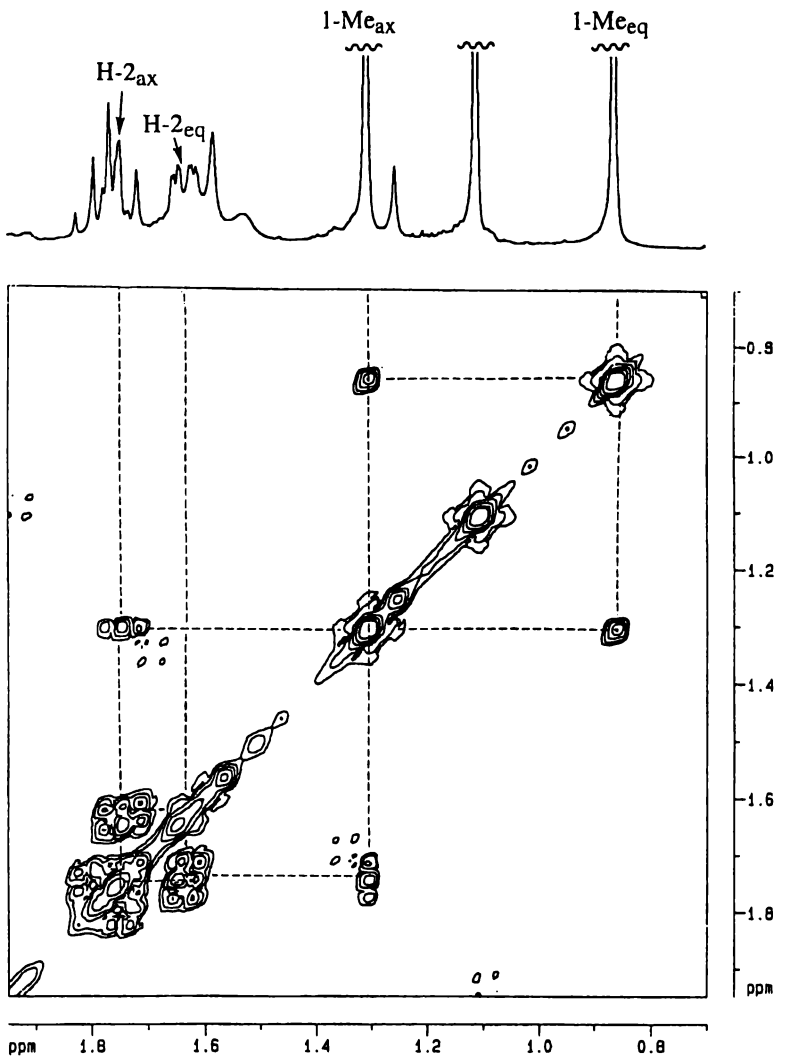


Figure 3.8. COSY-45 spectrum of **22a** ((*S*)-MPA ester of **22**) showing connectivities in the region δ 0.70–1.95 ppm. Structure **22** indicates the W-arrangement of H-2_{ax} and 1-Me_{ax} (**bold**).

Coupling information in the cross-correlation peak from 1-Me_{ax} indicated that H-2_{ax} was a triplet (J = 12.5 Hz), in accord with large geminal (H-2_{ax}–H-2_{eq}) and axial-axial (H-2_{ax}–H-3) couplings of an axial proton from a cyclohexane ring. The COSY spectrum indicated that the ddd at δ 1.63 arose from H-2_{eq}. Coupling constants of 12.5, 4.6 and 1.8 Hz observed for this multiplet were consistent with

geminal (H-2_{eq}–H-2_{ax}), equatorial-axial (H-2_{eq}–H-3_{ax}) and W-couplings (H-2_{eq}–H-4_{eq}), respectively, of an equatorial proton.

A cross-peak near the diagonal in the region δ 1.72–1.82 indicated that multiplets from H-4_{ax} and H-4_{eq} were resolved, although the multiplicities could not be determined from the correlation peak. The resolution-enhanced proton spectrum (Figure 3.9) indicated a triplet at δ 1.79 and possibly a ddd at approximately δ 1.74, arising from H-4_{ax} and H-4_{eq}, respectively. A coupling of 1.8 Hz was observed for the ddd attributed to H-4_{eq}, consistent with the W-coupling interaction observed for H-2_{eq}.

In most cases, ^{1}H chemical shifts of methylene protons can be confirmed by ^{1}H-^{13}C correlation. This information could not be extracted from the standard HSQC spectrum (Kay *et al.*, 1992) due to close proximity of C-2 and C-4 resonances (*ca* 0.1 ppm). The HSQC experiment was repeated with improved resolution along the ^{13}C axis, allowing distinction of the correlation peaks from protons attached to C-2 and C-4 (Figure 3.9). This spectrum confirmed the assignments made by scalar and COSY ^{1}H-^{1}H coupling interactions.

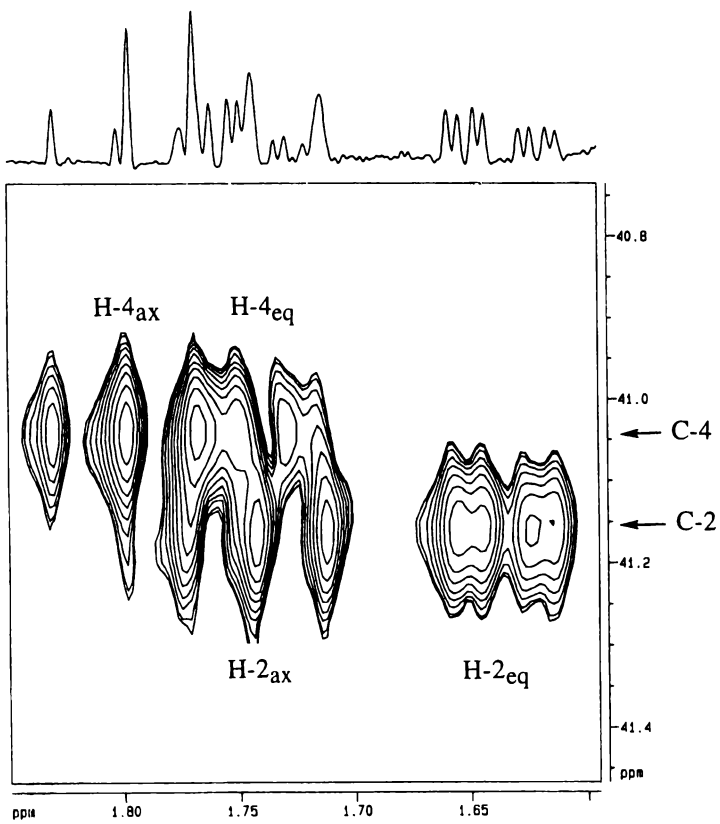


Figure 3.9. The reduced-window HSQC spectrum of **22a**. The resolution-enhanced ^{1}H spectrum is plotted above.

Assignment of proton resonances from the cyclohexane ring was also corroborated by NOE-difference experiments (Figure 3.10). Irradiation of 1-Me_{eq} gave rise to enhancements at δ 1.63 (ddd) (2.1%) and δ 1.75 (t) (2.2%), which confirmed those resonances as arising from H-2_{eq} and H-2_{ax}, respectively (Figure 3.10(b)). Irradiation of the equatorial methyl group at C-5 resulted in enhancements at δ 1.74 (ddd) (2.1%) and 1.79 (t) (3.3%), attributed to H-4_{eq} and H-4_{ax}, respectively (Figure 3.10(c)). Enhancements in the hydroxyl signals (δ 1.51) were also observed in that difference spectrum, in addition to those from an impurity (δ 1.59 and 1.92). The NOE-difference spectrum obtained by irradiation of axial H-3 (Figure 3.10(d)) confirmed those assignments. Enhancements of similar intensities were observed for the two equatorial proton resonances at δ 1.63 (4.7%) and 1.74 (3.9%). Signals at δ 1.80 and 1.77 are strong NOEs arising from a minor impurity. NOE-difference spectra (c) and (d) in Figure 3.10 confirmed the chemical shift of H-4_{eq}.

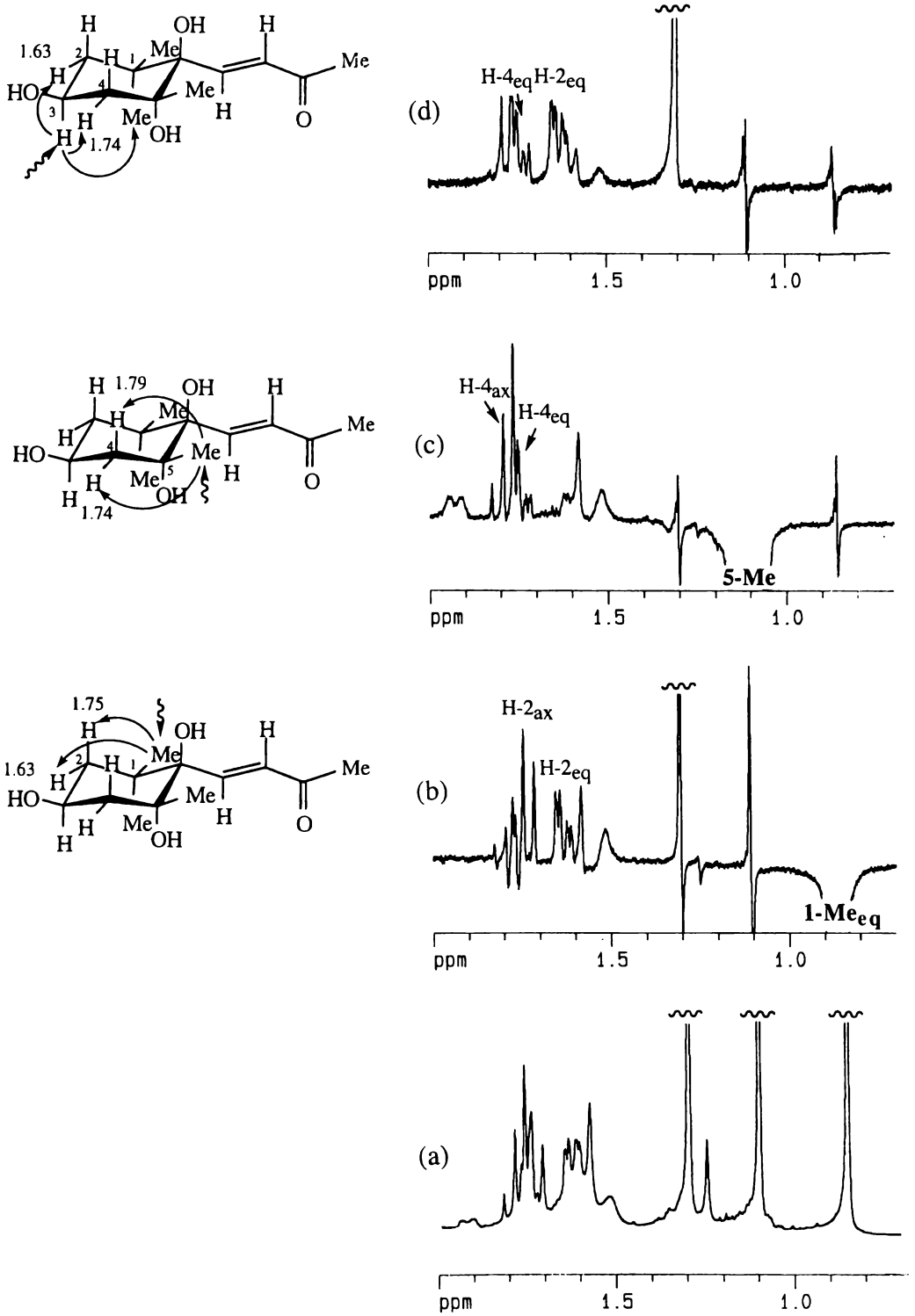


Figure 3.10. Partial ^{1}H spectrum (a) and NOE-difference spectra (b)-(d) confirming assignment of proton resonances for **22a.**

The *trans*-coupled olefinic protons, H-7 and H-8, exhibited a typical AB-type pattern, with coupling constants of 14 Hz. In compounds with a β end-group, the doublet of H-7 can be readily distinguished from that of H-8 by its larger line width. This is caused by unresolved couplings of H-7 to 5-Me and H-4 (Englert, 1995). These coupling interactions were observed in the COSY spectra of the β-ionone derivatives synthesised in Chapter Two.

Differentiation between H-7 and H-8 is not straightforward in cases where the C-5–C-6 double bond is replaced by an epoxide or diol functionality. In this instance, the high-field (δ 6.31) and low-field (δ 7.26) components of the AB-type resonance have the same line widths. The observation of a cross-peak in the long-range ^{1}H-^{13}C correlation spectrum (HMBC) caused by a three-bond coupling ($^{3}J_{CH}$) between H-8 and C-10, distinguished these protons.

The difference in chemical shifts of olefinic protons α- and β- to a carbonyl group is rationalised by the resonance effect (Figure 3.11). The signals of carbons at the α position, with slightly enhanced electron density as seen in the resonance structure in Figure 3.11, are at relatively high-field, whereas those at the β postion, with decreased electron density, are shifted down-field (Breitmaier and Volter, 1987). Similar trends are observed for attached protons. This resonance effect is observed five or more double bonds away in carotenoids with terminal carbonyl groups (Englert, 1995).

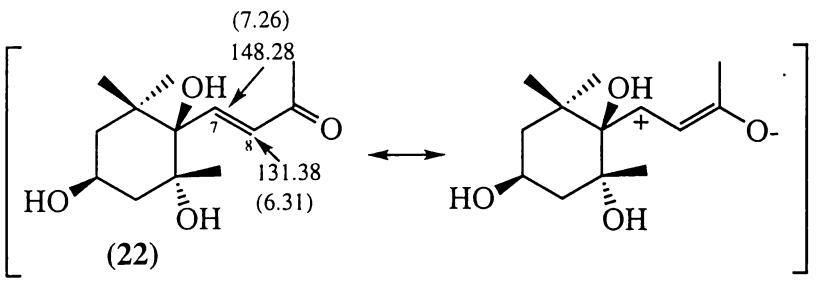


Figure 3.11. ^{13}C and ^{1}H chemical shifts for olefinic carbons and protons of **22**. Delocalisation of the conjugated system results in a deshielding of the β-olefinic carbon (C-7) and proton.

The ^{1}H NMR spectrum of (*R*)-MPA ester **22b** was assigned in a manner similar to that described for **22a**. Three distinct proton resonances were observed in the region δ 1.48–1.91, attributed to the four methylene protons. H-2_{ax} appeared as a triplet (J = 12.3 Hz) at δ 1.66 and H-2_{eq}, a ddd at δ 1.48 with coupling constants of 12.3, 4.2 and 1.3 Hz. ^{1}H-^{13}C and ^{1}H-^{1}H correlations confirmed that the doublet at δ 1.91 arose from H-4_{ax} and H-4_{eq}. These protons

are magnetically equivalent, each showing three-bond couplings (8.4 Hz) to H-3. A similar doublet was observed for H-2_{ax}/H-2_{eq} in parent alcohol **22** (Chapter Two). Assignment of ^{1}H resonances for **22b** was corroborated by NOE-difference experiments. Full ^{1}H and ^{13}C NMR data of **22a** and **22b** are reported in the Experimental Section of this Chapter.

3.2.4. Assignment of the Absolute Stereochemistry of 22

Interpretation of the ^{1}H Diamagnetic Shielding Observed for 22a/22b

The success of the NMR configurational correlation model for diastereoisomeric MPA esters (Dale and Mosher, 1973; Trost *et al.*, 1986) has been demonstrated for a number of structurally diverse secondary alcohols (Section 3.1.1). The model for MPA esters features the methoxy and carbonyl groups eclipsed (*synperiplanar*) with the C_α-H bond. Upfield shifts are predicted for substituents which eclipse the phenyl ring.[1] Thus, ^{1}H signals from H_a, H_b, H_c of the (*S*)-MPA ester appear at higher field than the same protons from the (*R*)-MPA derivative. Similarly, the ^{1}H signals from H_x, H_y, H_z of the (*S*)-MPA ester appear at lower field than those from the (*R*)-MPA ester (Figure 3.12).

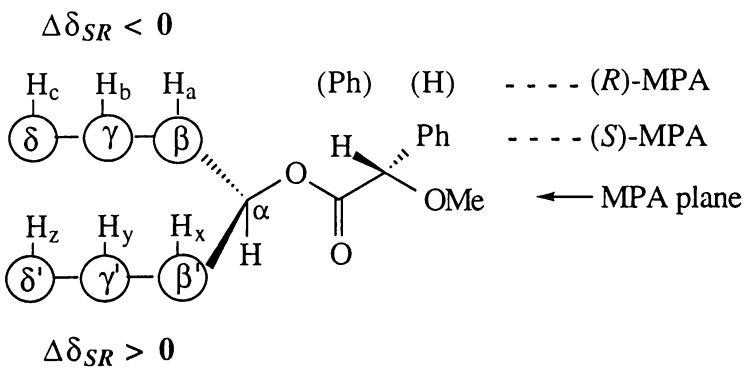


Figure 3.12. Extended Mosher method applied to MPA esters. The model shown is based on that proposed for MTPA esters (Ohtani *et al.*, 1991).

[1] Under the influence of a strong magnetic field, the diamagnetic flow of electrons from the phenyl group produces a local magnetic field at neighbouring protons. The net effect is a upfield shift of protons in the spatial cone above or below the the plane of the phenyl ring, and a downfield shift of protons in the plane of the ring (Johnson and Bovey, 1958; Haigh and Mallion, 1980).

Chemical shift differences ($\Delta\delta_{SR} = \delta S\text{-}\delta R$) were calculated for each proton of the diastereoisomeric MPA derivatives in order to determine whether all protons with positive and negative $\Delta\delta_{SR}$ values are arranged in a systematic manner either side of the ester plane (Ohtani *et al.*, 1991). These values are shown in Table 3.2.

Table 3.2. ^{1}H chemical shift differences ($\Delta\delta_{SR}$) observed for (*S*)- and (*R*)-MPA esters of **22.**

	Chemical shift (ppm)		
Proton	(*S*)-MPA **22a**	(*R*)-MPA **22b**	$\Delta\delta_{SR}$
1-Me$_{eq}$	0.86	0.82	+0.04
5-Me	1.10	1.15	-0.05
1-Me$_{ax}$	1.31	1.28	+0.03
H-2$_{eq}$	1.63	1.48	+0.15
H-4$_{eq}$	1.74	1.91	-0.17
H-2$_{ax}$	1.75	1.66	+0.09
H-4$_{ax}$	1.79	1.91	-0.12
H-10	2.30	2.30	0.00
H-3	5.29	5.29	0.00
H-7	7.26	7.26	0.00
H-8	6.31	6.31	0.00

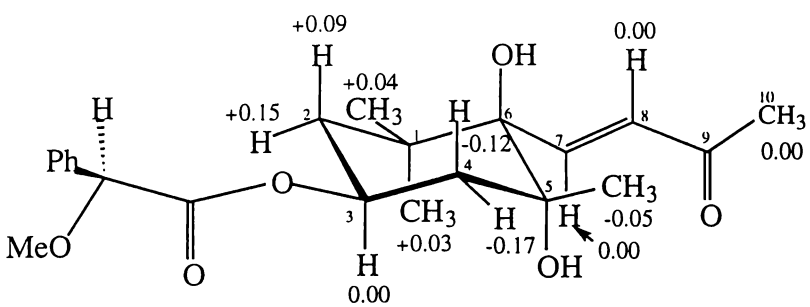


Figure 3.13. Representation of *(S)*-MPA ester **22a** showing the Mosher-Trost conformation of the MPA moiety. Preferential shielding of the (*S*)-MPA moiety at C-4 and C-5 substituents is indicated by negative $\Delta\delta_{SR}$ values at these protons.

The differential shielding observed for *(S)*-**22a** and *(R)*-**22b** MPA esters was rationalised by selecting the enantiomer depicted in Figure 3.13. Using that enantiomer and a conformation of the (*S*)-MPA moiety in which the methine proton, carbonyl and methoxy groups were *synperiplanar*, the predicted

anisotropic effect of the phenyl group was greatest for substituents at C-4 and C-5. Similarly, the maximum anisotropic effect of the *(R)*-MPA phenyl ring was predicted for C-2 and C-1 substituents.

Experimental data were consistent with these predictions. Negative $\Delta\delta$ values were observed for H-4_{eq} (-0.17) and H-4_{ax} (-0.12), and a relatively small negative $\Delta\delta$ value was observed for 5-Me (-0.05), consistent with preferential shielding of these protons by the phenyl substituent of the (*S*)-MPA group. Positive $\Delta\delta$ values were observed for H-2_{ax} (+0.09), H-2_{eq} (+0.15), 1-Me_{ax} (+0.03), and 1-Me_{eq} (+0.04), consistent with these groups being preferentially shielded by the phenyl substituent of the (*R*)-MPA. $\Delta\delta$ values reflected diminishing shielding effects of the phenyl substituent with distance, with protons in the plane of the cyclohexane ring exhibiting greater differential shielding compared with those above or below the ring (i.e axial substituents). Differential shielding predicted for the opposite enantiomer of **22** was inconsistent with observed $\Delta\delta$ values.

The zero $\Delta\delta$ values observed for protons of the side-chain (H-7, H-8 and H-10) were consistent with their distance form the phenyl group, and their approximate position in the ester plane. Protons attached to the esterified carbon, such as H-3 in **22a/22b**, exhibit negligible differential shielding as they lie in the ester plane. Differences in chemical shifts of this proton (H_α) are attributed to variation in the conformations of either (*S*)- or (*R*)-acid moieties, brought about by steric interaction (Ohtani *et al.*, 1991).

A survey of the literature revealed that for a variety of MTPA and MPA derivatives of secondary alcohols displaying a range of steric possibilities at the chiral centre, the magnitude of $\Delta\delta_{H\alpha}$ was in the range δ 0.00–0.09. Generally, the largest $\Delta\delta_{H\alpha}$ values were observed for compounds in which the difference of steric size of β-substituents was the greatest. Zero $\Delta\delta_{H\alpha}$ values were observed for compounds in which the β-substituents were identical, consistent with that observed for **22a/22b**.

The ^1H differential phenyl anisotropy of **22a** and **22b** indicated an *S* absolute configuration at C-3. As the relative stereochemistry of the parent alcohol **22** had been established by NMR and X-ray crystallography (Tan, 1989), the absolute structure was specified as (3*S*,5*R*,6*R*)-3,5,6-trihydroxy-5,6-dihydro-β-ionone. Suitable crystals of **22a** and **22b** were not obtained for corroboration of this assignment by X-ray crystallography.

Interpretation of ^{13}C Diamagnetic Shielding Observed for 22a/22b

^{13}C NMR spectra of esters **22a** and **22b** were assigned by ^{1}H-^{13}C correlation spectroscopy. Protonated carbons were assigned using the ^{1}H spectrum and HSQC correlations, and in instances where resonances were poorly resolved (i.e. C-2 and C-4), HSQC spectra with reduced spectral windows were acquired (e.g. Figure 3.9). HMBC correlations from 5-Me and 1-$Me_{ax/eq}$ allowed assignment of ^{13}C resonances from C-5 and C-6 (Figure 3.14). ^{13}C chemical shifts were obtained from the 100 MHz ^{1}H-decoupled ^{13}C NMR spectra and were measured to ±0.01 ppm, corresponding to a digital resolution of *ca* 0.4 Hz per point.

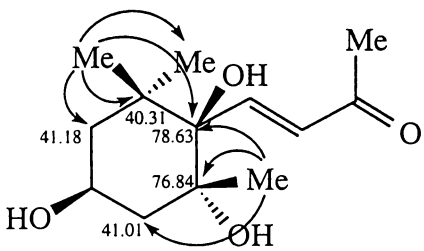


Figure 3.14. HMBC correlations observed for **22a**.

The sign of Δδ values observed for ^{13}C nuclei were consistent with those of ^{1}H nuclei of the cyclohexane ring, namely positive for C-1 and C-2 and their attached protons, and negative for C-4 and C-5, and their attached protons (Figure 3.15). Similarly, the magnitude of differential shielding for ^{13}C and ^{1}H nuclei on that ring diminished with increasing distance from the ester moiety. Differential shielding at the β-carbons (C-2 and C-4) was of greater magnitude than at the corresponding attached protons.

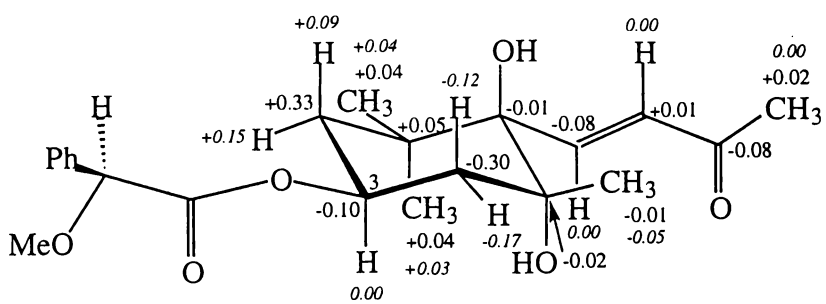


Figure 3.15. Comparison of ^{13}C and ^{1}H (*italics*) $\Delta\delta_{SR}$ values (in ppm) for **22a/22b**. (*S*)-MPA ester **22a** shown.

Some inconsistencies in $\Delta\delta$ values were observed between ^{13}C and ^{1}H nuclei. A $\Delta\delta$ value of -0.10 was observed at C-3, in contrast to the zero $\Delta\delta$ value observed for its attached proton. The magnitude and signs of $\Delta\delta$ values observed for side-chain carbons were non-zero, and of greater magnitude than expected on the basis of the diminishing diamagnetic affects observed at ^{13}C nuclei on the cyclohexane ring. The zero $\Delta\delta$ values observed for protons on the side-chain were consistent with their distance from the ester moiety and position in the ester plane.

Few studies comment on the diamagnetic ^{13}C shifts of MTPA or MPA esters. Intramolecular anisotropic magnetic fields play an important part in proton shielding. These effects are considered relatively insignificant in ^{13}C NMR (Levy and Nelson, 1972; Breitmaier and Volter, 1987), as ^{13}C chemical shifts occur over a far greater range (*ca* 200 ppm) than proton chemical shifts (*ca* 12 ppm). However, resolution of small diastereoisomeric ^{13}C chemical shifts has improved with use of higher fields in NMR spectroscopy.

Kanger *et al.* (1992) noted that ^{1}H and ^{13}C differential shielding of two diastereoisomeric MPA esters followed similar trends. Pehk *et al.* (1993) also showed that for several MTPA and MPA derivatives of allylic, homoallylic and acetylenic secondary alcohols, comparable trends were seen for ^{1}H and ^{13}C chemical shifts. ^{13}C differential chemical shifts of similar magnitude to those of ^{1}H nuclei were observed, ranging in magnitude from 0.1–0.01 ppm. Shielding at carbon nuclei close to the ester moiety was greater than that observed for the attached protons, consistent with that observed in the present study. Pehk *et al.* (1993) observed some irregularities for the carbons adjacent to the esterified carbon, however they were reported not to 'invalidate the general trends'. Reasons for those inconsistencies were not given.

The $\Delta\delta$ values observed for ^{13}C nuclei of the enone system in **22a/22b** suggested that factors other than diamagnetic shielding were operative in this case. It was assumed that temperature and concentration effects were negligible. Diastereoisomeric intermolecular interactions may account for the non-zero $\Delta\delta$ values observed at these carbon nuclei. The anisotropic effects associated with the carboxyl and/or carbonyl oxygen atoms may affect chemical shifts of the esterified carbon (C-3), and those adjacent to this carbon (e.g. Pehk *et al.*, 1993).

3.2.5. Stereochemistry of the 3,5,6-Trihydroxy-β End-Group in Carotenoids

The present number of naturally occurring carotenoids isolated from various sources is in excess of 600 (Britton *et al.*, 1995a). However, carotenoids possessing the 3,5,6-trihydroxy-5,6-dihydro-β end-group are seldom reported. Highly polar carotenoids from orange juice were first described as trihydroxy carotenoids by Curl (1954) and subsequently by Gross *et al.* (1979). The existence of the 3,5,6-trihydroxylated end-group was confirmed in hetereoxanthin (**141**) (Table 3.3), a carotenoid isolated from algae (Buchecker and Liaaen-Jensen, 1977). Since the isolation and characterisation of **141**, a number of 3,5,6-trihydroxy carotenoids have been reported from plant sources (Table 3.3). These highly oxygenated carotenoids are thought to contribute to plant colouration, but have also been implicated as intermediates in the catabolism of carotenoids to lower molecular cleavage products (Eugster, 1985).

The absolute stereochemistry of the 3,5,6-trihydroxy-β end-group of heteroxanthin (**141**), was elucidated as 3*S*,5*S*,6*S*, by a combination of chemical correlation, H-bonding studies, ^{1}H NMR and CD (Buchecker and Liaaen-Jensen, 1977). In that study, **141** was prepared by partial synthesis from diadinoxanthin (**142**), which itself was converted to diatoxanthin (**122**) of known 3*R*,3'*R* configuration (DeVille *et al.*, 1969). A correlation between CD and chirality of a specific centre in 3,5,6-trihydroxy-β end-groups was not established.

The same absolute stereochemistry was reported for the 3,5,6-trihydroxy-β end-group identified in matraxanthin (**143**), a carotenoid isolated from a Japanese clam (Matsuno and Sakaguchi, 1983). It was claimed that **143** and violaxanthin (**31**) had the same chirality at C-3, on the basis of CD spectra (*vide infra*). The *cis* relative configuration at C-3 and C-5 was determined by H-bonding studies, whilst the *trans* configuration at C-5–C-6 was determined by H-bonding data, and experimentally by acetonide or silylate formation, in a manner similar to that reported for elucidation of hetereoxanthin (**141**).

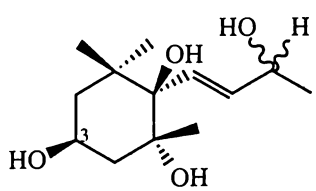
The *cis* relative configuration of C-3 and C-5 hydroxyl groups of both hetereoxanthin (**141**) and matraxanthin (**143**) were subsequently revised to *trans*, and consequently, the absolute configurations amended to 3*S*,5*R*,6*R* following the synthetic work of Buchecker *et al.* (1984) (Scheme 2.7). The absolute stereochemistry predicted for **143** was fortuitously correct, as the CD data of

violaxanthin (**31**) reflects the stereochemistry of the 5,6-epoxy functionality, rather than that at C-3 (e.g. violaxanthin and 3,3'-epiviolaxanthin have similar CD curves (Buchecker and Noack, 1995)). Revision of the absolute configurations of these carotenoids highlighted the inadequacies of H-bonding data for establishing the relative configurations of hydroxyl groups in cyclohexane rings.

Carotenoids with 3,5,6-trihydroxy-β end-groups have been isolated from various species of rose. The absolute configurations of this end-group in latoxanthin (**144**) and latochrome (**145**) were confirmed as 3*S*,5*R*,6*R* by chemical correlation with violaxanthin (**31**) (Märki-Fischer *et al.*, 1984). The same absolute configuration was identifed in karpoxanthin (**146**), by chemical correlation with (*9Z*)-*cis*-antheraxanthin (Märki-Fischer and Eugster, 1985).

Collectively, the above studies indicate that the absolute configuration of 3,5,6-trihydroxy-β end-groups is the same, irrespective of source. C-6 epimers of this end-group have been reported, although it was suggested that they were artifacts, produced *via* C-6 carbocations in an S_N1-type epoxide opening (Buchecker and Liaaen-Jensen, 1977; Buchecker *et al.*, 1984). Table 3.3 summarises the chiralities of 3-hydroxy-, 3-hydroxy-5,6-epoxy- and 3,5,6-trihydroxy carotenoids referred to in this Chapter.

The present study indicated that 3,5,6-trihydroxy-ionone **22** had the same chirality (3*S*,5*R*,6*R*) as that established for naturally occurring 3,5,6-trihydroxy-β carotenoid end-groups (Table 3.3). Triol **22** was the first reported degradation product of 3,5,6-trihydroxy-β carotenoids (Tan, 1989). More recently, 3,5,6-trihydroxy-5,6-dihydro-β-ionol (**147**) has been reported from another plant source (Pérez *et al.*, 1996). Ionol **147** has the same absolute configuration as **22**.



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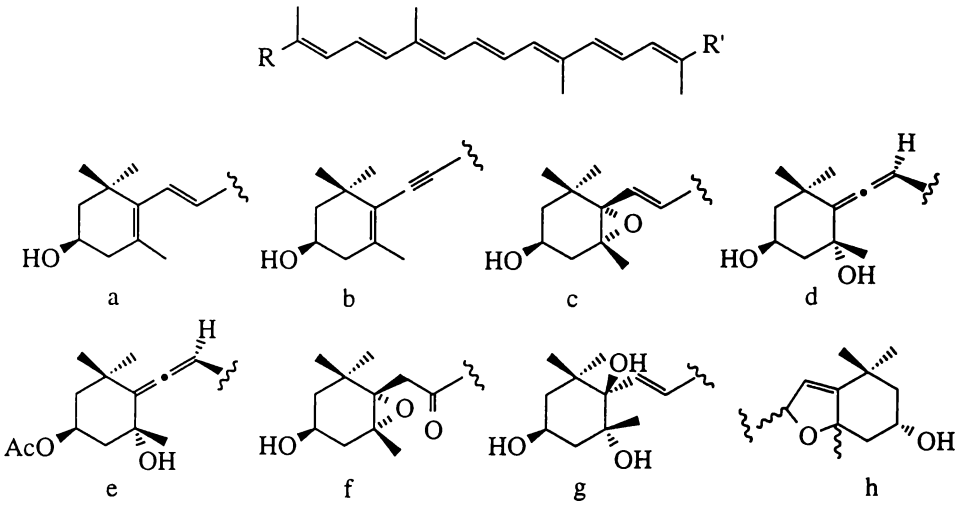


Table 3.3. Absolute configurations of xanthophyll carotenoids.

Carotenoid		Absolute Configuration
Monohydroxy Xanthophylls		
Zeaxanthin (**30**)	R = a, R' = a	3*R*,3'*R* [a]
Diatoxanthin (**122**)	R = a, R' = b	3*R*,3'*R* [a]
Epoxy Xanthophylls		
Violaxanthin (**31**)	R = c, R' = c	3*S*,5*R*,6*S*, 3*S*',5'*R*,6'*S* [b]
Fucoxanthin (**119**)	R = e, R' = f	3*S*,5*R*,6*R*, 3'*R* [c]
Neoxanthin (**32**)	R = d, R' = c	3*S*,5*R*,6*R*, 3'*S*,5'*R*,6'*S* [d]
Diadinoxanthin (**142**)	R = c, R' = b	3*S*,5*R*,6*S*, 3'*R* [e]
Trihydroxy Xanthophylls		
Heteroxanthin (**141**)	R = g, R' = b	3*S*,5*R*,6*R*, 3'*R* [f]
Matraxanthin (**143**)	R = g, R' = g	3*S*,5*R*,6*R*, 3'*S*,5'*R*,6'*R* [f]
Lactoxanthin (**144**)	R = g, R' = c	3*S*,5*R*,6*R*, 3'*S*,5'*R*,6'*S* [g]
Lactochrome (**145**)	R = g, R' = h	3*S*,5*R*,6*R*, 8'*S*/8'*R* [g]
Karpoxanthin (**146**)	R = g, R' = a	3*S*,5*R*,6*R*, 3'*R* [h]

[a] DeVille *et al.*, 1969, Bartlett *et al.*, 1969, Hlubucek *et al.*, 1974; [b] Bartlett *et al.*, 1969; [c] DeVille *et al.*, 1969; [d] Hlubucek *et al.*, 1974; [e] Buchecker and Liaaen-Jensen, 1977; [f] Buchecker *et al.*, 1984; [g] Märki-Fischer *et al.*, 1984; [h] Märki-Fischer and Eugster, 1985.

Cleavage of the C-9–C-10 double bond of carotenoids, which gives rise to C-13 ionols, ionones, and related aroma compounds, appears to be the preferred bio-oxidative pathway in plant tissues (Chapter One). Primary cleavage products of commonly encountered carotenoids, such as 3-hydroxy-β-ionone (**59**) from zeaxanthin (**30**), 3-hydroxy-5,6-epoxy-5,6-dihydro-β-ionone from violaxanthin (**31**), and grasshopper ketone (**121**) from neoxanthin (**32**), have been identified from various natural products (e.g. Wahlberg and Enzell, 1987; Winterhalter, 1996). In all cases examined, these compounds, and their derivatives, exhibit the same absolute stereochemistry as the assumed carotenoid precursors (Winterhalter, 1996).

Derivation of the 3,5,6-trihydroxy-β end-group from 3-hydroxy-5,6-epoxy-β end-group has been suggested (Eugster, 1985). The absolute configurations of naturally occurring 3,5,6-trihydroxy end-groups suggest their biosynthesis from the 3-hydroxy-5,6-epoxy-β end-group occurs *via* stereoselective epoxide opening, with retention of configuration at C-5 and inversion at C-6. This step may be enzyme-mediated or result from stereoselective acid-catalysed epoxide opening (Chapter Two).

Two diastereoisomeric 3-hydroxy-5,6-epoxy-β-ionones **23** and **24** were identified in the thyme honey extractives (Chapter Two). The relationship of these epoxides to **22** is uncertain. It is likely those epoxides would give rise to a mixture of diastereoisomeric 3,5,6-trihydroxy-β-ionones upon epoxide opening. Tan *et al.* (1990) observed a minor component which exhibited the same MS characteristics as **22**, consistent with it being a diastereoisomer of **22**. The absolute configuations of the epoxides and minor triol component could not be deduced due to their exceedingly small concentrations in the extractives.

Diastereoisomeric epoxides may be produced by non-enzymatic epoxidation of an optically active 3-hydroxy-β-ionone or parent carotenoid. The β-ring of carotenoids, and in particular that of β-carotene, are known to undergo epoxidation at C-5–C-6, especially in the presence of organic peroxides (Britton, 1991). The predominance of diastereoisomer **22** in the thyme honey extractives suggested that one epoxide is formed in preference to the other.

3.3. Conclusions

The absolute stereochemistry of **22** was deduced by analysis of the differential ^{1}H NMR chemical shifts ($\Delta\delta_{SR}$) of (*S*)- and (*R*)-MPA derivatives. ^{1}H and ^{13}C NMR of the ester derivatives indicated the parent compound was a single enantiomer. Assignment of ^{1}H and ^{13}C spectra was achieved using ^{1}H-^{1}H and ^{1}H-^{13}C correlation spectroscopy. The systematic arrangement of $\Delta\delta_{SR}$ values observed for MPA derivatives of **22** indicated the *S* configuration at C-3, and therefore an absolute configuration of 3*S*,5*R*,6*R*, given the established relative configuration of **22**.

^{13}C differential shifts were generally consistent with those of ^{1}H nuclei. Carbon nuclei of the enone system exhibited $\Delta\delta$ values which suggested that shielding and deshielding effects, other than those arising from diamagnetic phenyl ring currents, were operative.

The 3*S*,5*R*,6*R* configuration of **22** is identical to that of 3,5,6-trihydroxy carotenoids found in plants. It was not possible to determine the relationship of triol **22** to the two diastereoisomeric epoxides found in the honey extractives.

3.4. Experimental

3.4.1. Honey Sample

A sample of commercial thyme honey *(Thymus vulgaris)* was obtained from Wilson Neill-Hororata Honey Exports Ltd. The predominant floral source was verified as thyme following comparison of the GCMS data of the Et_2O extractives of this honey sample with those documented for unifloral thyme honey (Tan, 1989).

3.4.2. Spectroscopic Methods

NMR spectra were obtained on a 400 MHz Bruker Avance DRX-400 spectrometer at 303 K. Spectra were acquired using standard Bruker pulse programs. ^{1}H spectra were obtained over a 6000 Hz window with 32 K data

points, giving a resolution of 0.19 Hz/point. ^{1}H resolution-enhancement was carried out with gaussian broadening (GB) of 0.25 and line broadening (LB) of -2. 100 MHz ^{1}H-decoupled ^{13}C spectra were obtained over a window of 24,700 Hz with 64 K points, giving a resolution of 0.38 Hz/point. ^{13}C chemical shifts are quoted to 2 d.p.[1] to distinguish diastereoisomeric resonances. Exponential multiplication was executed giving 0.1 Hz linewidth for ^{1}H and 2 Hz for ^{13}C spectra.

Homonuclear correlation spectra were established by the COSY-45 experiment, with data collection and transformation as for DQFCOSY (Chapter Two). Referencing is as reported in Chapter Two. Hetereonuclear correlation was established by HSQC, optimised for an average $^{1}J_{CH}$ of 145 Hz (Δ = 1.72 ms). The reduced-window HSQC experiments were run with resolution of 1.17 Hz/point in the ^{1}H dimension and 1.97 Hz/point in the ^{13}C dimension. The HMBC experiment was obtained with a delay (D2) of 80 ms optimised for an average ^{n}J of 6.25 Hz.

3.4.3. Isolation of 22

Diethyl ether extractives of thyme honey (600 g) were obtained by liquid-liquid extraction, according to the method of Tan (1989). The extractives were reduced in volume and chromatographed (x 2) by PLC using 1:1 and 1:2 Et_2O:PE as solvent. The polar band (R_f = 0.15) on the plate was identified as triol **22** (10 mg) by MS and ^{1}H NMR.

3.4.4. Esterification Procedure for Preparation of MPA Esters

DCC (4.2 mg, 0.021 mmol) was added to a solution of parent alcohol (4.2 mg, 0.017 mmol), (*S*) or (*R*)-MPA (3.2 mg, 0.019 mmol) and DMAP (0.2 mg, 0.0017 mmol) in $CHCl_3$ (5 ml) and the reaction mixture was stirred at RT for 2 h. (quantitative conversion of the parent alcohols to the esters was evident from TLC). The dicyclohexylurea (DCU) was removed by filtration, the filter cake washed with 2 x 5 ml $CHCl_3$, and combined filtrates were washed with 2M HCl (2 x 10 ml), saturated sodium bicarbonate (15 ml) and saturated brine (15 ml)

[1] Reproducibilty to only 1 d.p. is common for ^{13}C resonances.

solutions. The organic phase was dried over $MgSO_4$, filtered and the solvent removed under vacuum to afford a colourless oil containing some suspended solid (DCU). The crude product was purified by PLC using 7:3 Et_2O:PE and the major band extracted with EtOAc, to give the esters as colourless oils.

(S)-MPA ester ***22a*** (R_f = 0.25), (76%). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.86 (3H, s, 1-Me_{eq}), 1.10 (3H, s, 5-Me), 1.31 (3H, s, 1-Me_{ax}), 1.60 (2H, s (br.), OH), 1.63 (1H, ddd, $J_{2eq,2ax}$ = 12.5, $J_{2eq,3}$ = 4.6, $J_{2eq,4eq}$ = 1.8 Hz, H-2_{eq}), 1.74 (1H, ddd, $J_{4eq,4ax}$ = 13.0, $J_{4eq,3}$ = 5.5, $J_{4eq,2eq}$ = 1.8 Hz, H-4_{eq}), 1.75 (1H, t, $J_{2ax,2eq}$ = $J_{2ax,3}$ = 12.5 Hz, H-2_{ax}), 1.79 (1H, t, $J_{4ax,4eq}$ = $J_{4ax,3}$ =13.0 Hz, H-4_{ax}), 2.30 (3H, s, H-10), 3.41 (3H, s, -CHPh(O<u>Me</u>)), 4.73 (1H, s, -C<u>H</u>Ph(OMe)), 5.29 (1H, m ($W_{1/2}$ = 22 Hz), H-3), 6.31 (1H, d, $J_{8,7}$ = 16.3 Hz, H-8), 7.26 (1H, d, $J_{7,8}$ = 16.3 Hz, H-7), 7.34-7.44 (5H, m, Ar-H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 25.44 (q, 1-Me_{ax}), 26.51 (q, 1-Me_{eq}), 27.38 (q, 5-Me), 27.50 (q, C-10), 40.30 (s, C-1), 41.01 (t, C-4), 41.18 (t, C-2), 57.40 (q, -CHPh(O<u>Me</u>)), 69.15 (d, C-3), 76.84 (s, C-5), 78.63 (s, C-6), 82.80 (d, -<u>C</u>HPh(OMe)), 127.20 (d, 2 x C-Ar), 128.65 (d, 2 x C-Ar), 128.72 (d, C-Ar), 131.38 (d, C-8), 136.40 (s, C-Ar), 148.28 (d, C-7), 170.37 (s, -O<u>C</u>OCHPh(OMe)), 197.90 (s, C-9). EIMS (**22a/22b**): *m/z* (rel. int.) 332 (1), 290 (1), 256 (1), 224 (23), 206 (5), 165 (3), 121 (CHPh(OMe), 100), 105 (4), 98 (5), 91 (9), 77 (14), 43 (36).

(R)-MPA ester ***22b*** (R_f = 0.28), (69%). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.82 (3H, s, 1-Me_{eq}), 1.15 (3H, s, 5-Me), 1.28 (3H, s, 1-Me_{ax}), 1.48 (1H, ddd, $J_{2eq,2ax}$ = 12.3, $J_{2eq,3}$ = 4.2, $J_{2eq,4eq}$ = 1.3 Hz, H-2_{eq}), 1.66 (1H, t, $J_{2ax,2eq}$ = $J_{2ax,3}$ = 12.3 Hz, H-2_{ax}), 1.80 (2H, s (br.), OH), 1.91 (2H, d, $J_{4ax/4eq,3}$ = 8.4 Hz, H-4_{ax} and H-4_{eq}), 2.30 (3H, s, H-10), 3.41 (3H, s, -CHPh(O<u>Me</u>)), 4.73 (1H, s, -C<u>H</u>Ph(OMe)), 5.29 (1H, m ($W_{1/2}$ = 22 Hz), H-3), 6.31 (1H, d, $J_{8,7}$ = 16.3 Hz, H-8), 7.26 (1H, d, $J_{7,8}$ = 16.3 Hz, H-7), 7.33-7.45 (5H, m, Ar-H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 25.40 (q, 1-Me_{ax}), 26.47 (q, 1-Me_{eq}), 27.39 (q, 5-Me), 27.48 (q, C-10), 40.25 (s, C-1), 40.85 (t, C-2), 41.31 (t, C-4), 57.39 (q, -CHPh(O<u>Me</u>)), 69.25 (d, C-3), 76.86 (s, C-5), 78.64 (s, C-6), 82.84 (d, -<u>C</u>HPh(OMe)), 127.17 (d, 2 x C-Ar), 128.64 (d, 2 x C-Ar), 128.71 (d, C-Ar), 131.37 (d, C-8), 136.38 (s, C-Ar), 148.36 (d, C-7), 170.41 (s, -O<u>C</u>OCHPh(OMe)), 197.98 (s, C-9).

Chapter Four

Isolation and Structural Characterisation of the Kamahines; Unusual Spiroketals found in Kamahi Honey

4.1. Introduction

4.1.1. New Zealand Kamahi and Rewarewa Unifloral Honeys

The extractable organic components of New Zealand honeys consist of a number of degraded carotenoids which demonstrate a high degree of floral dependency (Chapter One). Tan (1989) demonstrated that the extractives of kamahi honey contained a number of apparently related compounds of unknown structure, exhibiting GCMS spectra similar to the 3,5,5-trimethylcyclohex-2-en-1-one species (Chapter One) identified in heather honey. These components appeared to be unique to the kamahi plant source; hence there was interest in identifying them for use as floral markers for that honey.

The GC profile of rewarewa honey exhibited similar peaks to those found in kamahi extractives, although concentrations were lower (Tan, 1989). This implied that compounds common to both honeys were derived from the kamahi floral source. Moar (1985) has demonstrated that New Zealand rewarewa honeys usually include contributions from other plant sources including kamahi, white clover and willow. Pollen grain analysis indicated that the rewarewa sample used in that study possessed a significant kamahi contribution. The occurrence of kamahi-specific compounds in a honey demonstrated to contain a kamahi component highlighted their potential use as markers for that floral source.

One of the compounds of interest as a floral marker for the kamahi floral source was present in relatively large quantities in both kamahi (*ca* 85 μg/g) and rewarewa (*ca* 40 μg/g) honeys, making isolation and structural elucidation by NMR possible. This dominant component exhibited similarities in mass spectral data to other compounds of unknown structure in kamahi honey. Structural elucidation of the dominant compound was potentially useful for identification of these other minor components.

4.2. Results and Discussion

4.2.1. Isolation of Kamahine

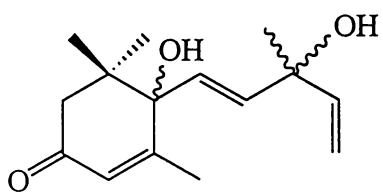
Diethyl ether extraction of an aqueous solution of kamahi honey (600 g) (Tan, 1989) was undertaken to isolate the dominant component of kamahi extractives in sufficient quantities for structural elucidation. GCMS of the extractives indicated the presence of the unidentified degraded carotenoid-like substances which had previously been documented by Tan (1989). The extract was subjected to column chromatography and following analysis of the fractions by GCMS, a fraction containing the major unknown component was obtained. This component was assigned the trivial name kamahine.

PLC failed to yield kamahine of reasonable purity for NMR analysis. GCMS indicated that a number of components had similar retention times, and coelution of some of these compounds with the compound of interest hindered attempts at purification. A concurrent investigation revealed sufficient quantities of kamahine could be isolated from the extractives of rewarewa honey (personal communication, Lu Yinrong). Bulk extraction of rewarewa honey (600 g) afforded a mixture of extractives which were separated by PLC. The major band was recovered from the PLC plate (*ca* 10 mg), and GCMS and NMR analysis showed it to be identical to the component of interest from kamahi honey. Further purification *via* PLC afforded a sample (>98% purity) suitable for NMR analysis.

4.2.2. Preliminary Investigation

Tan (1989) suggested that the kamahine component was a mixture of at least three isomers of formula $C_{15}H_{22}O_3$. Attempts to identify the structure of

kamahine were limited to analysis of ^{1}H and ^{13}C NMR spectra obtained from a small quantity (1 mg) of sample (Tan, 1989). The ^{13}C NMR data, whilst complicated by some of the carbon atoms appearing as clusters of peaks of similar chemical shift, showed three signals (δ 195.5, 195.2 and 195.0) attributable to the presence of a conjugated carbonyl group. Signals at *ca* δ 5.9 in the ^{1}H NMR spectrum were typical of the conjugated H-2 signal of 3,5,5-trimethylcyclohex-2-en-1-one derivatives, similar to that already established for a series of degraded carotenoid-like substances detected in heather honey samples (Tan *et al.*, 1989a). A cluster of signals in the region of δ 4.6 to 5.6 indicated the presence of a number of olefinic (non-conjugated) protons. These preliminary spectral data led to tentative assignment of the kamahi component as *cis/trans-S/R* isomers of structure **(148)**.



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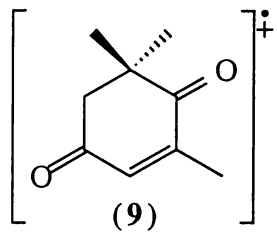
Non-polar capillary GCMS carried out in the present study also indicated the kamahine component was a mixture of three isomers, as manipulation of the GC temperature programme resulted in partial resolution of the broad kamahine peak into three peaks. The three isomers were inseparable by preparative techniques, including reverse-phase HPLC.

The ^{1}H and ^{13}C NMR spectra of the newly isolated kamahine component confirmed that the three compounds were diastereoisomeric. Spectrum editing using the DEPT sequence showed that each isomer possessed four methyl, one methylene, four methine and five quaternary carbons. In addition, two of the diastereoisomeric kamahines appeared to interconvert in deuterochloroform solution, whilst the remaining kamahine appeared to be stable. The three diastereoisomers were named kamahines A (**25a**), B (**25b**) and C (**25c**) for the purposes of this investigation.

High-resolution electron-impact GCMS showed a molecular ion at *m/z* 268.1307 corresponding to an elemental composition of $C_{14}H_{20}O_5$ (calculated *m/z* for $C_{14}H_{20}O_5$ = 268.1311). Low resolution EIMS showed a peak at 251 ($M^{+\cdot}$-17), suggesting a loss of a tertiary hydroxyl radical, and a base peak at *m/z* 152 consistent with a 2,6,6-trimethylcyclohex-2-en-1,4-dione fragment ion $[(\mathbf{9})]^{+\cdot}$.

Diketone **9** is also found in the extractives of kamahi honey. Ions at *m/z* 152, 137, 124 and 109 displayed by **9**, were common to kamahine and other components of unknown structure from this honey (Tan, 1989).



(9)

Chemical ionisation (CI) displayed the appropriate MH^+ (M+1) quasi-molecular ion at *m/z* 269 and a significant ion at *m/z* 251 attributable to loss of a water molecule. The latter ion was interpreted as the MH^+ ion by Tan (1989), giving rise to a molecular ion of *m/z* 250, consistent with the proposed structure (**148**). The CI mass spectrum also exhibited a base peak at *m/z* 153.

Acetylation of kamahine using standard acetylation procedures (pyridine/acetic anhydride), followed by PLC, afforded two fractions. One of these was shown to be a mixture of monoacetylated-kamahines A (**149a**) and B (**149b**), whereas the other fraction was pure monoacetyl-kamahine C (**149c**). The ^{13}C NMR spectrum of **149c** exhibited 16 carbon signals consistent with the presence of only one of the isomers, including the additional signals at δ 170.8 and δ 21.3 consistent with the addition of one acetyl group to the molecule. ^1H NMR revealed an extra methyl resonance at δ 2.12 attributed to the acetate ester. A parent ion was not observed by GCMS, although the highest mass ion observed was *m/z* 268 corresponding to a loss of a ketene (CH_2CO) fragment from the molecule.

The molecular formula of the acetyl derivative ($C_{16}H_{22}O_6$) required that the total number rings and sites of unsaturation equal six. Three sites of unsaturation were readily identified from the ^{13}C NMR spectrum. A singlet carbon at δ 170.8 was indicative of an ester carbonyl and a downfield singlet at δ 194.6 revealed the presence of an α,β-unsaturated ketone (support for the presence of this functionality in the molecule was the IR band at 1665 cm^{-1}). Two olefinic resonances (δ 125.4 (d) and 164.8 (s)) revealed the presence of a trisubstituted carbon-carbon double bond. The lack of evidence for additional unsaturated functional groups indicated that compound **149c** was tricyclic.

The initial evaluations of NMR and mass spectral data indicated that kamahines **25a-25c** were diastereoisomeric, and that they possessed a ring system derived from 2,6,6-trimethylcyclohex-2-en-1,4-dione (**9**). Although a number of such derivatives, described as degraded carotenoids, had been identified in heather honeys (Tan, 1989; Tan *et al.*, 1989a), it was apparent that the kamahines did not correspond to a known degraded carotenoid, or *nor*-sesquiterpenoid.

4.2.3. Characterisation of Acetate 149c

^{1}H and ^{13}C NMR Spectroscopy

The ^{1}H NMR spectrum of acetate **149c** recorded in $CDCl_3$ contained resonances able to be assigned to five methyl groups, including an acetate ester, an olefinic, and three aliphatic methyl groups. Two of the aliphatic methyl groups (δ 1.01 and 1.27) were attached to quaternary carbons, whilst the third downfield methyl (δ 2.12), was consistent with an acetate ester group. A three proton doublet at δ 1.10 was indicative of an aliphatic secondary methyl group. A downfield methyl signal at δ 2.06 displaying a small coupling, was probably a methyl group attached to an olefinic carbon atom.

An AB pattern was observed at δ 1.66/2.09 and assigned to a pair of non-equivalent methylene protons, and a resonance at δ 2.51 was attributed to a methine proton adjacent to an oxygenated carbon. The downfield chemical shift (δ 3.98) of a doublet suggested that one proton was at an oxygenated site, and a further doublet at δ 5.85 suggested the presence of an acetal or olefinic methine. The chemical shift of an additional signal at δ 5.91 supported the presence of an olefinic proton. A broad hydroxyl signal was apparent in this spectrum, indicating that one hydoxyl group in the molecule had not been acetylated. This indicated the possibility that this hydroxyl group was tertiary.

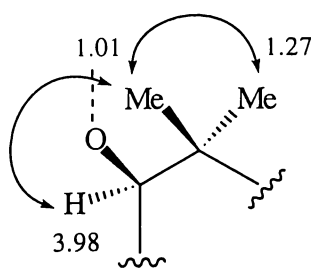
The ^{1}H-decoupled ^{13}C NMR spectrum also recorded in $CDCl_3$ showed the presence of 16 carbon atoms. Multiplicity editing using the DEPT sequence indicated the presence of five methyl, a methylene, four methine and six quaternary carbons. A downfield peak at δ 194.6 was assigned to an unsaturated ketone group, whilst two other signals at δ 164.8 (s) and δ 125.4 (d) were consistent with the olefinic carbons α- and β- to the carbonyl group, respectively. A methine carbon resonance at δ 103.6 suggested the presence of an acetal functional group,

and a singlet carbon at δ 114.6 indicated the presence of an olefinic or ketal group. Two carbon signals at δ 84.9 (s) and δ 89.9 (d) represented two carbon sites that had hydroxyl or ethereal oxygens attached. The remaining signals belonged to aliphatic methyl (x5), methylene (x1), and methine (x1) carbon atoms.

^{1}H-^{1}H Correlation

Detailed analysis of the ^{1}H spectrum of **149c** was undertaken using the ^{1}H-^{1}H DQFCOSY experiment (Bax and Freeman, 1981). ^{1}H-^{1}H connectivity maps of the different spin systems were identified in order to build up the various proton bearing fragments in the molecule. In each connectivity map, *J*-values obtained from the resolution-enhanced ^{1}H NMR spectrum were used to facilitate the analysis.

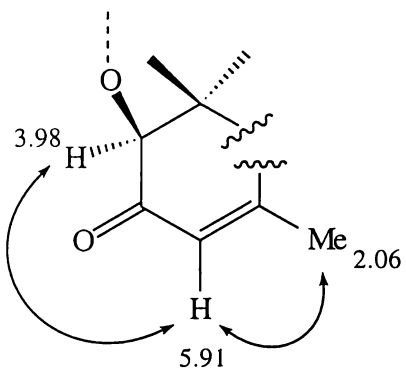
Coupling interactions were readily obtained from the DQFCOSY spectrum (Figure 4.4). Two of the aliphatic methyl groups (δ 1.01 and δ 1.27) exhibited mutual ^4J coupling, indicating that they were geminal with respect to each other. The proton appearing at δ 3.98 exhibited ^4J coupling to one of the geminal methyl groups (δ 1.01), suggesting that this proton was *anti*-1,2-diaxial with respect to this methyl group (fragment A, Figure 4.1) (*cf.* Figure 3.8).



A

Figure 4.1. Sub-structure A showing ^{1}H-^{1}H connectivities between methyl groups (δ 1.01 and 1.27) and methine proton (δ 3.98).

The COSY spectrum indicated that the proton at δ 3.98 was also coupled to the olefinic proton (δ 5.91), although only one resolvable ^4J coupling (^4J 2.2 Hz) was observed for this proton in the resolution-enhanced ^{1}H NMR spectrum (the COSY experiment can provide additional coupling information to that obtained from a resolution-enhanced ^{1}H NMR spectrum due to resolution of couplings less than the ^{1}H signal linewidths). The olefinic proton (δ 5.91) appeared as a doublet of quartets in the ^{1}H spectrum, exhibiting ^4J coupling constants of 2.2 and 1.5 Hz. Off-diagonal connectivities shown in the COSY spectrum indicated coupling of this proton to both the oxygenated methine at δ 3.98 (^4J = 2.2 Hz) and the olefinic methyl group at δ 2.06 (^4J = 1.5 Hz) leading to constitution of fragment B (Figure 4.2).



B

Figure 4.2. Sub-structure B linking fragment A to the protons at δ 5.91 and 2.06 of the enone system.

The W-type long-range coupling between the methine proton (δ 3.98) and conjugated olefinic proton (δ 5.91) parralleled that documented for the cyclohexenone ring of abscisic acid derivatives (Harada, 1973). This type of coupling, which is typical of that which occurs between protons which flank a carbonyl group (Tan, 1989), suggested that the proton at δ 3.98 was equatorial. Thus, the four-bond coupling between this proton and one of the adjacent methyl groups (Figure 4.1) was not a typical W-type interaction.

An additional independent spin system was identified in the COSY spectrum. In addition to mutual ^2J coupling (13.0 Hz), the methylene protons (δ 1.66 and 2.09) exhibited ^3J couplings (9.9 and 7.3 Hz, respectively) to a methine proton (δ 2.51), indicating that the methylene and methine protons were adjacent (Figure 4.3). The carbon bearing the methylene protons appeared to be attached to a quaternary carbon as no other ^{1}H-^{1}H coupling interactions were discernable with these protons.

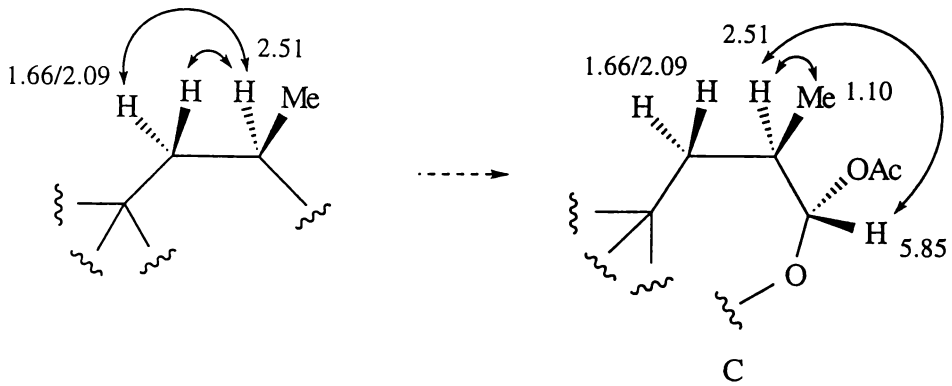


Figure 4.3. Fragment C represents an additional spin system present in the kamahine structure.

The methine proton also exhibited ^3J coupling to the aliphatic methyl group (δ 1.10) which appeared as a doublet with ^3J = 6.9 Hz in the ^{1}H spectrum, and an additional ^3J coupling to the proton at δ 5.85. The chemical shift of the doublet at δ 5.85, and the lack of further coupling interactions to this proton supported the presence of an acetal functional group. The above observations indicated that the methine proton was geminal to the methyl group (δ 1.10) and adjacent to the acetal functionality (fragment C, Figure 4.3). The coupling between the methylene proton at δ 2.09 and methine proton at δ 5.85 observed in the COSY spectrum was attributed to a W-type interaction. A contour plot of the DQFCOSY spectrum of **149c** indicating connectivities is shown in Figure 4.4.

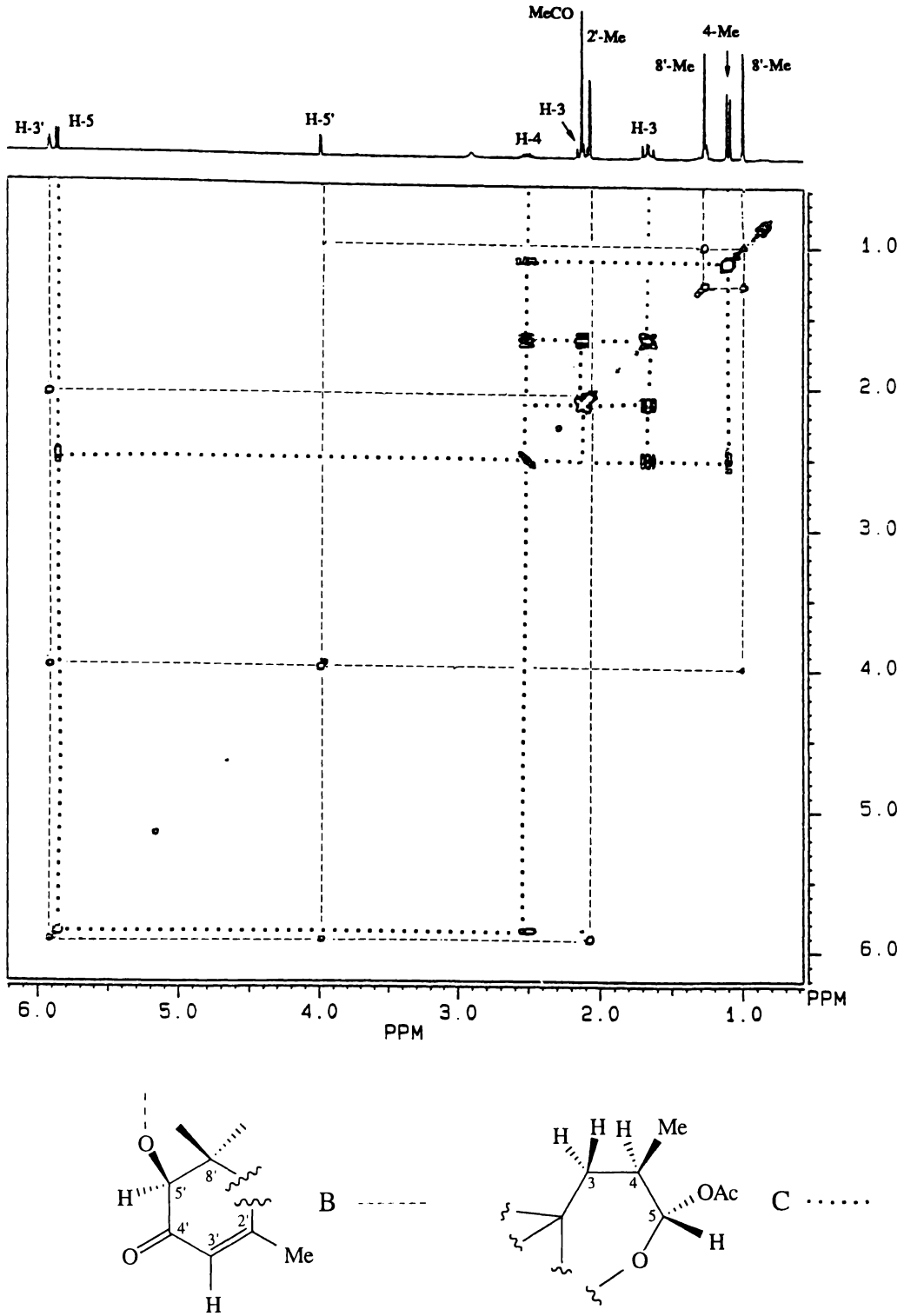


Figure 4.4. DQFCOSY spectrum of acetate **149c**. The independent spin systems B and C are identified from the off-diagonal connectivities shown. IUPAC numbering of the kamahine ring system is shown.

Heteronuclear 2D Correlation

Fragments B (Figure 4.2) and C (Figure 4.3), established by homonuclear coupling, accounted for five methyl groups, one methylene, four methine and four of the six singlet carbon signals present in the ^{13}C NMR spectrum of **149c**. The inverse-mode Heteronuclear Multiple Quantum Coherence (HMQC) (Bax and Subramanian, 1986) spectrum of **149c** depicted in Figure 4.6, shows assignment of the protonated carbon atoms of the ^{13}C NMR spectrum (F_1 axis). Expansion of the region of the spectrum exhibiting the methyl cross-correlation peaks showed sufficient resolution for assignment.

Four of the six singlet carbon resonances were assigned to quaternary carbons present in fragments B and C using chemical shift as the criterion. The signal appearing at δ 52.0 was attributed to the carbon attached to the geminal methyls, the resonance at δ 164.8 to the substituted alkene, and carbonyl signals at δ 170.8 and 194.6 to the acetate and α,β-unsaturated ketone groups, respectively. The chemical shift of the singlet carbon at δ 84.9 suggested that this carbon was oxygenated. The singlet resonance at δ 114.6 was consistent with the presence of a ketal functionality.

Two tentative structures for acetate **149c** were proposed to account for the ^1H and ^{13}C NMR characteristics (Figure 4.5). Both structures incorporated the two independent *J*-coupled fragments B and C established through the homonuclear 2D experiment. Two quaternary carbons, those resonating at δ 84.9 and 114.6 were required to link the two independent spin systems together. Structure D possessed the spiroketal functionality, and tertiary hydroxyl group consistent with earlier speculation. Structure E exhibited the fused tetrahydropyran ring incorporating a hemiketal functionality.

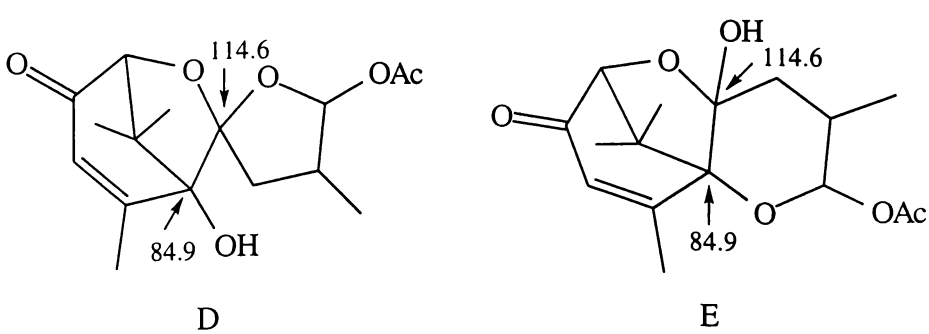


Figure 4.5. Proposed structures for monoacetyl-kamahine C (**149c**).

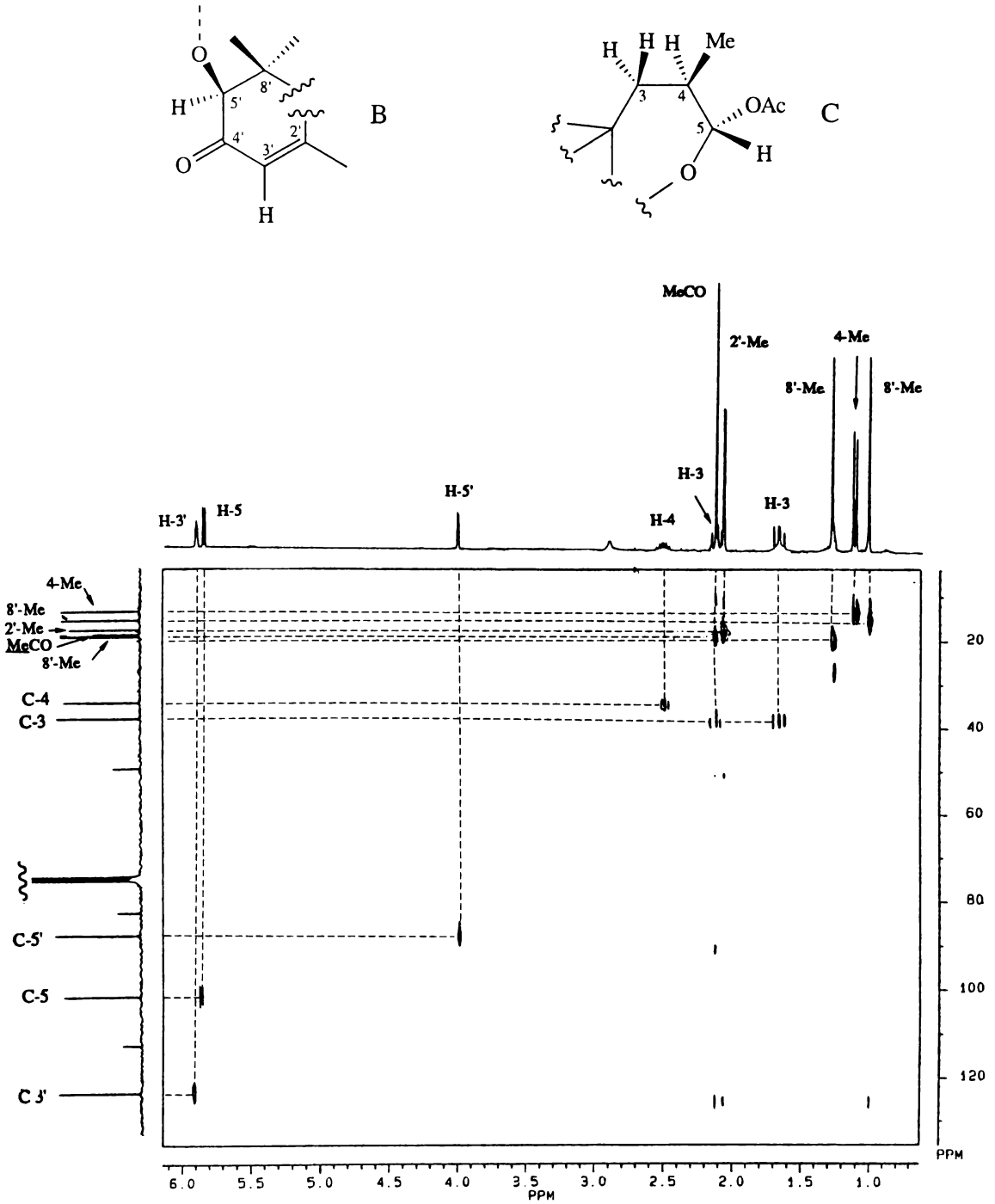


Figure 4.6. Above: Sub-structures B and C established from homonuclear correlations. Below: HMQC spectrum of acetate 1**49c** showing assignment of protonated carbons (δ 5–135 ppm).

Detection of direct one-bond interactions ($^1J_{CH}$) in the conventional mode XHCORR (Bax and Morris, 1981), or inverse-mode HMQC or HSQC (Kay *et al.*, 1992) experiments, allows unambiguous assignment of all protons to their respective carbon atoms (except in the instance of overlapping proton resonances). The direct ^1H-^{13}C correlation experiment, however, does not provide any connectivity information, apart from that contained in the cross peaks themselves. In addition, it does not provide information about the stereochemical relationship of protons or rings.

Connectivity information can be derived using several different NMR experiments. As indicated above, the use of the COSY experiment, in combination with analysis of multiplet patterns and coupling constants obtained from the resolution-enhanced ^1H NMR spectrum, enables determination of proton connectivities, and hence indirectly, ^{13}C-^{13}C connectivity. HOHAHA (Homonuclear Hartmann-Hahn) or TOCSY (Total Correlation) spectroscopy (Bax and Davis, 1985) also provides proton-proton connectivities, both direct and relayed. To some extent, connectivity patterns can be deduced from NOE experiments.

Direct ^{13}C-^{13}C connectivity information can be determined through the INADEQUATE (Incredible Naural Abundance Double Quantum Transfer) experiment (Bax *et al.*, 1981); however sample quantity requirements are a severe limitation in this case. The long-range heteronuclear shift correlation experiment, or the more sensitive heteronuclear multiple bond correlation (HMBC) experiment (Bax and Summers, 1986) provide connectivities between protons with more distant carbon atoms. The long-range ^1H-^{13}C connectivities detected in these experiments provide valuable structural and assignment information.

Contour plots of various regions of the HMBC spectrum demonstrating ^1H-^{13}C connectivities within the kamahine structure are depicted in Figures 4.11 and 4.12. The HMBC experiment is designed to remove cross peaks arising from direct $^1J_{CH}$ couplings, giving rise to long-range $^2J_{CH}$ and $^3J_{CH}$ couplings only. Four-bond $^4J_{CH}$ couplings are not generally observed. An additional feature of the HMBC spectrum, exemplified in Figure 4.11, are the more intense correlations presented by the methyl groups, and higher levels of t_1-noise associated with these groups (this is due to incompletely suppressed signal from protons not coupled to the ^{13}C nuclei).

The effect of t_1-noise can also be alleviated to a certain extent by using plots of different contour levels to indicate the correlations of interest. The contour level displayed in Figure 4.11, whilst exhibiting considerable t_1-noise, was chosen to reveal both intense and weak correlations. The suppression of t_1-noise can be partially achieved post-processing with baseline correction (e.g. SUB2), but is now achieved routinely by z-axis field gradients applied during the HMBC experiment.

The long-range ^1H-^{13}C couplings observed in the HMBC spectrum supported the proposed constitution of fragments B and C (Figures 4.2 and 4.3). The geminal methyl groups exhibited ^2J correlation to the singlet carbon at δ 52.0 and ^3J correlations to carbons δ 89.9 and 84.9, indicating that the geminal methyl groups were adjacent to both the oxygenated methine carbon and a carbon bearing an oxygen substituent (Figure 4.7).

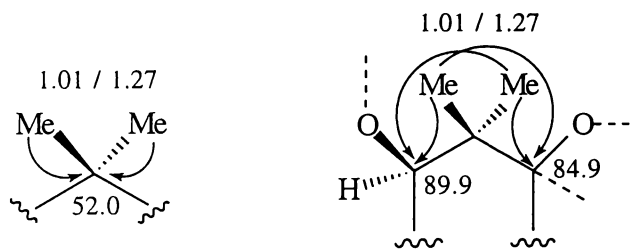


Figure 4.7. Correlations observed for the geminal methyl groups.

In addition, correlation of the olefinic methyl group (δ 2.06) to both olefinic carbons (δ 125.4 and 164.8) and the singlet carbon at δ 84.9 was evidence which placed the substituted olefinic carbon adjacent to the oxygenated carbon (Figure 4.8).

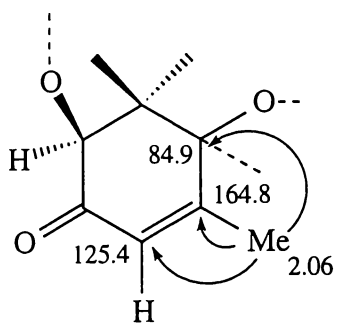
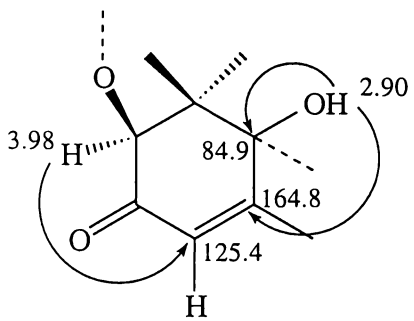


Figure 4.8. Correlations from the olefinic methyl group at δ 2.06.

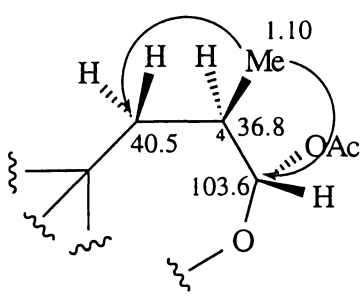
Cross-correlation peaks were observed from the hydroxyl proton to the carbons at δ 84.9 and 164.8 (Figure 4.9). These key long-range ^{1}H-^{13}C correlations were not detected in the conventional experiment (XHCORR). The former correlation was either a ^{2}J or ^{3}J coupling in proposed structures D and E (Figure 4.5), respectively. However, the latter correlation was consistent with the hydroxyl group being adjacent to the olefinic methyl group, which was predicted in stucture D only. The foregoing evidence led to formulation of fragment F (Figure 4.9). The three-bond coupling of the oxygenated methine (δ 3.98) to the olefinic carbon (δ 125.4) confirmed the previously established configuration of atoms shown in Figure 4.9. Correlations from the methyl and hydroxyl groups are indicated on the HMBC spectrum showing connectivities from protons in the region δ 0.00 to 3.00 ppm (Figure 4.11).



F

Figure 4.9. ^{1}H-^{13}C connectivities implicating fragment F. Correlations from the hydroxyl (δ 2.90) and methine proton (δ 3.98) are shown in Figures 4.11 and 4.12, respectively.

Correlations from the doublet methyl at δ 1.10 to the anomeric (δ 103.6), methylene (δ 40.5) and methine (δ 36.8) carbons provided independent support for fragment C (Figure 4.10). Correlations from the methine proton (C-4) were not observed. Summers *et al.* (1986) indicated that long-range connectivities from protons exhibiting a broad multiplet structure are more difficult to observe in the HMBC experiment.



C

Figure 4.10. Long-range ^{1}H-^{13}C correlations supporting fragment C.

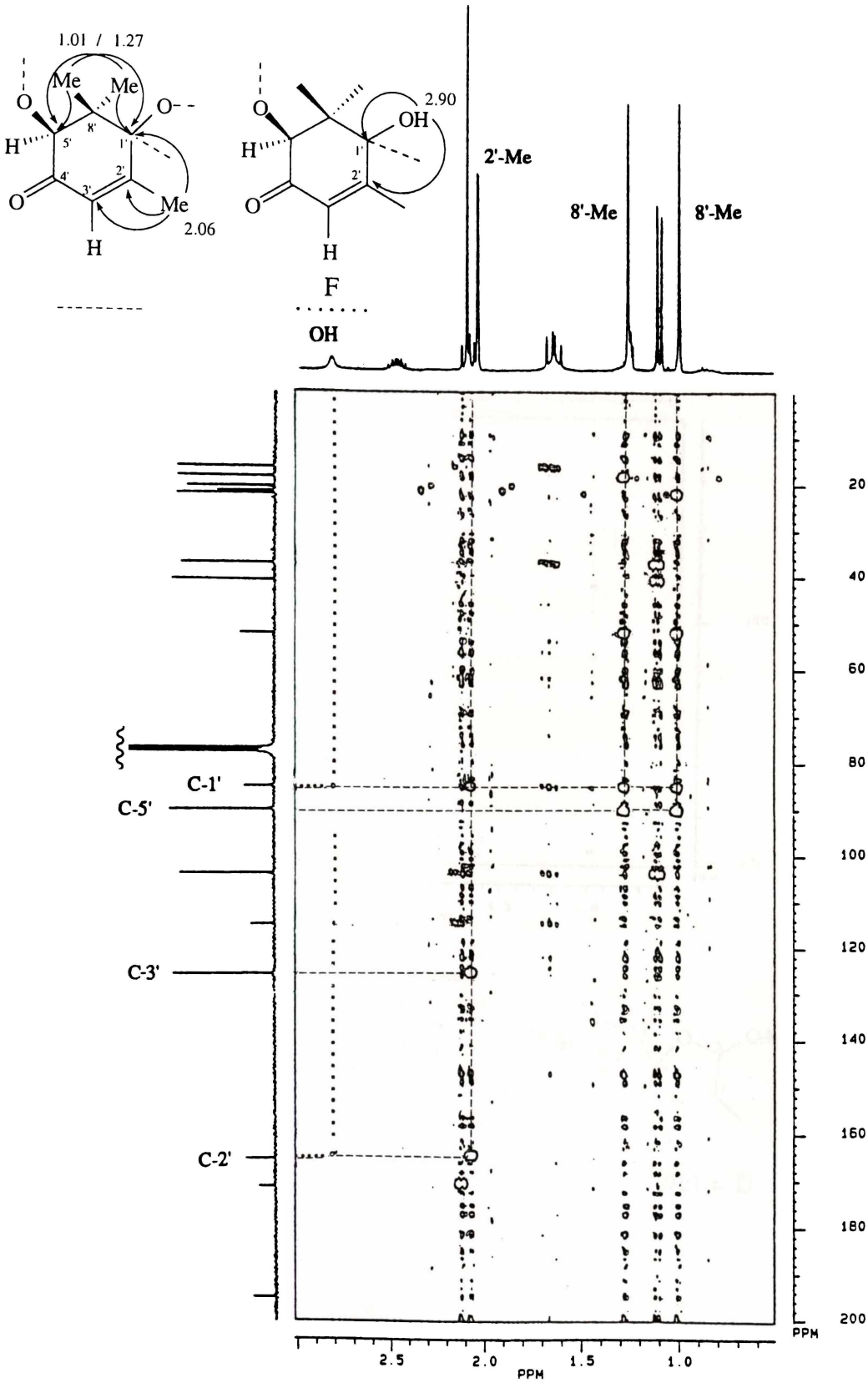


Figure 4.11. HMBC spectrum of **149c** in the region δ 0.00–3.00 ppm. Long-range correlations from methyl groups at δ 1.01, 1.27 and 2.06 and the hydroxyl group implicated fragment F. The correlations shown are depicted on the structures above.

Several long-range connectivities establsihed the structural identity of **149c** from the independent spin systems C and F. Shown in Figure 4.12 are cross-correlation peaks from the oxygenated methine (δ 3.98) of cyclohexenone system F and the acetal methine (δ 5.85) of fragment C, to the singlet carbon at δ 114.6.

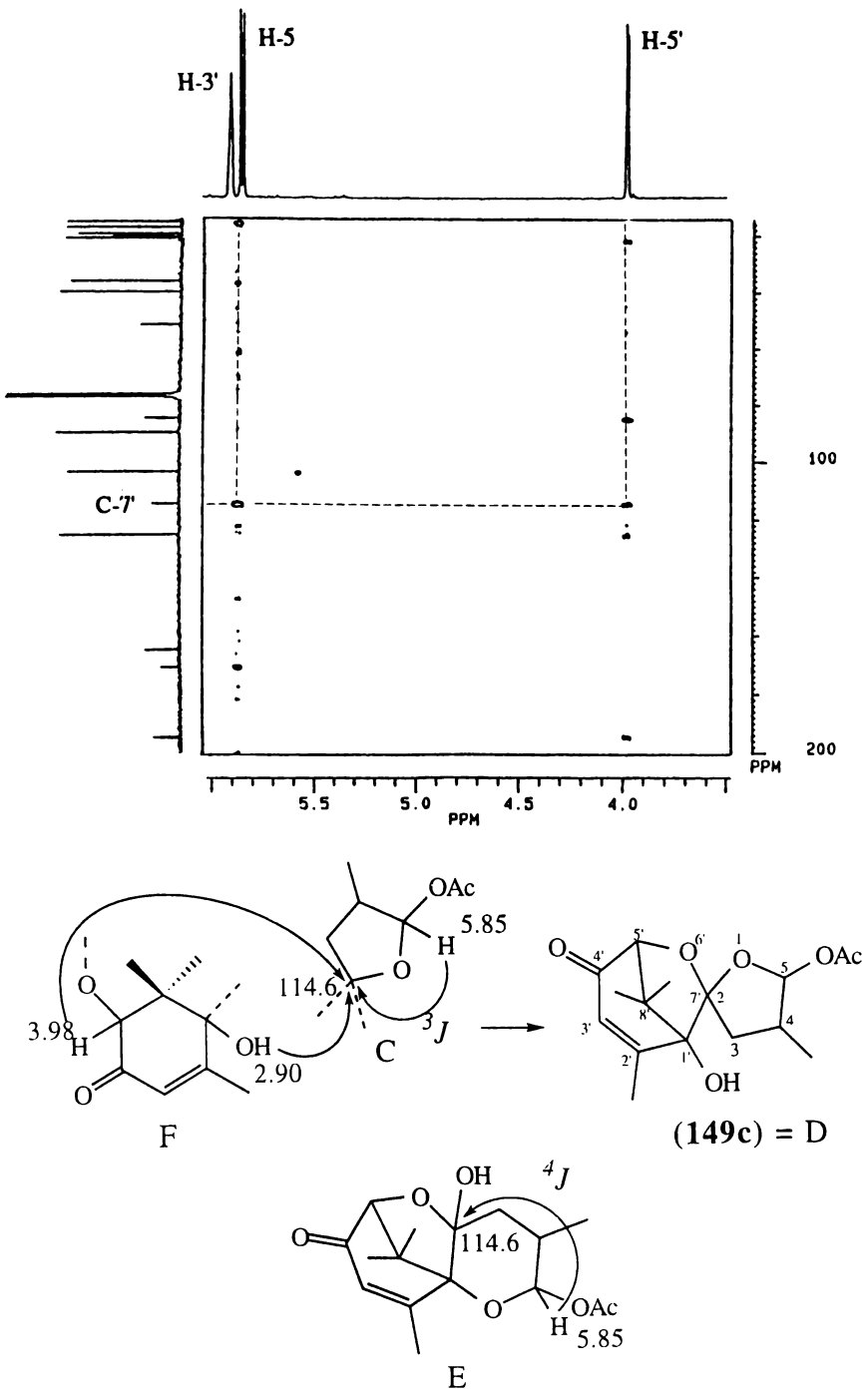


Figure 4.12. HMBC spectrum of **149c** showing correlations from protons in the region δ 3.50–6.00 ppm. Connectivity between fragments C and F was determined by long-range ^1H-^{13}C correlations from protons at δ 3.98 and 5.85.

The coupling observed between the acetal methine (δ 5.85) and quaternary carbon (δ 114.6) established that the two fragments were linked through a ketal functionality. The structure containing the tetrahydrofuran ring (structure D) would be required to exhibit a three-bond coupling, whilst the corresponding correlation in the structure containing the tetrahydropyran ring (structure E) was a four-bond coupling (Figure 4.12). This evidence, in addition to the ^{3}J correlation of the hydroxyl group to the unsaturated carbon (δ 164.8) discussed previously (Figure 4.9), discounted structure E. Kamahine **149c** therefore, possessed the tricyclic skeleton exhibiting the five-membered tetrahydrofuran ring and not the six-membered tetrahydropyran ring.

Several other protons showed correlations to the spiroketal carbon, including the tertiary hydroxyl proton and the methylene protons of the tetrahydrofuran ring (Figure 4.13). In addition, a three-bond correlation of one of the methylene protons (δ 1.66) to the carbon bearing the hydroxyl group was observed. These correlations are seen in the HMBC spectrum shown in Figure 4.11. Collectively, these connectivities did not distinguish structures D and E, although they corroborated the gross structure of kamahine **149c**.

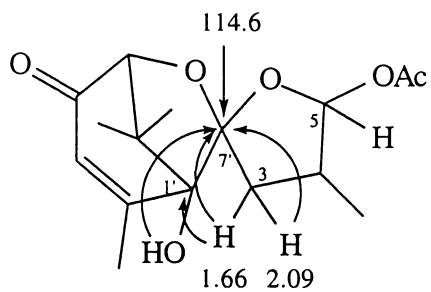


Figure 4.13. Long-range ^{1}H-^{13}C correlations from protons of the tetrahydrofuran ring which support the structure **149c**.

The structural information extracted from long-range ^{1}H-^{13}C correlations demonstrated that monoacetyl-kamahine C (**149c**) exhibited a spiro[furan-2,7'-oxabicyclo[3.2.1]octane] ring system. Acetate **149c** is the first reported member of this ring system.

NOE-Difference Experiments

The gross structure of monoacetyl-kamahine C (**149c**) was established by 2D ^{1}H-^{1}H and ^{1}H-^{13}C correlation experiments. A series of NOE-difference experiments were performed to determine the stereochemical orientation of the spirotetrahydrofuran ring, and the relative configurations of the substituents on that ring. Through-space interactions were also required to assign the ^{1}H resonances of 8-Me_{ax} and 8-Me_{eq}.

The 300 MHz NOE-difference spectrum of **149c**, obtained by irradiation of 2'-Me, showed a significant enhancement in the signal at δ 1.60, attributed to one of the methylene protons (H-3_a). This indicated that the spirotetrahydrofuran ring was oriented such that C-3 was *cis* to the enone system (Figure 4.14). The weak NOE induced in the 4-Me signal suggested that this substituent was *trans* to O-6'.

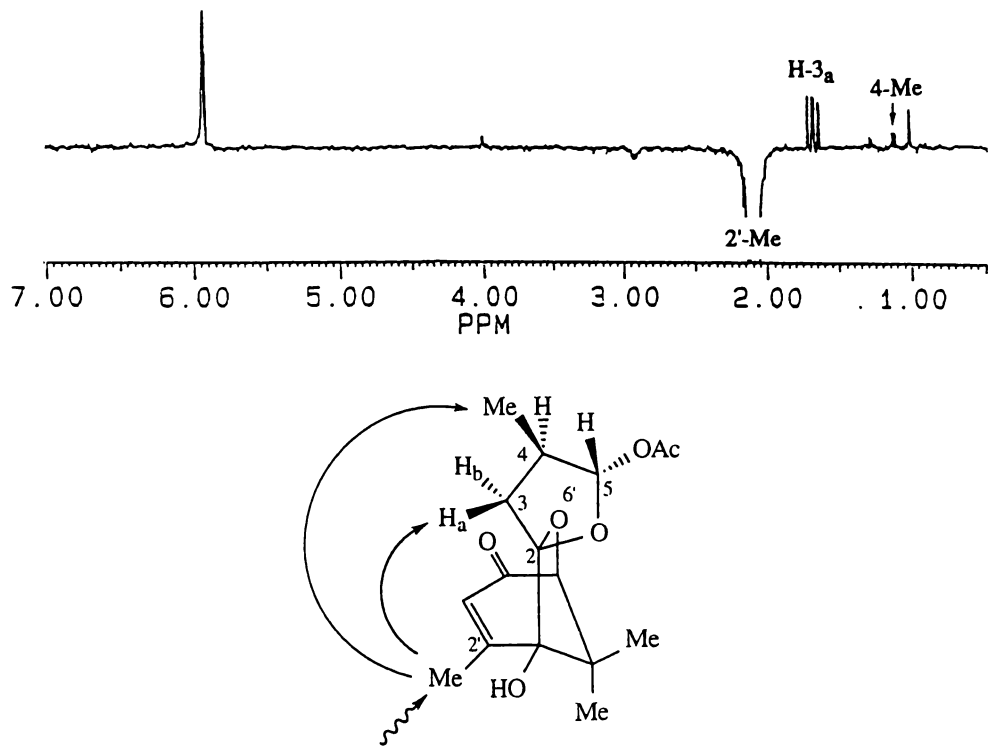


Figure 4.14. NOE-difference spectrum of a degassed sample of **149c** following irradiation of 2'-Me. Key NOE enhancements indicating the relative orientation of the enone functionality and spirotetrahydrofuran ring are shown.

The difference spectrum (Figure 4.14) was obtained from a degassed sample. The exclusion of paramagnetic materials, principally oxygen, results in improvement of NOEs (Noggle and Shirmer, 1971). The enhancement in NOEs is attributed to removal of competing spin-lattice relaxation pathways. Degassing was carried out in an NMR tube using the freeze-thaw-pump technique described by Noggle and Shirmer (1971).

The possibility existed that the irradiation of 4-Me was not completely selective owing to the slight overlap with the multiplet attributed to H-3_b. However, in a separate NOE experiment, saturation of the H-3_b signal resulted in strong enhancements for both H-3_a and H-4 signals; since no observable enhancement was observed for H-4 following irradiation of 2'-Me, it was assumed that no saturation of H-3_b had occurred. The positive enhancement between 2'-Me and H-3_a was substantiated by the reciprocal NOE-difference spectrum carried out at 400 MHz. The enhancements in H-3_b and 2'-Me observed in that experiment are seen in the insert shown in Figure 4.15 (a). The above results confirmed the *cis* relationship between C-3 and 2'-Me of the enone system, establishing the relative configuration at C-2 depicted.

The equatorial position of H-5' meant that distinction of the two 8-Me resonances, on the basis of a W-coupling interaction (Figure 4.1/4.2), was not possible. Irradiation of H-3' gave an NOE for the methyl signal at δ 1.01, identifying this signal as arising from 8-Me_{ax}. In addition, irradiation of H-5' gave NOEs at 8-Me_{ax} and 8-Me_{eq}, with the larger NOE occurring at 8-Me_{eq}. Internuclear distances, calculated from the MM2*-minimised structure of **149c**, indicated that H-5' was closer to 8-Me_{eq} (Table 4.2). Thus, NOE results indicated that H-5' exhibits a four bond coupling to the axial C-8' methyl group. Full NMR data for monoacetyl-kamahine C (**149c**) are presented in Table 4.1.

The NOE-difference spectra and structures demonstrating the relative configurations of the methyl and acetoxy substituents of the tetrahydrofuran ring of **149c** are shown in Figure 4.15. Irradiation of H-3_a (δ 1.66) (Figure 4.15(a)) induced a relatively strong enhancement in the 4-Me substituent establishing a *cis* relationship between these substituents. Irradiation of 4-Me (Figure 4.15(b)) led to moderate enhancements in the H-4 and H-3_a resonances and a strong enhancement in the H-5 resonance. These NOEs were consistent with the methyl group being closest to H-3_a and H-5.

Table 4.1. NMR data for monoacetyl-kamahine C (**149c**).

Carbon	$\delta^{13}C$	$\delta^{1}H$ (J Hz)	COSY	HMBC (H→C)
4-Me	16.1 (q)	1.10 (d, ^{3}J = 6.9 Hz)	H-4	3, 4, 5
8'-Me_{ax}	18.1 (q)	1.00 (s)	8'-Me_{eq}	8'-Me_{eq}, 8', 1', 5'
2'-Me	20.3 (q)	2.06 (d, ^{4}J = 1.5 Hz)	H-3'	1', 2', 3'
MeCO	21.3 (q)	2.12 (s)		
8'-Me_{eq}	21.8 (q)	1.27 (s)	8'-Me_{ax}	1', 5', 8', 8'-Me_{ax}
4	36.8 (d)	2.51 (m)	H-5, H-3_a, H-3_b,4-Me	5
3	40.5 (t)	H3_a: 1.66 (dd, ^{2}J = 13.0, ^{3}J = 9.9 Hz), H3_b: 2.09 (dd,^{2}J = 13.0, ^{3}J = 7.3 Hz)	H-4	3_a→1', 7', 4, 4-Me, 5; 3_b→ 7', 4, 4-Me, 5
8'	52.0 (s)			
1'	84.9 (s)	2.90 (s(br.), OH)		2', 7'
5'	89.9 (d)	3.98 (d, ^{4}J = 2.2 Hz)	H-3'	1', 3', 4', 7', 8'-Me_{eq}
5	103.6 (d)	5.85 (d, ^{3}J = 4.8 Hz)	H-4	4, 7'
7'	114.6 (s)			
3'	125.4 (d)	5.91 (dq, ^{4}J = 2.2, ^{4}J = 1.5 Hz)	2'-Me, H-5'	2'-Me, 1'
2'	164.8 (s)			
MeCO	170.8 (s)			
4'	194.6 (s)			

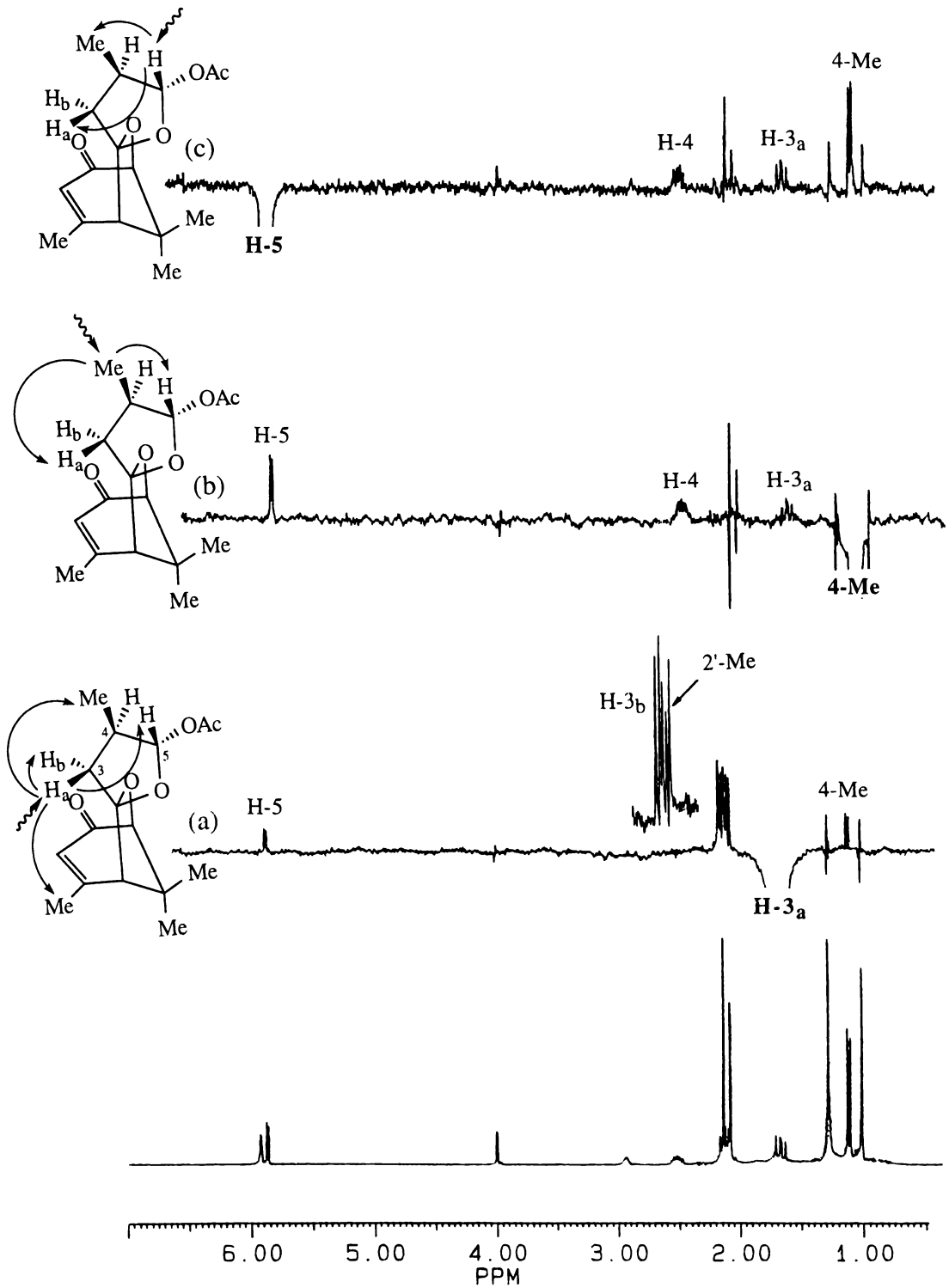


Figure 4.15. ^{1}H NMR spectrum (300 MHz) and NOE-difference spectra of **149c** showing the relative configurations of C-4 and C-5 substituents. The insert in spectrum (a) shows the NOEs observed in the 400 MHz spectrum.

Supporting the *cis* configuration of 4-Me and H-5 substituents was the relatively strong NOE induced in the 4-Me signal and weaker NOE observed for H-4 following irradiation of H-5 (δ 5.85) (Figure 4.15(c)). An NOE enhancement was also observed across the tetrahydrofuran ring at the H-3_a proton (the reciprocal NOE is observed in difference spectrum, Figure 4.15(a)). Table 4.2 summarises the relative magnitude of NOE enhancements observed for **149c**. Internuclear distances calculated from the MM2*-minimised structure are also given for comparison with the NOE intensities.

Table 4.2. Observed NOEs and MM2*-calculated internuclear distances for acetate **149c**.

Irradiated proton	Observed NOEs (relative intensity[1]/internuclear distance (Å)[2])
4-Me	H-4 (s, 2.4), H-3_a (m, 2.5), H-5 (s, 2.3)
8'-Me_{ax}	8'-Me_{eq} (s, 2.3), H-5 (w, 2.9)
2'-Me[3]	H-3' (s, 2.4), H-3_a (s, 2.3), 4-Me (w, 4.3), 8'-Me_{ax} (w, 3.0)
8'-Me_{eq}	8'-Me_{ax} (s, 2.3), H-5' (s, 2.4)
H-4	4-Me (s, 2.4), H-3_b (m, 2.4), H-5 (m, 3.1)
H-3_a	H-3_b (s, 1.9), 4-Me (m, 2.4), H-5 (w, 3.1)
H-3_b	H-3_a (s, 1.9), H-4 (m, 2.4)
H-5'	8'-Me_{eq} (s, 2.4), 8'-Me_{ax} (m, 2.9)
H-5	4-Me (s, 2.3), H-4 (m, 3.1), H-3_a (m, 3.1)
H-3'	2'-Me (s, 2.4), 8'-Me_{ax} (w, 3.0)

[1] Relative intensity of NOEs are assigned s = strong, m = medium and w = weak.

[2] Internuclear distances were calculated from the MM2*-minimised structure using MacroModel.

[3] NOE-difference experiment was carried out on a degassed sample.

4.2.4. Crystal Structure of Acetate 149c

Crystallisation of compound **149c** was induced during the course of the NMR investigation. Colourless plates suitable for a single-crystal X-ray diffraction study were obtained from hexane:chloroform (9:1) following slow cooling.

A perspective and stereo-drawing of the solved structure is presented in Figure 4.16. Crystallographic data including bond distances and bond lengths involving non-hydrogen atoms are tabulated in Appendix One. The structure and relative stereochemistry of the X-ray structure were identical to the NMR-derived structure, with O-1 of the tetrahydrofuran ring lying *trans* to O-4' of the enone system. Both the acetoxy and the methyl substituents of the tetrahydrofuran ring were *trans* with respect to each other as determined by NOE experiments. The conformation of the molecule, including that of the acetoxy substituent, observed for both the X-ray crystallographic and MM2*-calculated structures were identical.

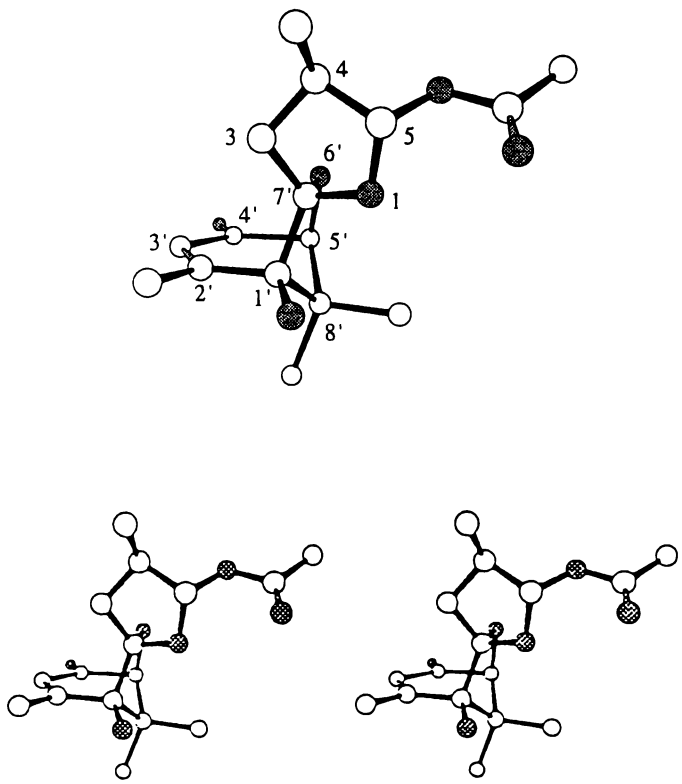


Figure 4.16. X-ray crystallographic structure of acetate **149c** showing IUPAC numbering. The structures below enable a stereo view of the ring system.

Several structural features of the kamahine molecule were apparent. The cyclohexenone ring exhibited a half-chair conformation resulting from the planarity of the enone system, whilst bonds to the carbon attached to the geminal methyl groups are twisted downwards 60° to the plane of the ring. The five-membered tetrahydrofuran ring was slightly non-planar, with a small degree of puckering (35°). This type of puckering is well documented for cyclopentane and its

heterocyclic analogues (e.g. Elliel, 1962). Two of the most commonly encountered puckered shapes, the envelope and half-chair, result in substantial decrease in ecplising strain between adjacent substituents. The spirotetrahydrofuran ring of the kamahine ring system adopts the envelope form.

Acetate **149c** crystallised in an asymmetric space group ($P2_12_12_1$). However it was not possible to determine the absolute configuration from the crystallographic data. Since the absolute configurations of the chiral centres were not determined, the set of configurations shown (Figure 4.16) for these centres is arbitrary. Determination of the absolute configurations of the parent kamahines **25a-25c** is the subject of Chapter Five of this thesis.

4.2.5. Characterisation of Acetates 149a and 149b

Acetylation of kamahine afforded two fractions following PLC. One fraction contained acetate **149c**, whilst the less polar fraction contained a 2:1 mixture of acetates **149a** and **149b**. The ^1H and ^{13}C NMR signals observed for the mixture of acetates were indicative of the tricyclic ring system established for **149c**. Multiple-elution PLC allowed separation of small quantities of acetates **149a** and **149b** which facilitated assignment of the ^1H and ^{13}C spectra. The ^1H and ^{13}C NMR data for **149a** and **149b** are documented in the Experimental Section of this Chapter. Spectra were assigned by techniques similar to those described for **149c**.

The relative stereochemistry of **149c** was successfully determined by the application of NOE-difference experiments. The stereochemistries of **149a** and **149b** were predicted by correlation of both the NOE and coupling constant data with that from the crystallographically-solved kamahine **149c**. The results of NOE-difference experiments showed that the relative configurations of the cyclohexenone system and spirotetrahydrofuran rings of all three diastereoisomers were the same, and that the diastereoisomerism arose from epimerisation of C-4 and C-5 in the spirotetrahydrofuran ring. Thus, for both **149a** and **149b**, irradiation of the methyl substituent from the enone functionality (2'-Me) induced an enhancement in H-3_a from the spirotetrahydrofuran ring. That result indicated that O-1 and O-4' were *trans* with respect to each other, as had been established for **149c**. Figure 4.17 shows the relevant NOE-difference spectrum obtained from the mixture of **149a** and **149b**.

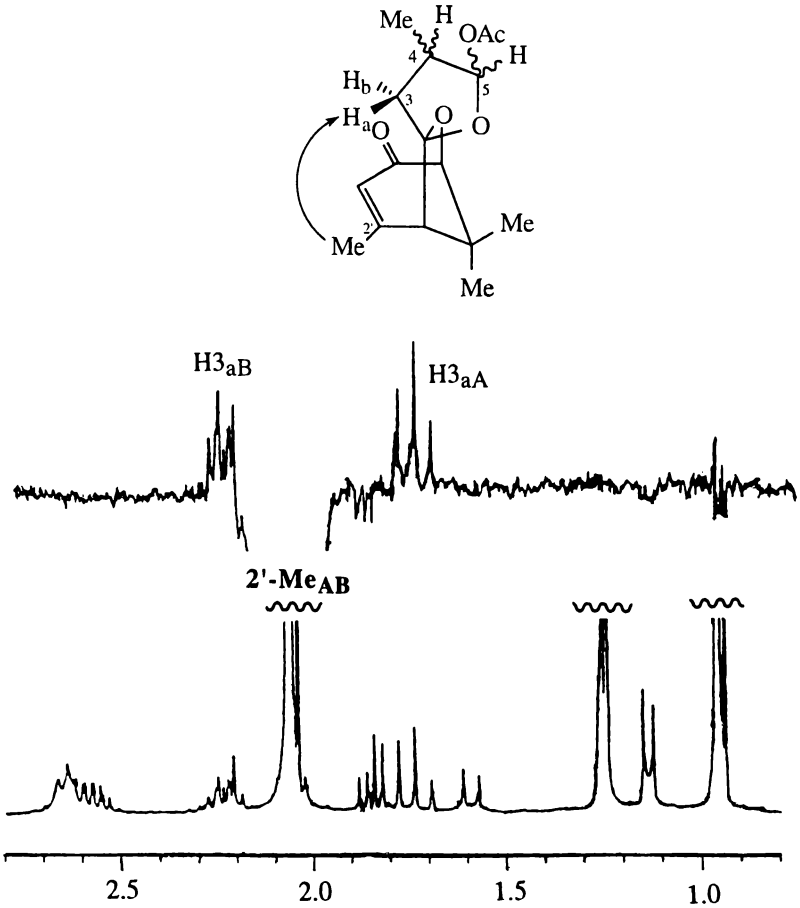


Figure 4.17. NOE-difference spectrum of the acetate mixture **149a** and **149b** indicating the orientation of the spirotetrahydrofuran ring relative to the enone system.

The NOE enhancements which determined the relative configurations of the C-4 and C-5 substituents of **149a** are shown in Figure 4.18. Irradiation of 4-Me (δ 0.98) (Figure 4.18(a)) gave a strong NOE at δ 2.59 (H-4) and a relatively strong enhancement at δ 1.80 (H-3_a), indicating a *cis* relationship between 4-Me and H-3_a substituents (due to overlap of the 4-Me and 8'-Me_{ax} signals, NOEs were also observed for protons in close proximity to 8'-Me_{ax}). In comparison, saturation of the same methyl group in **149c** (*cf.* Figure 4.15(b)), resulted in similar relative enhancements for H-4 and H-3_a signals. That result was consistent with the *cis* orientation of 4-Me and H-3_a in **149c**.

A *cis* relationship between 4-Me and the C-5 acetate group was established for **149a**, demonstrating that kamahines **149a** and **149c** were epimeric at C-5. Irradiation of H-4 of **149a** induced a moderate NOE at the H-3_b proton (δ 1.60) (Figure 4.18(b)), confirming that these two substituents were *cis*, an identical configuration to that established for **149c**.

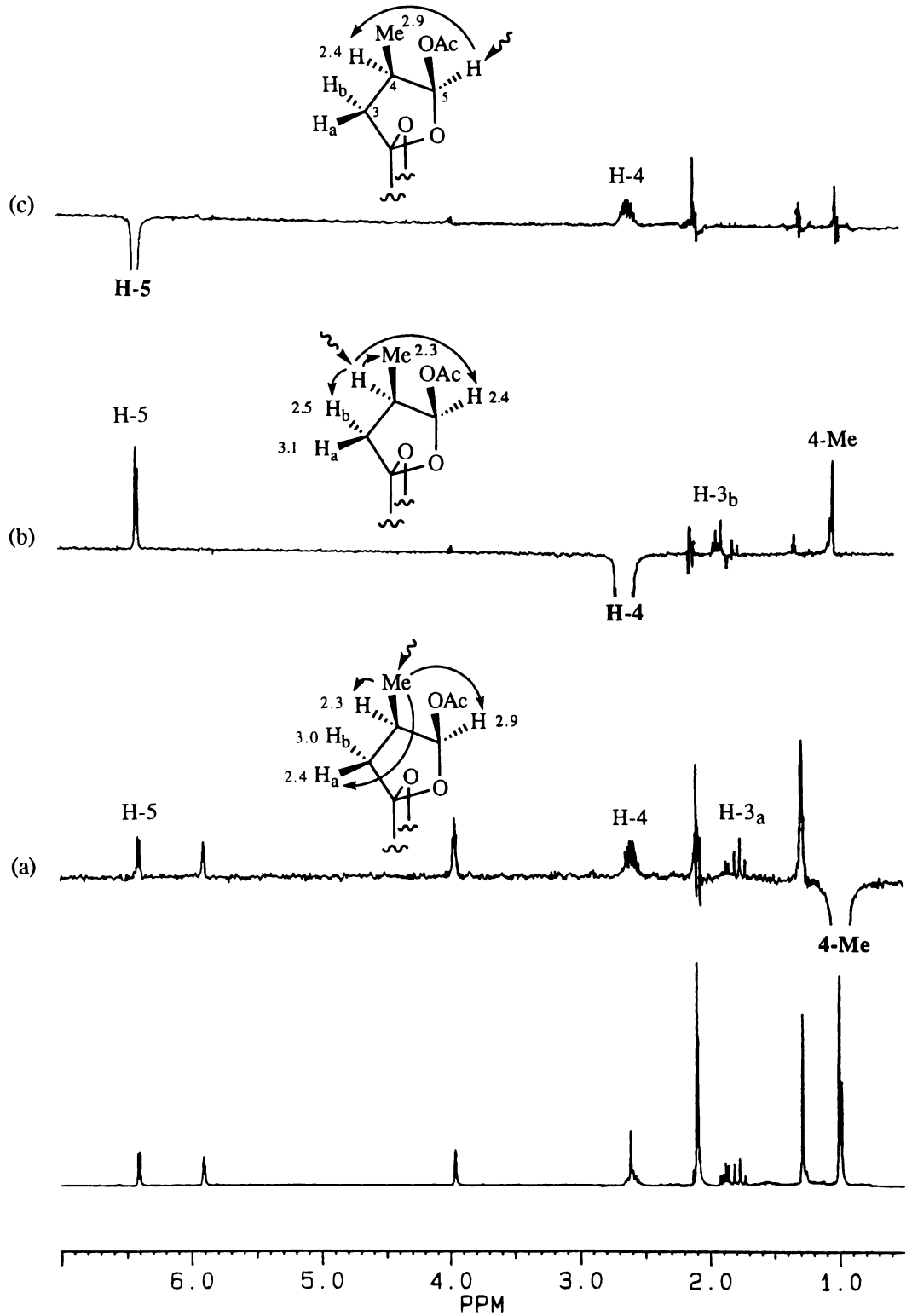


Figure 4.18. ^{1}H NMR spectrum and NOE-difference spectra of **149a** showing the relative configurations of the spirotetrahydrofuran substituents. NOEs and MM2*-calculated internuclear distances from the irradiated proton are depicted on the structures.

Difference spectrum (Figure 4.18(b)) also indicated the H-4 was in close proximity to H-5. The reciprocal NOE spectrum supported this configuration of substituents; saturation of the H-5 signal (δ 6.39) resulted in a strong enhancement at H-4 (Figure 4.18(c)). These NOEs were consistent with the MM2*-calculated internuclear distances for this diastereoisomer.

The stereochemical relationship between C-3 and C-4 substituents of acetate **149b** were not easily deduced from NOE-difference experiments due to overlap of the H-4 and H-3_a signals. Irradiation of 4-Me showed a relatively strong enhancement for the multiplet at δ 2.25 attributed to both the H-4 and H-3_a resonances, and a moderate enhancement for the doublet signal attributed to H-3_b (Figure 4.19(a)). The former enhancement was assumed to arise principally from the geminal proton H-4, expected to be in closer proximity to the methyl group (Table 4.3). The relative enhancements of H-4(/H-3_a) and H-3_b resonances observed in this difference spectrum, therefore, implied that H-3_b was closest to 4-Me. By comparison, irradiation of 4-Me from **149c** (*cf.* Figure 4.15(b)) showed enhancements of similar intensities for H-4 and H-3_a, consistent with H-3_a being closest to 4-Me. Acetates **149b** and **149c** were therefore epimeric at C-4.

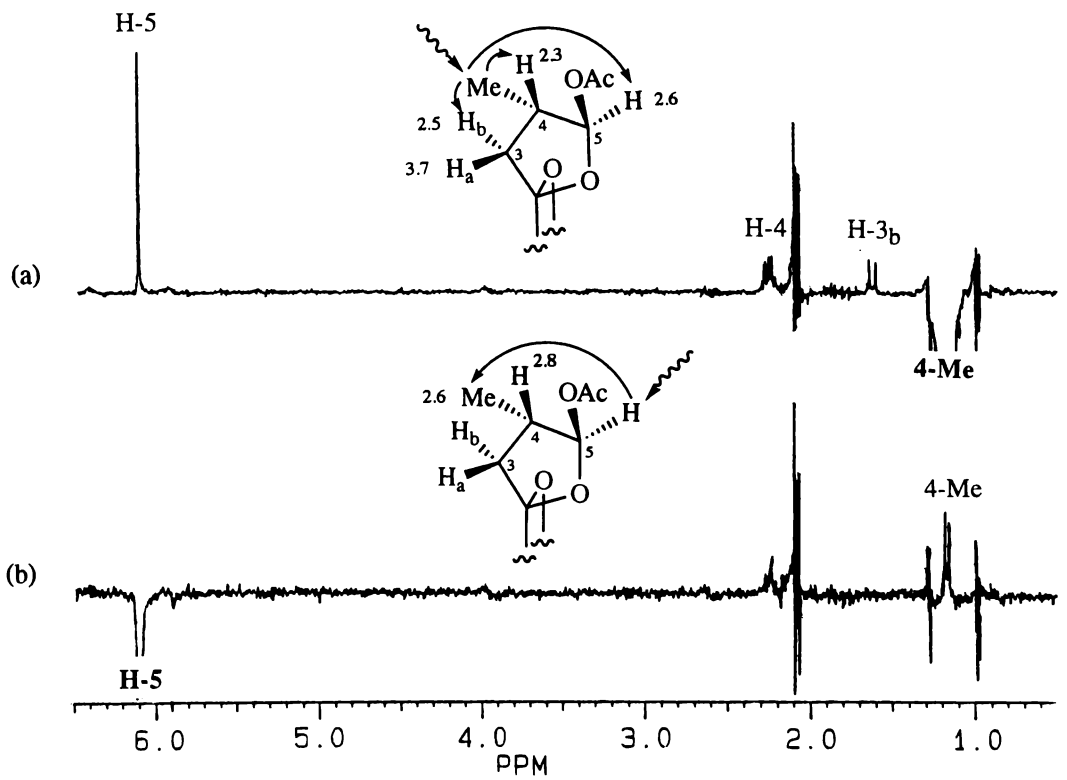


Figure 4.19. NOE-difference spectra of acetate **149b** demonstrating the relative configurations at C-4 and C-5.

Table 4.3. Internuclear distances and NOE predictions for acetate **149b**.

Proton Pair	Internuclear Distance[1] (Å)	Predicted NOE
(4-Me - H-4)	2.35	s
(4-Me - H-3_a)	3.71	w/nill
(4-Me - H-3_b)	2.57	s/m
(4-Me - H-5)	2.60	s/m

[1] Calculated from the MM2*-minimised structure and MacroModel.

s = strong, m = medium, w = weak.

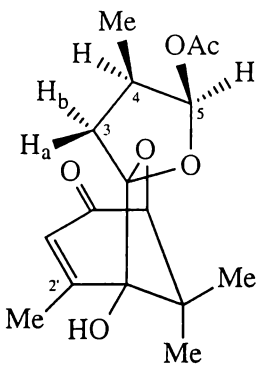
A *trans* relationship between the methyl and acetate groups was established for **149b**. Saturation of the H-5 signal resulted in a strong enhancement for 4-Me and a weak NOE for H-4 (Figure 4.19(b)). This NOE-difference spectrum was similar to that observed for **149c**, which also exhibited a *trans* relationship between these substituents. Given that the spirotetrahydrofuran ring orientation was identical in all isomers, the configurations at C-4 and C-5 of **149b** were therefore epimeric with **149c**.

The magnitude of spin-spin coupling constants for the methylene, methine and anomeric protons of the spirotetrahydrofuran ring confirmed the relative configurations of **149a-149c** determined by NOE experiments. The MM2*-calculated structures were used for the analysis of the relationship of torsion angles between vicinal H atoms and ^3J values on the spirotetrahydrofuran ring, using the NMR module of MacroModel. This software bases calculations on the Karplus relationship (Karplus, 1963) and the parameters derived by Haasnoot *et al.* (1980). The observed versus calculated coupling constants for acetates **149a-149c** are reported in Table 4.4. The predicted dihedral angles based on the relative configurations determined by NOE experiments gave coupling constants comparable (within 1.2 Hz) to those calculated from high-resolution ^{1}H NMR spectra. The relative configurations of the three diastereoisomers are depicted in Figure 4.20.

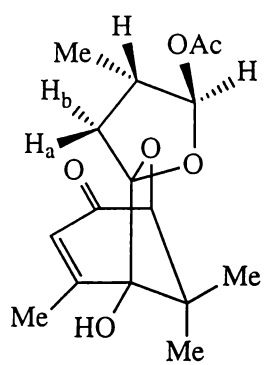
Table 4.4. Observed versus calculated coupling constants for acetates **149a-149c.**

Isomer	Dihedral angle (ø)		Calculated[1] coupling constant (Hz)	Observed coupling constant (Hz)
149a	(3_a-4)	-166.3	11.8	12.6
	(3_b-4)	-44.4	5.7	6.7
	(4-5)	41.8	4.2	4.8
149b	(3_a-4)	-33.7	7.2	7.5
	(3_b-4)	87.0	0.9	0.0
	(4-5)	-87.6	1.0	0.0
149c	(3_a-4)	-162.6	11.5	9.9
	(3_b-4)	-40.1	6.4	7.3
	(4-5)	142.4	6.0	4.8

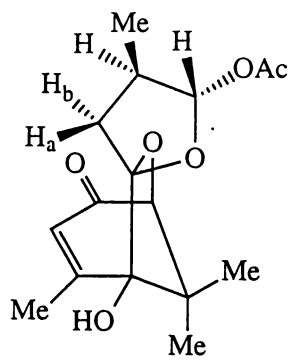
[1] Coupling constants were calculated using MM2*-minimised structures and the NMR module of MacroModel.



(149a)



(149b)



(149c)

Figure 4.20. Relative configurations of kamahine acetates **149a-149c.**

4.2.6. Kamahines 25a-25c

Kamahines **25a-25c** were obtained from kamahi and rewarewa honeys as an inseparable mixture. Although manipulation of the temperature programming during GCMS analysis indicated some separation of the three diastereoisomers,

their separation on a preparative scale was difficult. Both multiple-elution PLC and preparative HPLC failed to yield isomerically pure samples of the kamahines. During the course of the investigation it became apparent that two of the kamahines were interconverting, further complicating the separation process.

The relationship of acetates **149a-149c** to the parent hemiacetals **25a-25c** appeared to be evident upon examination of the ^1H NMR spectra. Figure 4.21 demonstrates that the relative chemical shifts and scalar coupling of the anomeric protons (H-5) allowed direct comparison of each of the diastereoisomeric hemiacetals **25a-25c** with an acetate (or acetal) derivative **149a-149c**. The hemiacetal resonances were slightly broadened due to geminal coupling to the hydroxyl group.

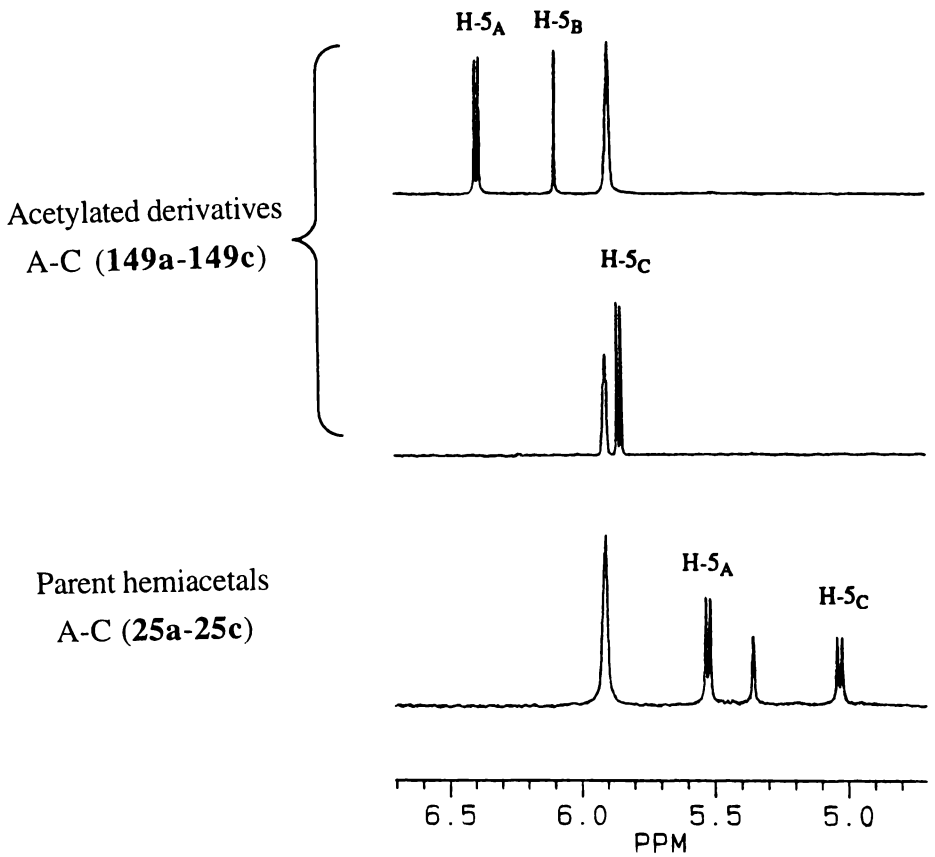


Figure 4.21. Correlation of acetal protons from **149a-149c** with hemiacetal protons of parent compounds **25a-25c**.

The above comparisons were based on the assumption that acetylation of the cyclic hemiacetal moiety does not bring about changes in the dihedral angles of protons from the spirotetrahydrofuran ring. Whilst in the majority of cases this assumption is probably valid, it was decided to investigate the effect of acetylation

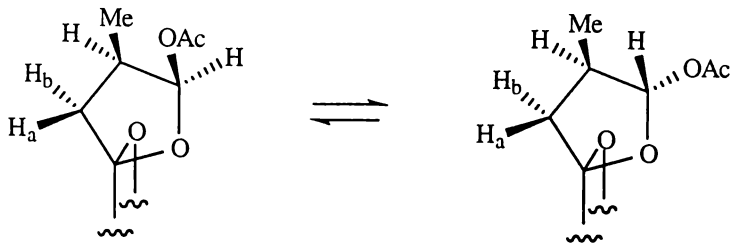
on the scalar coupling patterns of the parent hemiacetals. Two alternative experiments were proposed for this purpose, namely, careful deacetylation of a single acetyl derivative, or acetylation of a single parent isomer.

It was anticipated that conventional means of ester hydrolysis using base or acid may result in opening of the cyclic acetal and consequently loss of the spirotetrahydrofuran ring system. This was observed experimentally. TLC showed loss of starting material when acetate **149c** was treated with NaOH/MeOH at elevated and RT. Use of the relatively weak base pyrollidine at RT afforded the same result.

The alternative method involved separation of a single hemiacetal isomer and its subsequent acetylation. An isomerically pure sample (*ca* 1 mg) of one of the diastereoisomers was obtained by multiple-elution PLC, following dissection of the kamahine band from the PLC plate. The other two diastereoisomers were the predominant components of the remainder of the PLC band and could not be separated by this means. ^{1}H NMR indicated that the single diastereoisomer exhibited a broad singlet (δ 5.35) attributed to the hemiacetal methine.

Microscale acetylation of the isomerically pure kamahine was monitored by ^{1}H NMR. Acetylation of the hemiacetal group resulted in the disappearance of the broad singlet at δ 5.35 and the appearance of a sharp singlet at δ 6.09, the acetal methine attributed to acetate **149b**. This result indicated that there was no observable change in coupling constant between protons H-4 and H-5 upon acetylation, and that direct correlation between the structures of acetates **149a-149c** and the parent hemiacetals **25a-25c** was justified.

Additional evidence supporting the direct correspondance of acetates **149a-149c** with hemiacetals **25a-25c** was the observation that the ratio of hemiacetals **25a**:**25c** in the mixture decreased over time, whilst the amount of hemiacetal **25b** appeared to remain constant. Interconversion of **25a** and **25c** appeared to reach equilibration after 24 h. A sample of acetate **149a**, when dissolved in $CDCl_3$, gradually epimerised to acetate **149c**; in d_6-acetone, this epimerisation was much slower. Presumably the $CDCl_3$ was sufficiently acidic to catalyse the epimerisation.



(149a) **(149c)**

Figure 4.22. Equilibration of **149a** and **149c**.

The epimerisation of kamahine **25a** to **25c** suggested that **25c** was the more stable diastereoisomer. MM2* calculations were consistent with that proposal. Comparison of the relative energies of **25a/25c** and the corresponding acetates **149a/149c** indicated **25c** and **149c** were the most stable diastereoisomers.

It was apparent that the relative stabilities of **25a** and **25c** could not be rationalised in terms of the anomeric effect. Substituents at the anomeric centres of **25a/25c** are quasi-axial/equatorial according to the definition of substituent positions proposed by Anet (1990). That relationship of substituent positions is well documented for five-membered rings (Kirby, 1983). The anomeric effect is described as a preference for the electronegative substituent of the anomeric centre to lie axial and is explained in terms of a preferred orientation of localised non-bonding electron pairs. As the electronegative substituent at the anomeric centre of tetrahydrofurans is in the same relationship to the lone pair on the ring oxygen whether in the quasi-axial or quasi-equatorial position, the conformational consequences of the anomeric effect are much less dramatic.

^{1}H and ^{13}C NMR data of **25a-25c** were obtained from the mixture. The resolution-enhanced ^{1}H spectrum gave sufficient spectral dispersion of most signals for coupling constant assignment. Some corroborative evidence for the assignments was gained from the ^{1}H NMR spectrum of the sample of isomerically pure **25b**. Off-diagonal connectivities from the anomeric protons of **25a** and **25c**, shown in the expanded region of the DQFCOSY spectrum (Figure 4.23), identified a sequence of connectivities identifying the respective C-4 and C-3 protons. The remaining set of connectivities identified the corresponding proton resonances from **25b**. Methylene protons ($H\text{-}3_a/H\text{-}3_b$) from each diastereoisomer were distinguished by their scalar couplings (multiplicity and coupling constants).

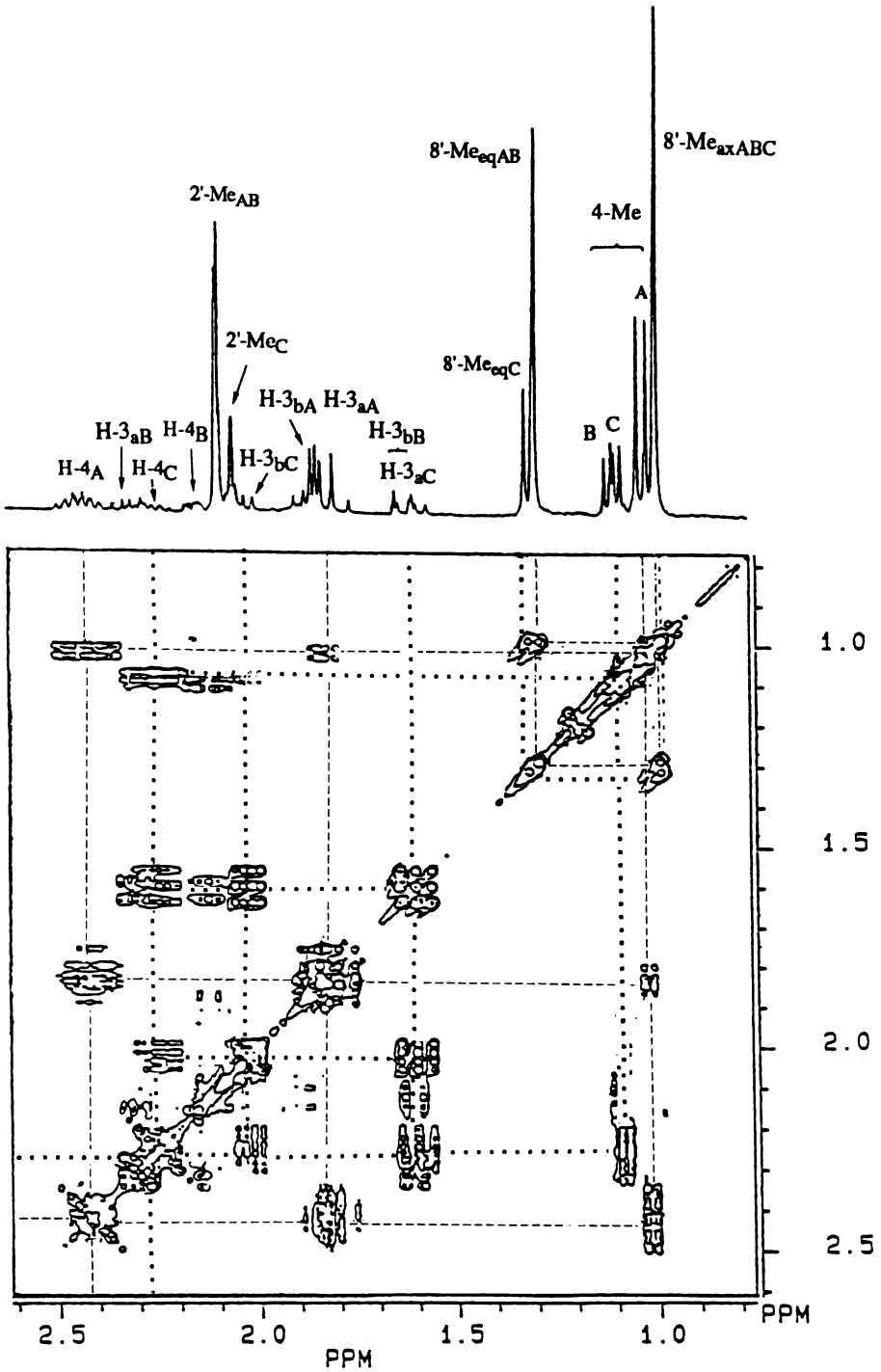


Figure 4.23. DQFCOSY spectrum of the mixture **25a**-**25c** in the region δ 0.80–2.60 ppm. Connectivities of **25a** (----) and **25c** (····) are shown.

Resonances from the cyclohexenone substituents (H-3', 8'-$Me_{ax/eq}$, 2'-Me, H-5') form an independent spin system in the kamahine structure. The olefinic proton (H-3') and 8-Me_{ax} of all three diastereoisomers exhibited the same chemical shift. Two sets of resonances were observed for 8'-Me_{eq} (δ 1.30 and 1.31), 2'-Me (δ 2.04 and 2.09) and H-5' (δ 3.95 and 3.97). The equilibration of isomers **25a** and **25c** in $CDCl_3$, which brought about changes in relative peak heights of ^1H NMR resonances was diagnostic in assigning those resonances. Interconversion of **25a** and **25c** resulted in increases in the relative peak heights of **25c** protons, including the set of distinct signals at δ 1.31, 2.04, and 3.97, identifying those signals as arising from **25c**. The different chemical shifts exhibited by cyclohexenone substituents 8'-Me_{eq}, 2'-Me and H-5' of **25c** relative to those of **25a** and **25b** was attributable to differences in the positions occupied by the acetate group (the relative configurations of the acetate group of **25c** and **25a/25b** are epimeric).

A 2D ^{13}C-^1H correlation spectrum of the mixture **25a-25c** and assignment of the protonated ^{13}C chemical shifts of **25a** and **25c** is shown in Figure 4.24. Singlet carbons were assigned on the basis of chemical shift. C-7' and C-1' of each isomer **25a-25c** exhibited different chemical shifts. These were assigned by comparison of their relative intensities, which were established in the ^1H spectrum (the ratio of diastereoisomers in $CDCl_3$ solution appeared to equilibrate after 24 h).

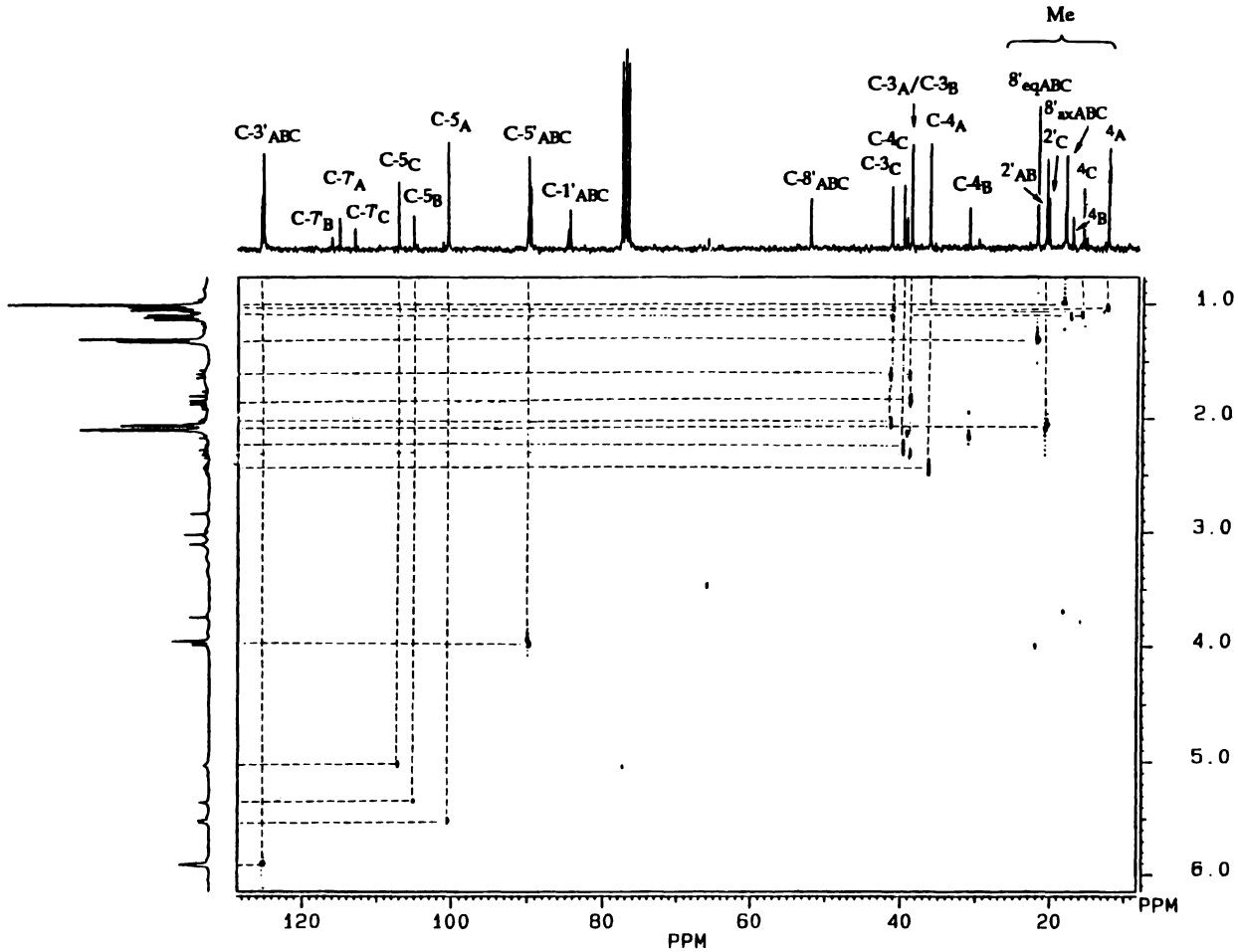
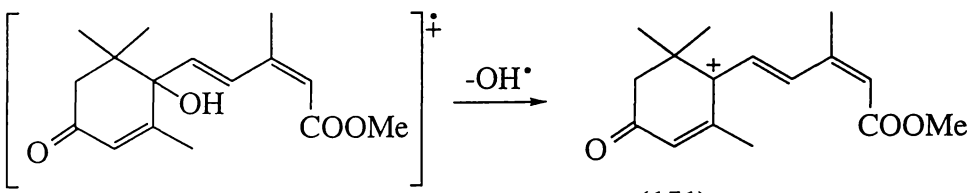


Figure 4.24. XHCORR spectrum of the mixture **25a-25c** showing assignment of the ^{13}C NMR spectrum. Correlations of **25a** and **25c** are shown.

4.2.7. Mass Spectral Interpretation

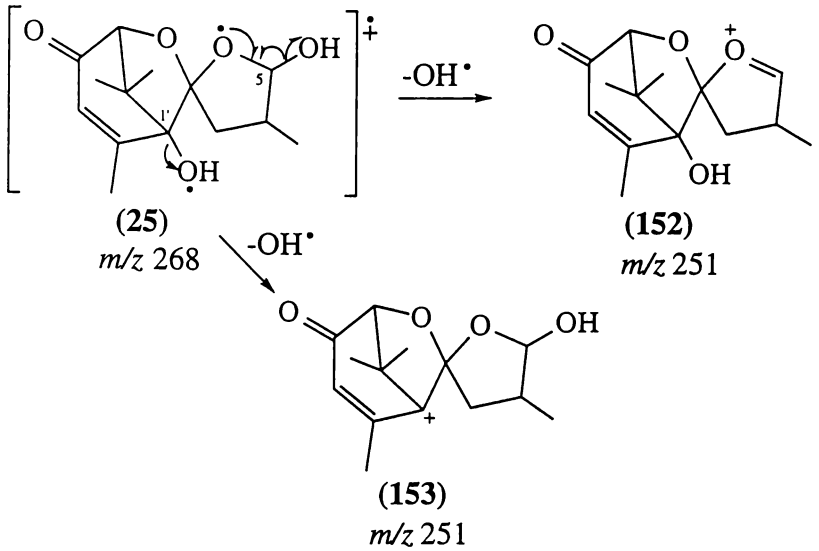
A molecular ion (*m/z* 268) was not observed for kamahine (**25**) in the EIMS; however CIMS showed the appropriate quasi-molecular ion (M+H) at *m/z* 269. The highest mass ion observed by EIMS was a weak ion at *m/z* 251. This was attributed to loss of an hydroxyl (OH) radical. Although elimination of a H_2O molecule is commonly observed for alcohols, especially when a suitable donor site is available, ejection of an OH radical may occur in instances where steric constraints dictate, or when the cleavage leads to a particularly stable fragment (Rose and Johnstone, 1982). The mass spectrum of methyl abscisate (**150**) (Tan, 1989), for example, consists of a weak *m/z* 261 ion consistent with loss of an OH radical. This gives rise to the extensively conjugated allylic cation **151** (Figure 4.25)



(150) *m/z* 278 **(151)** *m/z* 261

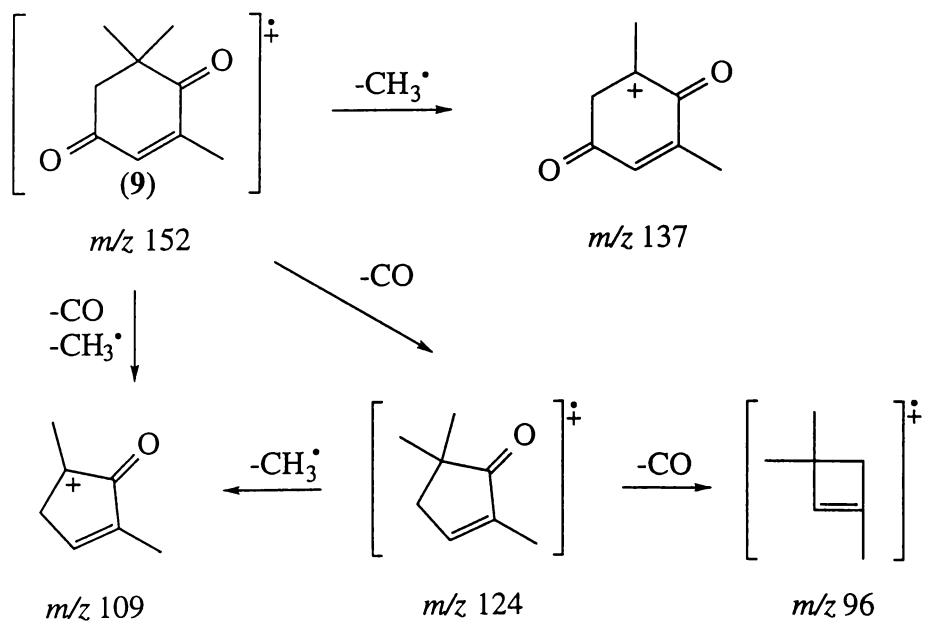
Figure 4.25. Loss of an hydroxyl radical from methyl abscisate (**150**).

Two pathways could account for loss of an OH radical from kamahine (**25**), both of which afford cations stabilised by oxygen or conjugation (Scheme 4.1). Loss of an OH radical from the hemiacetal functionality (C-5) gives the stable oxonium ion **152**. The *m/z* 251 ion observed in the mass spectra of the acetate derivatives could be attributed to the same oxonium ion, through loss of 59 (CH_3COO) from the molecular ion (*m/z* 310). Alternatively, the highly conjugated cation **153** results from extrusion of an OH radical from the tertiary position (C-1').



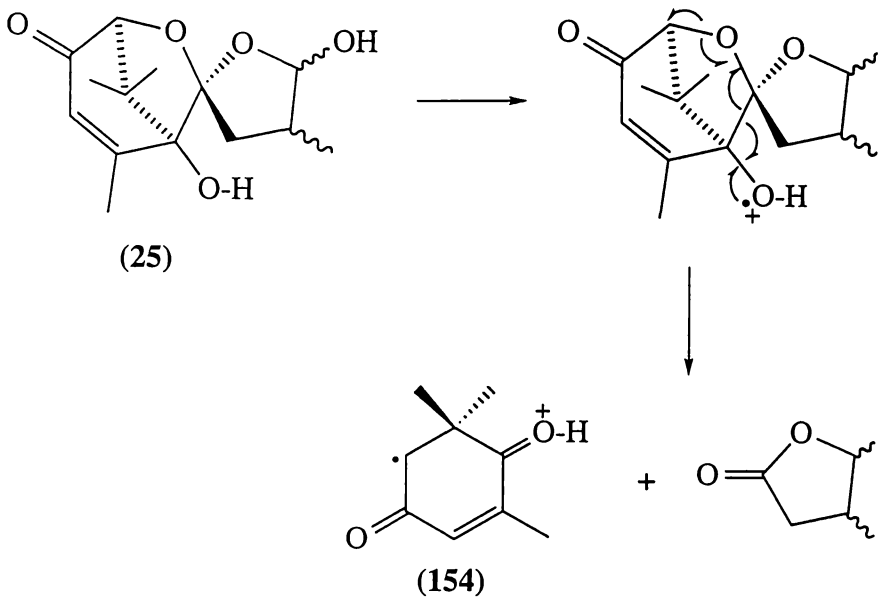
Scheme 4.1. Loss of an OH radical from kamahine (**25**) to give the *m/z* 251 ion. Both fragmentations are depicted on the kamahine ring system.

The EIMS of kamahine consisted of a base peak at *m/z* 152 and significant ions at *m/z* 137, 124 and 109. Similar fragments are observed in the EIMS of 3,5,5-trimethylcyclohex-2-en-1,4-dione (**9**), which exhibits weak ions of *m/z* 152 ($M^{+\cdot}$), 137 ($M^{+\cdot}$ - Me), 124 ($M^{+\cdot}$ - CO), 109 ($M^{+\cdot}$ - 43), a significant ion at *m/z* 96 ($M^{+\cdot}$ - 2 x CO). Scheme 4.2 depicts the possible fragmentation pathway to these ions. Initially, the *m/z* 152 ion observed in the EIMS of kamahine was attributed to formation of the dione fragment $[\mathbf{9}]^{+\cdot}$.



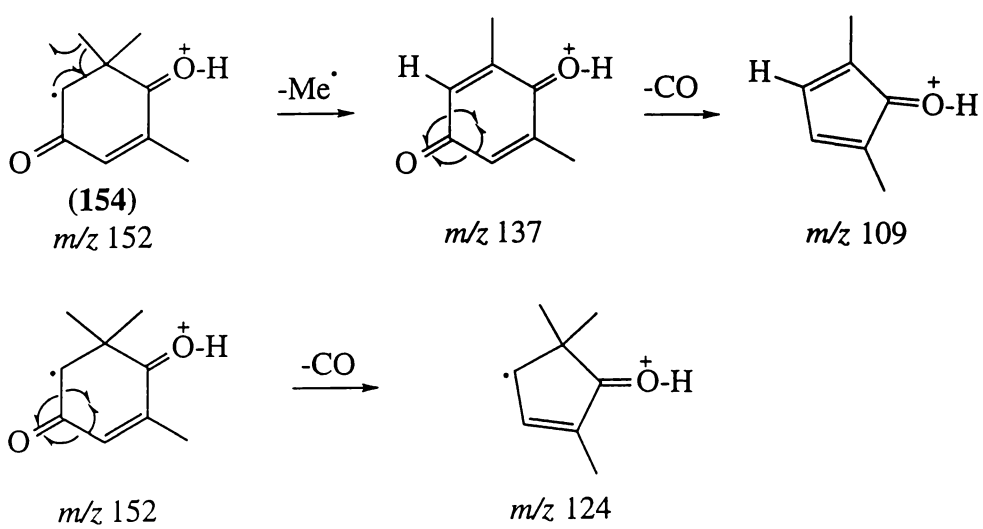
Scheme 4.2. Proposed mass spectral fragmentation of 3,5,5-trimethylcyclohex-2-en-1,4-dione (**9**).

Following elucidation of the structure of kamahine, the *m/z* 152 ion was attributed to formation of radical **154** (Scheme 4.3), rather than the dione fragment $[\mathbf{9}]^{+\cdot}$ (Scheme 4.2). Upon loss of an electron from the tertiary hydroxyl group, fragmentation of the kamahine ring system could occur *via* the events shown in Scheme 4.3, giving rise to the highly stable oxonium ion.



Scheme 4.3. Proposed fragmentation of kamahine (**25**) giving rise to the *m/z* 152 ion.

Loss of a methyl radical followed by CO from the *m/z* 152 fragment would give rise to fragments at *m/z* 137 and 109, respectively. Fragments of *m/z* 124 and 96 arise from *m/z* 152 fragment by sequential loss of two CO molecules. Mass fragmentation pathways of **25** are proposed in Scheme 4.4.



Scheme 4.4. Proposed mass fragmentation pathways of kamahine (**25**).

The occurrence of 3,5,5-trimethylcyclohex-2-en-1,4-dione (**9**) in the extractives of kamahi honey (Tan 1989) could be explained by thermal decomposition of kamahine (**25**). GCMS analysis of the mixture of acetates **149a/149b** gave rise to an early-eluting peak identified as dione **9**. The ratio of diketone to acetate changed upon re-analysis. This observation supports a recent suggestion that **9** is a thermal artifact, produced by splitless injection to the GC column (Rowland *et al.*, 1995). Rowland *et al.* (1995) suggested that 3,5,5-trimethylcyclohex-2-en-one derivatives in the honey extractives were likely sources of **9**.

4.2.8. Kamahi Plant Extraction

The extractable organic constituents of honeys are believed to be derived principally from the floral extractives. This is particularly true of marker compounds, as it is doubtful their uniqueness could be due to differences between hives (i.e. bee physiology), or in processing practice.

Investigation of kamahi plant material was carried out as part of the present study. Selected-ion GCMS failed to identify kamahine, or its likely precursors, in the chloroform extractives of leaves and flowers of that plant. The profile of extractives appeared to contain a complex mixture of high molecular weight components which were probably triterpenes, in addition to fatty acids and hydrocarbons, although none of the components was positively identified. As this investigation was designed to identify the component of interest, further evaluation of the extractives was not pursued. Nectar samples were not analysed due to difficulties in obtaining sufficient quantities of flowers for extraction.

Preliminary investigations of this nature may not give a good indication of the plant's role in determining the extractive profiles of unifloral honeys. The same technique was used by Tan *et al.* (1988; 1989a) who found little relationship between the extractable organic components of manuka flowers and that of the resulting honey. Simple soaking of the flowers may be inappropriate; this method appears to favour extraction of the major substances from the flower, such as triterpenes and components of plant waxes, which may obscure trace components present in the nectar. Quantities of nectar are often as small as 1μL per flower (Erickson *et al.*, 1979). Simple solvent extraction of a small quantity of nectar, as carried out in a more recent study, gave promising results in terms of identifying potential honey components (Rowland *et al.*, 1995).

The possibility remains that some components of the nectar may be hydrophilic, and therefore are not extracted by the methods employed. Glycosidic precursors of volatile monoterpenes and C-13 *nor*-isoprenoids are well known constituents of natural products (Chapter One). Many carotenoid metabolites are most abundant at, and subsequent to, the time of degradation of the cell wall. However, the isolation of glycosides of C-13 degraded carotenoids from the green leaves suggests that some are also produced in the intact cell (Wahlberg and Enzell, 1987). Hydrophobic organic substances are believed to be transported in glycosidic form within the plant (Enzell, 1985). The biological roles of many of these metabolites is uncertain (Wahlberg and Enzell, 1987).

Glycosidic components are water soluble and often difficult to isolate and separate. These problems have been overcome by procedures which allow selective retention of the highly polar constituents, such as C_{18} reversed-phase (C_{18}RP) adsorbent (e.g. Engel and Tressl, 1983; Winterhalter, 1990) or Amberlite XAD-2 resin (Strauss *et al.*, 1987b). Other methods have been developed for separation of these mixtures, such as droplet countercurrent chromatography (DCCC) (Strauss *et al.*, 1987b). These methods have been successful in elucidating flavour precursors of natural products, such as wine and fruits.

Processing techniques, such as pH adjustment and heating may influence flavour by hydrolysis of glycosides to flavour compounds (monoterpenes and *nor*-isoprenoids) (Wilson *et al.*, 1984). The pH of honeys, a factor often controlled by the bee's physiology (Maga, 1983), and heating during post-harvest processing, may affect the profile of extractives obtained from a honey in a similar manner.

4.2.9. Biological Activity

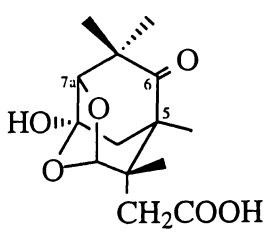
As a large number of organic compounds are synthesised by various plants, many exhibiting marked pharmacological activity (White, 1975), it is not surprising that occasionally such materials are found in honey. The antibacterial effects of honey have been long recognised. Although the activity attributed to the hydrogen peroxide content is well documented (White 1975), there are reports indicating additional activity, thought to arise from biologically active substances derived from the plant source (White, 1975 and cited references; Tóth, 1987). In most of these studies, organic extractives were analysed for activity as a whole and individual substances contributing to the activity were not identified.

The relationship of biological activity to the occurrence of certain extractables in New Zealand honeys has not been established with any certainty, although Tan (1989) indicated a significant proportion of the activity appears to be retained in the aqueous phase, possibly as glycosides. Comprehensive research of the volatile organic and non-volatile components of honey, and their biological activities would be beneficial as knowledge of their relationship remains obscure at this stage.

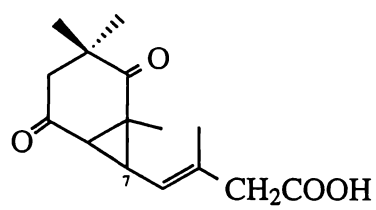
As kamahine was a natural product possessing a new ring system, investigation of its biological properties was indicated. Kamahine showed no discernable antimicrobial, antiviral or cytotoxic effects in the series of assays commonly carried out to assess biological activity (Experimental Section).

4.2.10. Other Degraded Carotenoids from New Zealand Honeys

Since the completion of this work, another novel component from kamahi honey has been isolated and characterised. Details of the structural elucidation of the methyl ester of meliracemoic acid are reported by Ede *et al.* (1993). A combination of heteronucleur NMR and NOE-difference experiments showed that meliracemoic acid possessed a substituted 2,5-methano-hexahydro-1,3-benzodioxalane skeleton (**26**). Both **25** and **26** possess C-14 skeletons and may be described as *nor*-sesquiterepenoids. More recently, ericinic acid (**27**) and its C-7 epimer, isoericinic acid, have been identified as constituents of the extractives of New Zealand erica honey (Hyink, 1998).



(**26**)



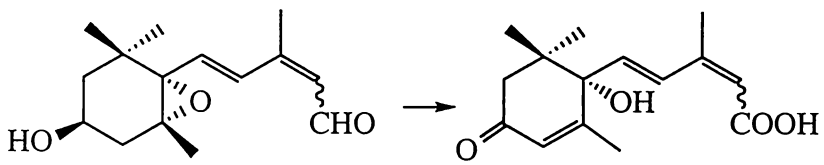
(**27**)

4.2.11. Biosynthetic Origin of Kamahine

The mechanisms giving rise to kamahine are not immediately obvious, as intermediates which could corroborate a proposed pathway have not been identified

in the honey extracts. A C-15 3,5,5-trimethyl-4-hydroxy-cyclohexen-2-en-one precursor is likely. This precursor may be derived from ABA, or a xanthophyll carotenoid which has undergone photochemical or enzymatic degradation.

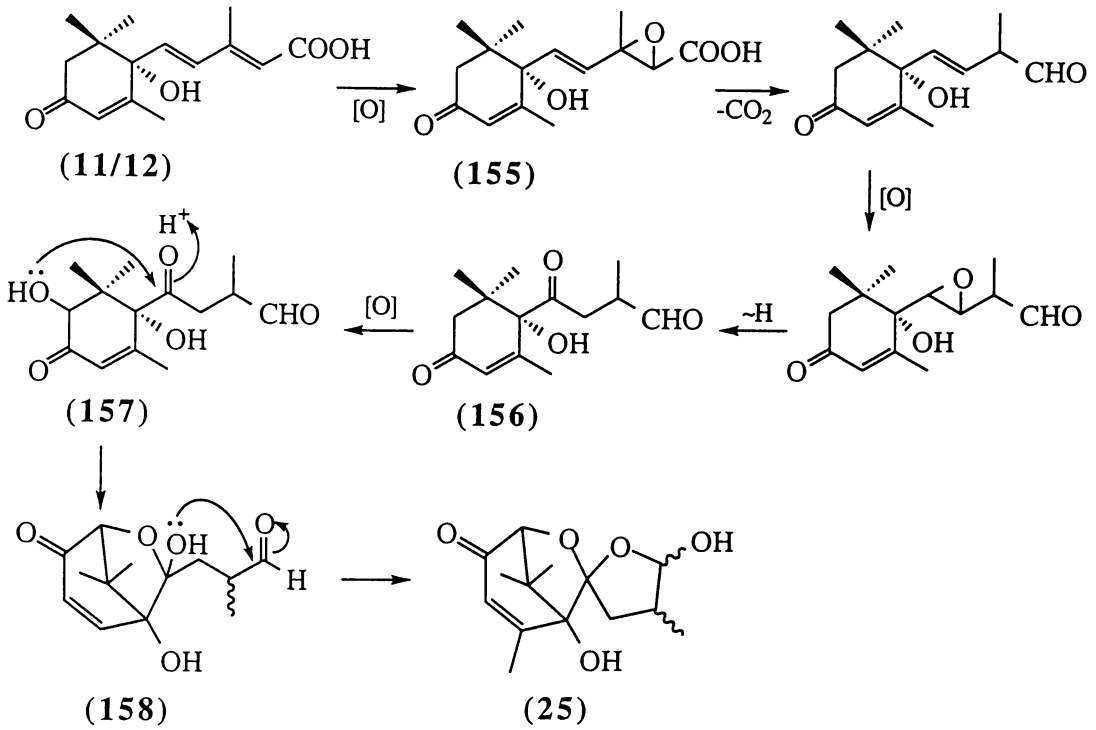
The C-15 degraded carotenoid *cis/trans*-xanthoxin (**124**) occurs naturally in a variety of plants (Morgan and Morgan, 1974), and may be formed by *via* photolysis (Taylor and Burden, 1970) or mild chemical oxidation (Burden and Taylor, 1970) of 3-hydroxy-5,6-epoxy carotenoids such as violaxanthin (**31**) and neoxanthin (**32**). There is increasing evidence that the biosynthesis of ABA (**11/12**) in higher plants is *via* enzymatic cleavage of the above carotenoids, through the intermediacy of **124** (e.g. Sindhu and Walton, 1987; Li and Walton, 1990; Faga, 1995 (cited references)) (Figure 4.26).



cis/trans-(**124**) (**11**)/(**12**)

Figure 4.26. Biosynthesis of ABA in plants *via* degradation of xanthophyll carotenoids.

Conditions within honey, such as the hydrogen peroxide content, pH, enzymatic activity and osmolarity may lead to novel α-hydroxylation and ketalisation reactions. Scheme 4.5 outlines a possible sequence of transformations of ABA (**11/12**) to kamahine **25**. In the proposed mechanism, loss of a carbon atom occurs *via* decarboxylation of epoxide derivative **155**. Generation of the hemiketal functionality requires oxidation of the side-chain double bond to a ketone (**156**), in addition to hydroxylation of the cyclohexyl ring α to the gem-dimethyl and ketone groups (**157**). The cyclic hemiacetal group constituting the spirotetrahydrofuran ring of **25** is formed following nucleophilic attack of the hemiketal hydroxyl group on the aldehyde functionality (**158**).



Scheme 4.5. Proposed transformation of ABA **11/12** to kamahine **25** in honey.

The structural similarities of kamahine (**25**) and meliracemoic acid (**26**) suggest a common precursor. In **26**, the residual side chain has undergone migration from its presumed original location, in addition to rearrangement within the side-chain. The side-chain migration (Wagner-Meerwein rearrangement) occurs in certain hydroxylated megastigmanes upon acid treatment (Stevens *et al.*, 1975; Strauss *et al.*, 1986), and photolysis (Ito *et al.*, 1997). These rearrangement products are found in wine (Sefton *et al.*, 1989). This type of rearrangement is possible in an acidic medium such as honey. Ericinic acid (**27**) has also undergone side-chain migration, although has not lost a carbon atom.

Kamahine and meliracemoic acid are unusual in having been oxidised α to the gem-dimethyl and keto groups (position C-5' in **25**, C-7_a in **26**). Various oxygenated metabolites of ABA have been identified from natural sources (Faga, 1993; 1995); however none feature the oxygenation observed in **25** and **26**. Carotenoids reviews (Britton, 1989; 1991; Faga, 1990; 1994) reveal that few carotenoids, or their derivatives, feature oxygenation at C-2. Hydroxyl substitution at C-2 does occur in carotenoids associated with insects (Davies, 1985); mono-oxygenase enzymes are believed to be the catalysts for such substitutions. These enzymes may be present in bee secretions.

One further consideration in proposing a precursor/product relationship is the aspect of uniqueness. ABA is present in all flowering plants (Rowland *et al.*, 1995), although it is only detected in certain honey types. NZ willow honeys contain relatively high concentrations of this plant hormone (Tan *et al.*, 1990), in contrast to trace concentrations detected in other New Zealand unifloral honeys, such as kamahi (Tan, 1989). Assuming the conditions within the honey, such as pH, H_2O_2 concentration, and enzyme activity, are independent of floral source, the confinement of kamahine to honeys containing a kamahi floral input is difficult to explain if an ABA source is invoked. Kamahine (**25**) and meliracemoic acid (**26**) may result from biosynthetic processes in the plant, rather than oxidative processes within the honey.

4.3. Conclusions

The dominant component in the diethyl ether extractives of New Zealand kamahi and rewarewa honeys was a mixture of three diastereoisomeric compounds **25a-25c** which could not be fully separated by PLC or HPLC. Compound **149c** was one of three diastereoisomeric monoacetyl-kamahines isolated from these extractives following acetylation. A combination of ^1H-^1H and ^1H-^{13}C 2D correlation and NOE-difference experiments led to deduction of the structure of monoacetyl-kamahine C (**149c**). X-ray crystallography corroborated the structure and relative stereochemistry. Acetate **149c** has the new tricyclic skeleton, 4,5-dihydro-1',5-dihydroxy-2'4,8',8'-tetramethylspiro[furan-2*(3)H*,7'[6']-oxabicyclo[3.2.1]oct[2']-en]-4'-one.

NOE-difference experiments indicated that the diastereoisomerism of monoacetyl-kamhines A-C (**149a-149c**) arose from epimerisation at C-4 and C-5 of the spirotetrahydrofuran ring. Monoacetyl-kamahine **149a** was epimeric with **149c** at C-5; these two diastereoisomers interconverted in $CDCl_3$. Monoacetyl-kamahine **149b** was epimeric with **149c** at C-4 and C-5.

The relationship between the acetyl derivatives **149a-149c** and the parent compounds **25a-25c** was established by microscale acetylation of **25b**. The anomeric protons of **149a-149c** and **25a-25c** were diagnostic in that their relative chemical shifts and scalar couplings were identical.

ABA or a 3-hydroxy-5,6-epoxy-β carotenoid are probable sources of the kamahines. Kamahine or its precursors were not found in extractives of the plant. Further research is required to ascertain the origins of extractable organic substances of honey, and to evaluate the transformations they undergo.

4.4. Experimental

4.4.1. Honey Extraction

Honey Samples

Bulk samples of kamahi and rewarewa honeys were obtained directly from the beekeepers. Kamahi honey was collected from the 1989 season and rewarewa honey was collected from the 1992 season. Pollen analysis and GCMS analysis of the Et_2O extractives of the kamahi honey showed it to be a mixture of rewarewa and kamahi floral sources (Wilkins *et al.*, 1995a). Honey samples were stored at 5°C prior to chemical analysis (1991-1992).

Bulk Extraction

Honey (600 g) was dissolved in distilled water (800 ml) and the resultant solution transferred to a liquid/liquid extractor. After 24 h of extraction with Et_2O, the extract was dried over anhydrous $MgSO_4$ and then concentrated to a volume of 5 ml under reduced pressure. The extractives were filtered through a column of $MgSO_4$ prior to chromatographic separation.

4.4.2. Analytical Techniques

GCMS analysis, spectroscopic techniques, and chromatographic methods were carried out as described in Chapter Two. Chemical-ionisation (isobutane) and high-resolution GCMS were obtained on the Kratos instrument of the Ruakura Agricultural Research Centre. HMQC and HMBC NMR experiments were performed on a Bruker AC300 spectrometer at 300 K in inverse mode on a 5 mm dual (^1H-^{13}C) probe using standard Bruker programs. Degassing prior to NOE-difference experiments was performed using the freeze-thaw method (Noggle and

Shirmer, 1971). Any experiments involving multiplet signals were performed with sequential irradiation of each line in the multiplet (Kinns and Saunders, 1984).

4.4.3. Isolation of Kamahines A-C:

The concentrated extractives (Section 4.4.1) were columned using 1:3 Et_2O:PE as solvent. The fractions were analysed by GCMS to identify those containing the component of interest. Subsequently, these fractions were further purified by PLC using the above solvent system at 1:1 then 1:3. The major band from the PLC plate (R_f = 0.50) was extracted with Et_2O and concentrated under reduced pressure to yield a colourless oil (15 mg) which was confirmed as kamahine by GCMS analysis. NMR data for the three diastereoisomeric kamahines was taken from the mixture.

Kamahine A (25a): ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.99 (3H, s, 8'-Me_{ax}), 1.03 (3H, d, $J_{4\text{-}Me,4}$ = 6.8 Hz, 4-Me), 1.30 (3H, s, 8'-Me_{eq}), 1.80 (1H, t, $J_{3a,3b}$ = $J_{3a,4}$ = 12.4 Hz, H-3_a), 1.87 (1H, dd, $J_{3b,3a}$ = 12.4, $J_{3b,4}$ = 6.6 Hz, H-3_b), 2.09 (3H, d, $J_{2'\text{-}Me,3'}$ = 1.6 Hz, 2'-Me), 2.45 (1H, m, H-4), 3.25 (1H, s(br.), OH), 3.95 (1H, d, $J_{5',3'}$ = 2.1 Hz, H-5'), 5.52 (1H, d, $J_{5,4}$ = 4.8 Hz, H-5), 5.90 (1H, dq, $J_{3',5'}$ = 2.1, $J_{3',2'\text{-}Me}$ = 1.6 Hz, H-3'). ^{13}C NMR (75.5 MHz) δ: 12.3 (q, 4-Me), 18.1 (q, 8'-Me_{ax}), 20.7 (q, 2'-Me), 21.9 (q, 8'-Me_{eq}), 38.7 (t, C-3), 39.2 (d, C-4), 52.0 (s, C-8'), 84.7 (s, C-1'), 89.9 (d, C-5'), 100.5 (d, C-5), 115.0 (s, C-7'), 125.1 (d, C-3'), 166.6 (s, C-2'), 195.0 (s, C-4').

Kamahine B (25b): ^{1}H NMR δ: 0.99 (3H, s, 8-Me_{ax}), 1.11 (3H, d, $J_{4\text{-}Me,4}$ = 6.8 Hz, 4-Me), 1.30 (3H, s, 8'-Me_{eq}), 1.63 (1H, dd, $J_{3b,3a}$ = 13.3, $J_{3b,4}$ = 1.8 Hz, H-3_b), 2.09 (3H, d, $J_{2'\text{-}Me,3'}$ = 1.6 Hz, 2'-Me), 2.15 (1H, m, H-4), 2.31 (1H, dd, $J_{3a,3b}$ = 13.3, $J_{3a,4}$ = 7.6 Hz, H-3_a), 3.25 (1H, s(br.), OH), 3.95 (1H, d, $J_{5',3'}$ = 2.1 Hz, H-5'), 4.18 (1H, s(br.), OH), 5.35 (1H, s(br.), H-5), 5.90 (1H, dq, $J_{3',5'}$ = 2.1, $J_{3',2'\text{-}Me}$ = 1.6 Hz, H-3'). ^{13}C NMR δ: 15.3 (q, 4-Me), 18.1 (q, 8'-Me_{ax}), 20.8 (q, 2'-Me), 22.0 (q, 8'-Me_{eq}), 38.7 (t, C-3), 39.2 (d, C-4), 51.9 (s, C-8'), 84.9 (s, C-1'), 90.0 (d, C-5'), 105.1 (d, C-5), 115.9 (d, C-7'), 125.9 (d, C-3'), 166.6 (s, C-2'), 195.5 (s, C-4').

Kamahine C (25c): ^{1}H NMR δ: 0.99 (3H, s, 8'-Me_{ax}), 1.09 (3H, d, $J_{4\text{-}Me,4}$ = 6.8 Hz, 4-Me), 1.31 (3H, s, 8'-Me_{eq}), 1.60 (1H, dd, $J_{3a,3b}$ = 13.1, $J_{3a,4}$ = 9.7 Hz, H-3_a), 2.03 (1H, dd, $J_{3b,3a}$ = 13.1, $J_{3b,4}$ = 6.9 Hz, H-3_b), 2.04 (3H, d, $J_{2'\text{-}Me,3'}$ =1.4 Hz, 2'-Me), 2.25 (1H, m, H-4), 2.97 (1H, s (br.), OH),

3.88 (1H, s(br.), OH), 3.97 (1H, d, $J_{5',3'}$ = 2.0 Hz, H-5'), 5.03 (1H, t, $J_{5,4}$ = $J_{5,OH}$ = 5.9 Hz, H-5), 5.89 (1H, dq, $J_{3',5'}$ = 2.0, $J_{3',2'\text{-Me}}$ = 1.4 Hz, H-3'). ^{13}C NMR δ: 15.7 (q, 4-Me), 18.2 (q, 8'-Me$_{ax}$), 20.4 (q, 2'-Me), 21.9 (q, 8'-Me$_{eq}$), 39.7 (d, C-4), 41.3 (t, C-3), 52.1 (s, C-8'), 84.4 (s, C-1'), 89.6 (d, C-5'), 107.1 (d, C-5), 112.9 (s, C-7'), 125.3 (d, C-3'), 165.5 (s, C-2'), 195.2 (s, C-4').

Kamahines A-C (taken from mixture **25a-25c**) IR: υ 3444 (OH), 2955 and 2877 (CH), 1672 cm^{-1} (C=O, α,β-unsaturated ketone). UV (EtOH): λ_{max} 206 (log ε 3.6), 243 nm (log ε 3.7). EIMS: *m/z* (rel. int.) 251 (M$^{+\cdot}$-17, 2), 222 (5), 179 (5), 170 (30), 152 (88), 137 (45), 109 (100), 89 (45), 83 (26), 71 (33), 69 (35), 55 (35), 43 (90). CIMS (isobutane): *m/z* (rel. int.) 269 (M$^{+\cdot}$+1, 3), 251 (40), 221 (11), 193 (10), 169 (12), 153 (100), 127 (10), 109 (16), 99 (55), 83 (18). High-resolution EIMS: Found *m/z* M$^{+\cdot}$ = 268.1307, calculated *m/z* M$^{+\cdot}$ for $C_{14}H_{20}O_5$ = 268.1311.

4.4.4. Acetylation of Kamahines A-C

Kamahines **25a-25c** (15 mg, 0.06 mmol) were acetylated using the standard acetylation procedure (Chapter Two). The resulting oil (10 mg) was plated using 1:2 Et_2O:PE to give two major bands:-

Band 1 (R_f = 0.4) contained *monoacetyl-kamahine C* ***(149c)*** (4 mg, 21%) as a colourless oil. ^{1}H NMR (300 MHz) δ: 1.00 (3H, s, 8'-Me$_{ax}$), 1.10 (3H, d, $J_{4\text{-Me},4}$ = 6.9 Hz, 4-Me), 1.27 (3H, s, 8'-Me$_{eq}$), 1.66 (1H, dd, $J_{3a,3b}$ = 13.0, $J_{3a,4}$ = 9.9 Hz, H-3$_a$), 2.06 (3H, s, $J_{2'\text{-Me},3'}$ = 1.5 Hz, 2'-Me), 2.09 (1H, dd, $J_{3b,3a}$ = 13.0, $J_{3b,4}$ = 7.3 Hz, H-3$_b$), 2.12 (3H, s, MeCO), 2.51 (1H, m, H-4), 2.90 (1H, s(br.), OH), 3.98 (1H, d, $J_{5',3'}$ = 2.2 Hz, H-5'), 5.85 (1H, d, $J_{5,4}$ = 4.8 Hz, H-5), 5.91 (1H, dq, $J_{3',5'}$ = 2.2, $J_{3',2'\text{-Me}}$ = 1.5 Hz, H-3'). ^{13}C NMR (75.5 MHz) δ: 16.1 (q, 4-Me), 18.1 (q, 8'-Me$_{ax}$), 20.3 (q, 2'-Me), 21.3 (q, MeCO), 21.8 (q, 8'-Me$_{eq}$), 36.8 (d, C-4), 40.5 (t, C-3), 52.0 (s, C-8'), 84.9 (s, C-1'), 89.9 (d, C-5'), 103.6 (d, C-5), 114.6 (s, C-7'), 125.4 (d, C-3'), 164.8 (s, C-2'), 170.8 (s, MeCO), 194.6 (s, C-4'). EIMS: *m/z* (rel. int.) 268 (M$^{+\cdot}$-CH_2CO, 3), 251 (M$^{+\cdot}$-CH_3COO, 9), 221 (5), 194 (3), 165 (4), 152 (100), 137 (21), 124 (68), 109 (39), 99 (30), 83 (9), 49 (13), 43 (47). IR: υ 3459 (OH), 2964 and 2922 (CH), 1738 (C=O, ester), 1682 cm^{-1} (C=O, α,β-unsaturated ketone). UV (EtOH): λ_{max} 212 (log ε 3.7), 250 nm (log ε 3.6).

Band 2 (R_f = 0.55) contained a 2:1 mixture of monoacetyl derivatives **149a** and **149b**, respectively (5.5 mg). The oil was replated using 3:7 Et_2O:PE to give two bands:-

*Monoacetyl-kamahine A (**149a**)* (R_f = 0.45) (3 mg, 16%) as a colourless oil; ^{1}H NMR (300 MHz) δ: 0.98 (3H, s, 8'-Me_{ax}), 0.98 (3H, d, $J_{4\text{-}Me,4}$ = 6.7 Hz, 4-Me), 1.27 (3H, s, 8'-Me_{eq}), 1.80 (1H, t, $J_{3a,3b}$ = $J_{3a,4}$ = 12.6 Hz, H-3_a), 1.88 (1H, dd, $J_{3b,3a}$ = 12.6, $J_{3b,4}$ = 6.7 Hz, H-3_b), 2.08 (3H, d, $J_{2'\text{-}Me,3'}$ = 1.6 Hz, 2'-Me), 2.09 (3H, s, MeCO), 2.59 (1H, m, H-4), 2.62 (1H, s(br.), OH), 3.94 (1H, d, $J_{5',3'}$ = 2.1 Hz, H-5'), 5.89 (1H, dq, $J_{3'5'}$ = 2.1, $J_{3',2'\text{-}Me}$ = 1.6 Hz, H-3'), 6.39 (1H, d, $J_{5,4}$ = 4.8 Hz, H-5). ^{13}C NMR (75.5 MHz) δ: 12.0. (q, 4-Me), 18.0 (q, 8'-Me_{ax}), 20.5 (q, 2'-Me), 21.2 (q, MeCO), 21.7 (q, 8'-Me_{eq}), 35.8 (d, C-4), 38.5 (t, C-3), 51.9 (s, C-8'), 84.4 (s, C-1'), 89.9 (d, C-5'), 99.5 (d, C-5), 115.8 (s, C-7'), 125.3 (d, C-3'), 165.3 (s, C-2'), 169.9 (s, MeCO), 194.5 (s, C-4'). IR (mixture of **149a** and **149b**): υ 3502 (OH), 2970 and 2933 (CH), 1734 (C=O, ester), 1683 cm^{-1} (C=O, α,β-unsaturated ketone). UV (**149a/149b**) (EtOH): λ_{max} 194 (log ε 3.7), 230 nm (log ε 3.8). EIMS (**149a/149b**): *m/z* (rel. int.) 268 ($M^{+\cdot}$-CH_2CO, 3), 251 ($M^{+\cdot}$-CH_3COO, 9), 221 (5), 194 (3), 165 (4), 152 (100), 137 (21), 124 (68), 109 (39), 99 (30), 83 (9), 49 (13), 43 (47).

*Monoacetyl-kamahine B (**149b**)* (R_f = 0.50) (2 mg, 11%) as a colourless oil; ^{1}H NMR (300 MHz) δ: 0.99 (3H, s, 8'-Me_{ax}), 1.16 (3H, d, $J_{4\text{-}Me,4}$ = 7.6 Hz, 4-Me), 1.29 (3H, s, 8'-Me_{eq}), 1.62 (1H, d, $J_{3b,3a}$ = 12.0 Hz, H-3_b), 2.06 (3H, s, MeCO), 2.08 (3H, d, $J_{2'\text{-}Me,3'}$ = 1.6 Hz, 2'-Me), 2.25 (1H, m, H-4), 2.25 (1H, dd, $J_{3a,3b}$ = 12.0, $J_{3a,4}$ = 7.6 Hz, H-3_a), 2.66 (1H, s(br.), OH), 3.96 (1H, d, $J_{5',3'}$ = 2.2 Hz, H-5'), 5.89 (1H, dq, $J_{3',5'}$ = 2.2, $J_{3',2'\text{-}Me}$ = 1.6 Hz, H-3'), 6.09 (1H, s, H-5). ^{13}C NMR (75.5 MHz) δ: 16.8 (q, 4-Me), 17.9 (q, 8'-Me_{ax}), 20.5 (q, MeCO), 21.3 (q, 2'-Me), 21.7 (q, 8'-Me_{eq}), 37.7 (t, C-3), 38.2 (d, C-4), 51.7 (s, C-8'), 84.9 (s, C-1'), 90.1 (d, C-5'), 104.6 (d, C-5), 117.2 (s, C-7'), 125.3 (s, C-3'), 165.3 (s, C-2'), 169.8 (s, MeCO), 194.7 (s, C-4').

4.4.5. Microscale Acetylation of 25b

A sample of isomerically pure **25b** was obtained by multiple-elution PLC using 1:1–1:4 Et_2O:PE as solvent. Dissection of the upper region of the band **25-25c** gave **25b**. A $CDCl_3$ solution of **25b** was transferred to an NMR tube and following ^{1}H NMR analysis, acetic anhydride and pyridine (10 μl each) were

added to the NMR tube by syringe. Progress of acetylation was monitored by ^1H NMR.

4.4.6. X-ray Crystallography of 149c

Clear colourless crystals of **149c**, m.p. 152-154°C, were obtained from hexane:chloroform 9:1 following slow cooling. Empirical formula $C_{16}H_{22}O_6$, M_r = 310.2, orthorhombic, space group $P2_12_12_1$; a = 8.660, b = 13.554 and c = 13.661 Å. U = 1603.5 $Å^3$, D_{calc} = 1.28 g cm^{-3}. Of the 1790 unique intensities, 1010 were used with $I \geq 2\sigma(I)$. Intensities were measured using Mo K_α radiation (λ = 0.71073 Å). The structure was solved by direct methods (SHELXS86 (Sheldrick, 1986)). All atoms were treated isotropically, and hydrogen atoms were included in calculated positions with common isotropic temperature factors for each type of hydrogen. The refinement (SHELX76 (Sheldrick, 1976)) converged with R = 0.0801, R_w = 0.0700 where $w = [\sigma^2F + 0.000525F^2]^{-1}$.

4.4.7. Kamahi Plant Extraction

Fresh kamahi flowers (x5), pods (x5) and leaves (1-2) were each soaked in chloroform (2 ml) for 24 h. The filtered solutions were concentrated and methylated prior to GCMS analysis.

4.4.8. Biological Testing

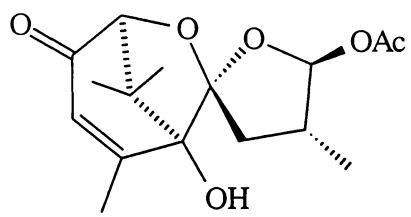
A sample of kamahine was submitted for biological testing at the Department of Chemistry, University of Canterbury by Ms. G Ellis. Kamahine showed no discernable antimicrobial or antiviral effects, and no cytotoxicity, including antitumour activity. Organisms used to test antimicrobial activity were bacteria, *E. coli, P. aeruginosa* and *B. subtilus*, and fungi, *C. albicans*, *T. mentagrophytes* and *C. resinae*. Antiviral and cytotoxic assays are carried out with BSC-1 cells (African Green Monkey kidney) infected with Herpes simplex type 1 virus (ATCC VR 733) and polio virus type 1 (Pfieser vaccine strain). Antitumour activity was tested against P388 (Murine Leukaemia) cells.

Chapter Five

Determination of the Absolute Stereochemistry of Kamahines A-C

5.1. Introduction

Chapter Four outlined the structure elucidation of diastereoisomeric kamahines **25a-25c** by 1- and 2D NMR techniques. X-ray crystollography of one of the diastereoisomeric acetyl derivatives substantiated the structures as the first reported compounds possessing the novel spiroketal ring system (**149c**). The crystallographic space group was determined as $P2_12_12_1$ suggesting that **149c** was chiral (or at least crystallised in chiral form). However, due to the absence of a heavy atom in the molecule, the absolute configuration could not be determined from the data.



(149c)

The absolute stereochemistry of kamahines **25a-25c** was required to complete their characterisation, and to gain some insight into their biosynthetic origin. The structure and absolute stereochemistry are the only source of information about the relationship of **25** to proposed precursors. Intermediates which could corroborate likely pathways, have not been identified in the honey or plant source. Xanthophyll carotenoids, or ABA, are putative biogenetic precursors of kamahine.

5.1.1. Methods of Absolute Stereochemistry Determination

Various methods for determining the absolute stereochemistry of chiral compounds are discussed in Chapter Three of this thesis. Analysis of the absolute configuration of kamahine by optical methods such as CD was not feasible due to the difficulties in obtaining complete separation of the diastereoisomers.

The NMR method using chiral derivatives represented a convenient chemical process for analysis of the absolute configuration of the kamahines. It was anticipated that conversion of the enantiomeric hemiacetals to diastereoisomeric acetals using a chiral derivatising agent would facilitate their separation, and subsequent NMR analysis. Moreover, if suitable crystals were obtained, this approach enables unequivocal determination of absolute stereochemistry by X-ray crystallography. Of considerable interest, was the application of this method to cyclic hemiacetals of the type present in kamahine, as similar determinations had not been reported previously.

5.2. Results and Discussion

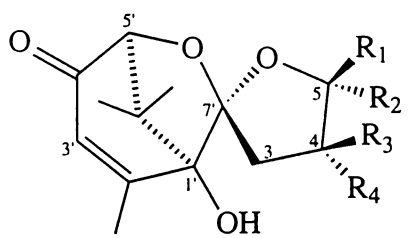
Applications of Mosher's protocol have generally been limited to assignment of the absolute configurations of secondary alcohols, and amines (Chapter Three). The general applicability of this methodology has been demonstrated for a variety of secondary alcohols covering various steric environments and degrees of polarity at the chiral centre. This suggests that the nature of the esterified alcohol has little influence on the conformational behaviour of the acid moiety.

Determination of the absolute stereochemistry of the kamahines required extrapolation of the correlation from esters of secondary alcohols to that of hemiacetals. Prior to extension of the correlation to new types of compounds, it is desirable that studies be carried out on model compounds of known configuration (Dale and Mosher, 1973) Indeed, various investigators have extended the correlation to new classes of compounds using this strategy (e.g. Ohtani *et al.*, 1991; Rieser *et al.*, 1992).

As an appropriate model compound of known configuration could not be prepared in the time available, use of the configurational correlation on the kamahines required caution. The value of the extended Mosher method, when applied to configurational assignment of compounds which cannot be easily subjected to another independent proof (Latypov *et al.*, 1995), is in the incorporation of a self-examining mechanism.

5.2.1. Preparation of MPA Esters of Kamahines A-C

In previous work, derivatisation of a secondary alcohol functionality with α-methoxyphenylacetic acid (MPA) using DCC and DMAP was found to occur rapidly and without racemisation of the acid (Chapter Three). The esterification of hemiacetals **25a-25c** under the same experimental conditions proceeded rapidly, affording three diastereoisomeric products (GCMS). ^{1}H NMR revealed that each product consisted of a single kamahine diastereoisomer and additional proton signals consistent with the addition of an MPA ester moiety. Separation of (*S*)-MPA esters **159a-159c** and (*R*)-MPA esters **160a-160c** by PLC gave sufficient quantities of each derivative for NMR analysis. NMR data for **160b** was extracted from the mixture **160a/160b**.



	R_1	R_2	R_3	R_4
25a	H	OH	H	Me
25b	H	OH	Me	H
25c	OH	H	H	Me
159a	H	(*S*)-χ	H	Me
159b	H	(*S*)-χ	Me	H
159c	(*S*)-χ	H	H	Me
160a	H	(*R*)-χ	H	Me
160b	H	(*R*)-χ	Me	H
160c	(*R*)-χ	H	H	Me

χ = MPA

The observation of three MPA ester products from each reaction, with each product giving rise to one set of ^{1}H and ^{13}C NMR signals, indicated that the three diastereoisomeric kamahines were single enantiomers. Furthermore, it was evident that racemisation of the MPA moiety was insignificant under the conditions used for the synthesis.

5.2.2. Characterisation of MPA Esters 159a-159c and 160a-160c

Assignment of the proton NMR signals and relative stereochemistry of the three parent kamahine diastereoisomers **25a-25c** was described in Chapter Four. Tentative assignment of the ^{1}H NMR spectra of the corresponding MPA derivatives was made from chemical shift and spin-spin coupling information summarised below. Where necessary, these assignments were confirmed by NMR techniques including 2D homonuclear correlation spectroscopy (COSY), and NOE-difference experiments.

The addition of an MPA moiety to the molecules was indicated by ^{1}H signals at approximately δ 3.45, 4.76 and 7.35-7.45 arising from the methoxy, methine and aromatic protons of the acid group, respectively. Each MPA derivative **159a-159c** and **160a-160c** exhibited proton NMR characteristics which were indicative of the parent kamahine ring systems. This comparison is shown for (*S*)-MPA ester **159a** and the corresponding acetate **149a** (Figure 5.1).

The ^{1}H signal from H-5, in particular, was diagnostic in that the multiplicity, scalar coupling values and relative chemical shifts for this proton in each of **159a-159c** and **160a-160c** were identical to the corresponding acetates **149a-149c**. This allowed direct correlation between an acetate and MPA derivative. Generally, the MPA group attains a conformation in which that methine proton does not lie within the shielding region of the phenyl group.

Protons H-3_a, H-3_b and H-4 were readily identified by their scalar coupling patterns. The resonance from H-3_a, which was identified as the proton of the spirotetrahydrofuran ring closest in space to the enone functionality in previous work (Section 4.2.3), was confirmed by NOE-difference experiments following irradiation of 2'-Me from each of **159a-159c** and **160a-160c**. NOE-difference experiments performed on these derivatives corroborated the relative configurations

of the tetrahydrofuran ring substituents determined for the corresponding acetates **149a-149c** (Chapter Four).

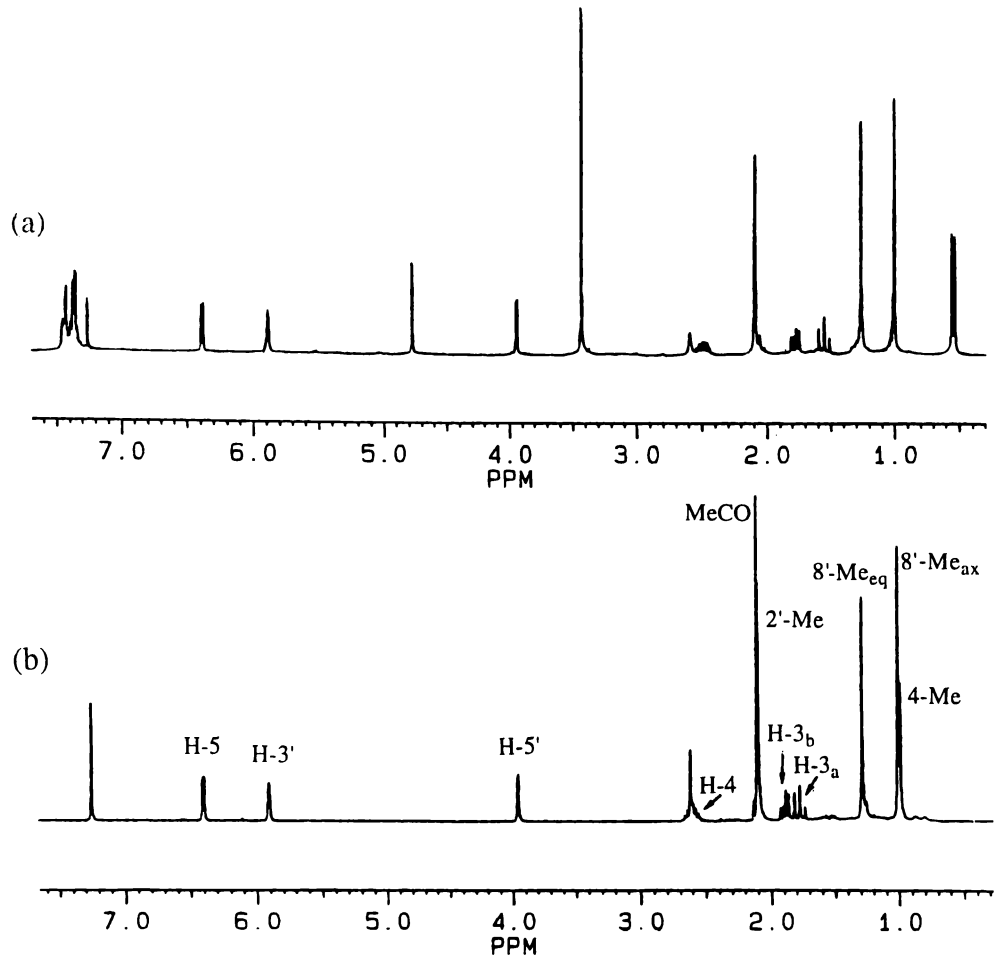


Figure 5.1. Comparison of the ^{1}H NMR spectra of (a) (*S*)-MPA ester **159a**, and (b) acetate **149a**.

Proton resonances characteristic of H-3', H-5', 4-Me, and 2'-Me were readily identified and the geminal methyl groups (8'-Me$_{ax/eq}$) were distinguished in the COSY spectra by the four-bond coupling between the axial methyl group and H-5' (Section 4.2.3). Carbon spectra were assigned using the XHCORR spectrum. Singlet carbons were well dispersed, allowing unambiguous assignment. ^{13}C chemical shifts were obtained from the ^{1}H-decoupled 300 MHz spectra, and are quoted to 2 d.p. to distinguish diastereoisomeric signals (Section 3.3.2). ^{1}H and ^{13}C data for (*S*)-**159a-159c** and (*R*)-**160a-160c** MPA derivatives (except ^{13}C NMR data for **160b**) are reported in the Experimental Section of this Chapter. Selected proton chemical shifts of the MPA esters are presented in Tables 5.1 and 5.2.

5.2.3. Absolute Stereochemistry of Kamahines A-C

Interpretation of Diamagnetic ^1H shifts Observed for 159c/160c

Chemical shift differences ($\Delta\delta = \delta S - \delta R$) (Table 5.1) were calculated for each proton of (*S*)-**159c** and (*R*)-**160c** MPA esters of **25c** in order to confirm that all the assigned protons with positive and negative $\Delta\delta$ values were arranged in a systematic manner either side of the ester plane (Ohtani *et al.*, 1991). The NMR configurational correlation scheme for MPA esters (Trost *et al.*, 1986) features an ester plane in which the methoxy and carbonyl substituents are approximately eclipsed with the methine proton attached to the esterified carbon (Chapter Three).

The non-planar geometry of the kamahine skeleton did not allow a simple representation of the 'opposite sides' of the molecule with respect to the ester plane. The spiro-linkage imparted a three-dimensional shape which complicated representation of the ester conformations for these molecules. For example, protons seemingly located on the left side of the ester plane in the two-dimensional structure actually existed on the right side of the plane in the three-dimensional structure. In interpreting the shielding effects therefore, the model building facility in MacroModel was used to generate the conformations of the MPA esters for both enantiomers of **159a-159c** and **160a-160c**, as would be predicted be Mosher-Trost rules.

Using the Mosher-Trost conformation of the MPA moieties, diamagnetic shielding observed for **159c/160c** was rationalised by selecting the enantiomer depicted in Figure 5.2/5.3. Large negative $\Delta\delta$ values were observed for H-5' (-0.32) and C-8' methyl groups (-0.28 and -0.15), and relatively small negative $\Delta\delta$ values for protons of the olefinic bond (-0.05 and -0.04) and methylene protons of the tetrahydrofuran ring (-0.03 and -0.03). The negative $\Delta\delta$ values of these protons were consistent with preferential shielding by the phenyl group of the (*S*)-MPA moiety. Positive $\Delta\delta$ values were observed for H-4 (+0.11) and 4-Me (+0.15), consistent with preferential shielding from the (*R*)-MPA moiety. In general, observed $\Delta\delta$ values showed systematic variations with respect to the ester plane, displaying the diminishing effects of the phenyl group with distance. MM2*-generated structures of (*S*)-**159c** and (*R*)-**160c** MPA esters of both enantiomers of kamahine C (**25c**) are shown in Figure 5.3.

Table 5.1 ^1H chemical shifts and $\Delta\delta_{SR}$ values for MPA esters **159c** and **160c** of **25c**.

	Chemical Shift (ppm)		
Proton	(*S*)-ester **159c**	(*R*)-ester **160c**	$\Delta\delta = \delta S - \delta R$
H-5'	3.60	3.92	-0.32
8'-Me_{eq}	0.89	1.17	-0.28
8'-Me_{ax}	0.83	0.98	-0.15
H-3'	5.85	5.90	-0.05
2'-Me	2.01	2.05	-0.04
H-3_a	1.58	1.61	-0.03
H-3_b	2.06	2.09	-0.03
4-Me	1.10	0.95	+0.15
H-4	2.55	2.44	+0.11
H-5	5.96	5.85	+0.11

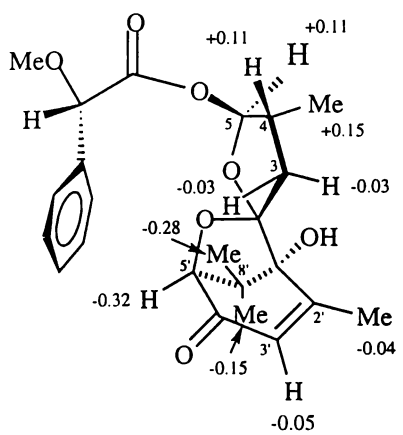


Figure 5.2. Representation of the MacroModel-generated conformation of *(S)*-MPA ester **159c** as predicted by Mosher-Trost rules. Included are the observed $\Delta\delta_{SR}$ values for ^1H resonances (ppm).

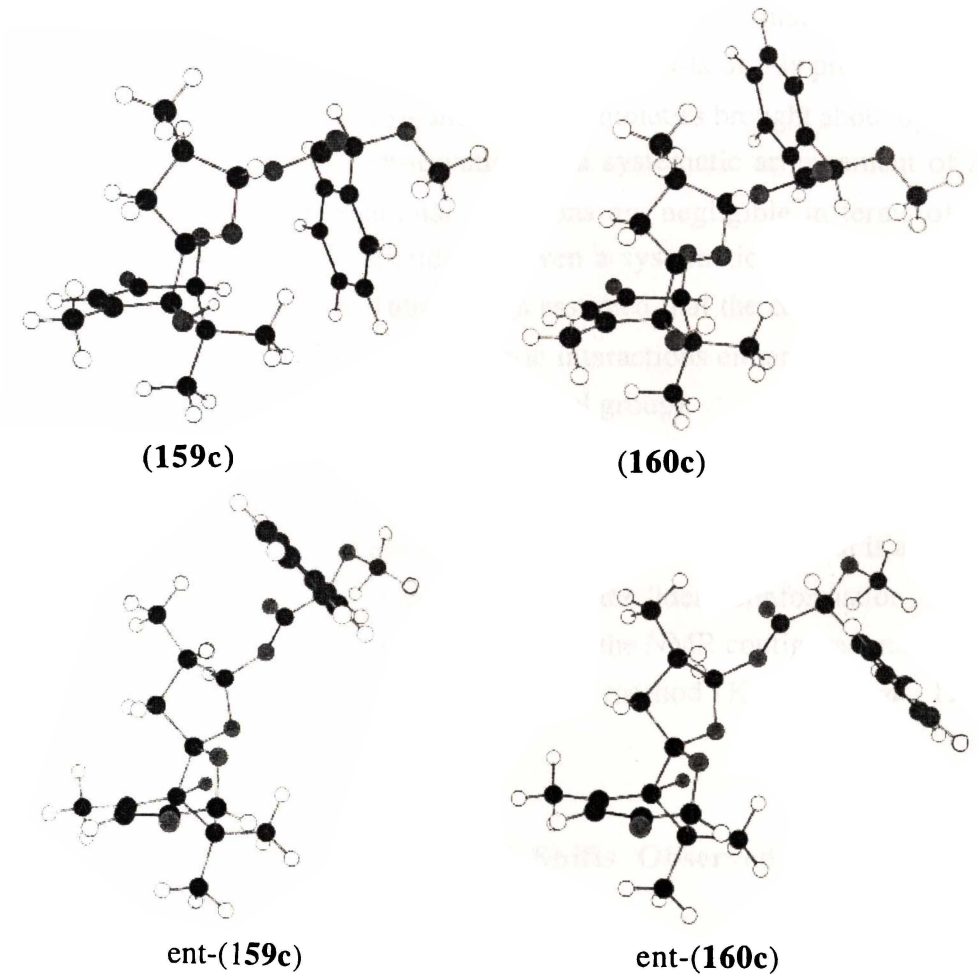


Figure 5.3. MM2*-generated structures of *(S)*-and *(R)*-MPA esters of **25c** (**159c** and **160c**, respectively) showing the Mosher-Trost conformation of the MPA moiety. The (*S*)-and (*R*)-MPA esters of the opposite enantiomer of **25c** are shown for comparison.

MacroModel-generated *(S)*- and (*R*)-MPA esters of the opposite enantiomer of **25c** predicted diamagnetic shielding which was inconsistent with experimental data (Figure 5.3). The phenyl group of the (*S*)-MPA moiety eclipsed C-4 substituents of the tetrahydrofuran ring, giving rise to predicted negative $\Delta\delta$ values for those protons (ent-(**159c**)). The phenyl group of the (*R*)-MPA moiety eclipsed H-5' and 8'-Me groups of the cyclohexenone ring, predicting positive $\Delta\delta$ values for those substituents (ent-(**160c**)).

The proton attached to the esterified carbon atom lies in the ester plane; therefore $\Delta\delta$ for this proton ($\Delta\delta_{Hester}$) is usually zero, or small in magnitude relative to other $\Delta\delta$ values. Differences in chemical shifts of this proton indicate conformational variations of the (*S*)- and (*R*)-ester moieties brought about by steric interactions (Chapter Three). Observation of a systematic arrangement of $\Delta\delta$ values suggests these conformational variations are negligible in terms of the regions of the molecule being shielded. Given a systematic distribution of $\Delta\delta$ values were observed for **159c/160c**, it was assumed that the $\Delta\delta$ value of +0.11 for H-5 was attributable to the different steric interactions either side of the ester plane (furan ring oxygen versus secondary methyl group).

In instances where the ester group experiences unfavourable steric interactions, irregularities in $\Delta\delta$ are observed (Kusumi *et al.*, 1988). It is assumed that the ester moiety is prevented from taking up the 'ideal' conformation. Thus, incorporation of a large number of data points into the NMR configurational model provides a self-examination mechanism for the method (Kusumi *et al.*, 1991; Ohtani *et al.*, 1991).

Interpretation of Diamagnetic ^{1}H Shifts Observed for 159a/160a and 159b/160b

Examination of the $\Delta\delta$ values for (*S*)-**159a** and (*R*)-**160a** MPA esters of kamahine A (**25a**) showed a systematic distribution of $\Delta\delta$ values (Table 5.2/Figure 5.4). The largest negative $\Delta\delta$ occurred at 4-Me (-0.38), and relatively small negative values were observed for its geminal partner H-4 (-0.07), and the adjacent protons, H-3_a and H-3_b (-0.05 and -0.05). Conversely, cyclohexenone substituents displayed positive $\Delta\delta$ values, the largest value observed at 8'-Me_{eq} (+0.13). Observed $\Delta\delta$ values were consistent with the diminishing shielding effect of the phenyl group with distance. Model (*S*)- and (*R*)- MPA derivatives of **25a** are shown in Figure 5.5. (*S*)- and (*R*)-MPA derivatives of the opposite enantiomer of **25a** predicted diamagnetic shielding which was inconsistent with experimental data. Comparison of (*S*)-MPA esters of both enantiomers is also shown in Figure 5.5.

Table 5.2. ^{1}H chemical shifts and $\Delta\delta_{SR}$ values for MPA derivatives **159a/160a** and **159b/160b**.

Proton	Chemical Shift (ppm)					
	Kamahine A (**25a**)			Kamahine B (**25b**)		
	159a	**160a**	$\Delta\delta_{SR}$	**159b**	**160b**	$\Delta\delta_{SR}$
8'-Me_{ax}	1.00	0.94	+0.06	1.00	0.95	+0.05
8'-Me_{eq}	1.27	1.14	+0.13	1.28	1.15	+0.13
2'-Me	2.10	2.01	+0.09	2.00	2.00	+0.00
H-5'	3.95	3.91	+0.04	3.96	3.92	+0.04
H-3'	5.90	5.87	+0.03	5.87	5.87	0.00
4-Me	0.54	0.92	-0.38	1.10	1.15	-0.05
H-4	2.50	2.57	-0.07	1.95	2.15	-0.20
H-3_a	1.55	1.60	-0.05	1.97	2.16	-0.19
H-3_b	1.76	1.81	-0.05	1.50	1.65	-0.15
H-5	6.36	6.39	-0.03	6.10	6.14	-0.04

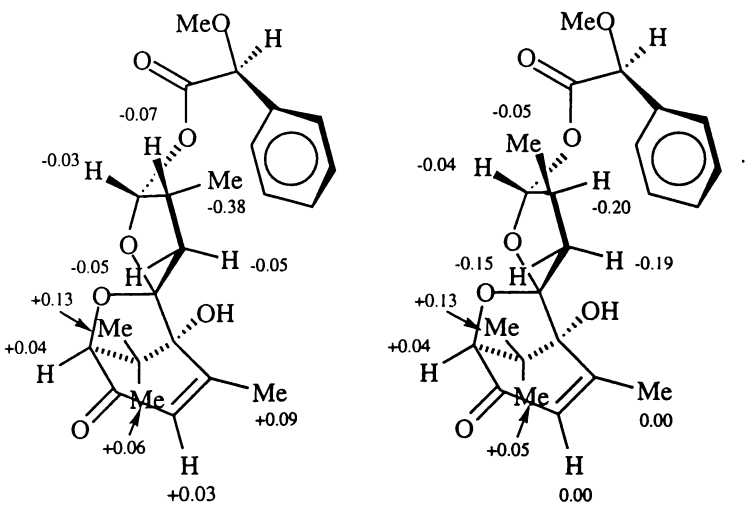
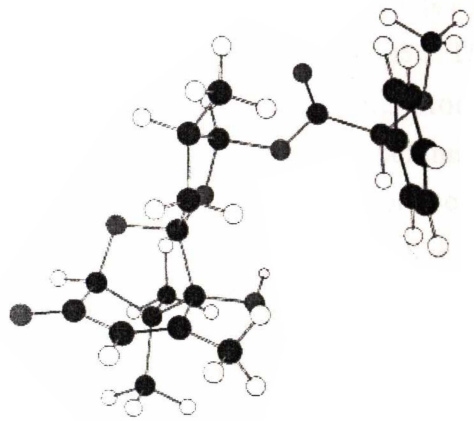
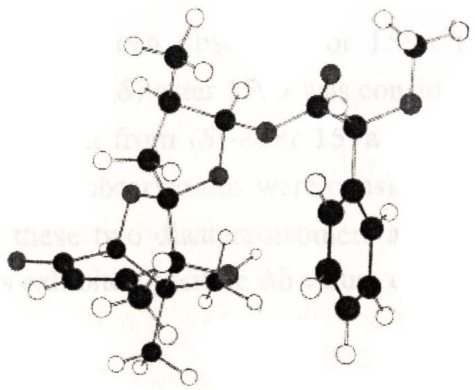


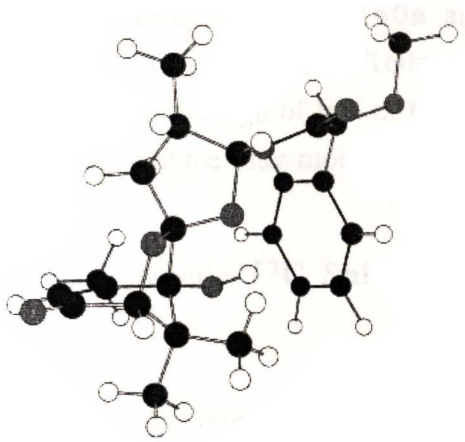
Figure 5.4. Representations of the MacroModel-generated conformations of (*S*)-MPA esters **159a** and **159b** according to Mosher-Trost rules. Included are the $\Delta\delta_{SR}$ values for the ^{1}H resonances (ppm).



(**159a**)



(**160a**)



ent-(**159a**)

Figure 5.5. Comparison of shielding effects predicted for (*S*)-**159a** and (*R*)-**160a** MPA esters of kamahine A (**25a**). The (*S*)-MPA ester of the opposite enantiomer (ent-(**159a**)) is shown below.

The trend of $\Delta\delta$ values observed for **159a/160a** indicated that the phenyl group of (*S*)- and (*R*)-MPA esters shielded different regions of the kamahine ring system to that observed for **159c/160c**. This was attributed to the opposite relative configurations of **159a/160a** and **159c/160c** at the ester carbon (C-5).

The relative stereochemistry of kamahine **25b** at the esterified carbon (C-5) was identical to kamahine **25a**. This suggested that **25b** would afford similar trends in differential shielding to **25a**, provided both compounds were the same

enantiomer. The arrangement of Δδ values observed for (*S*)- and (*R*)-MPA esters of **25b** (**159b/160b,** respectively) was indeed consistent with the enantiomer established for both **25a** and **25c** (Table 5.2). Negative Δδ values were observed for the tetrahydrofuran ring protons as had been observed for **159a/160a** derivatives. The extent of shielding at H-4 from (*S*)-ester **159b** was considerably greater than that observed for the same proton from (*S*)-ester **159a**, whilst an opposite trend was observed for 4-Me. Those observations were consistent with the established epimeric relationship of these two diastereoisomers at the C-4 position. Cyclohexenone ring substituents exhibited positive Δδ values displaying similar trends to that observed for **159a/160a**.

Differential shifts of -0.03 and -0.04 ppm were observed for H-5 of **159a/160a** and **159b/160b**, respectively, in comparison to +0.11 observed for the same proton of **159c/160c**. As **159a/160a** and **159b/160b** exhibit opposite relative stereochemistry to that of **159c/160c** at the esterified carbon (C-5), the differences in magnitude and sign of H-5 of those derivatives may reflect the different steric environments of the ester moieties.

Interpretation of Diamagnetic ^{13}C Shifts Observed for 159c/160c and 159a/160a

^{1}H chemical shifts of diastereoisomeric MPA (or MTPA) esters are influenced in a regular manner by the diamagnetic effect of the phenyl ring. A systematic arrangement of differential chemical shifts (Δδ) is obtained, reflecting the diminishing ring current effect with distance. A similar effect was observed for ^{13}C nuclei of **159c/160c** and **159a/160a**, however some inconsistencies in Δδ values were also observed (Figure 5.6).

The Δδ value of C-3 in **159c/160c** was +0.24, in contrast to the small negative Δδ values observed for the attached protons, $H\text{-}3_a$ and $H\text{-}3_b$. A large negative Δδ was observed at C-5. Positive Δδ values at C-1' and C-4' were inconsistent with the trend of negative Δδ values observed for carbons and protons on that ring. In **159a/160a**, ^{13}C differential shifts of the enone functionality, and C-4 and C-5, were inconsistent with predicted diamagnetic shielding effects.

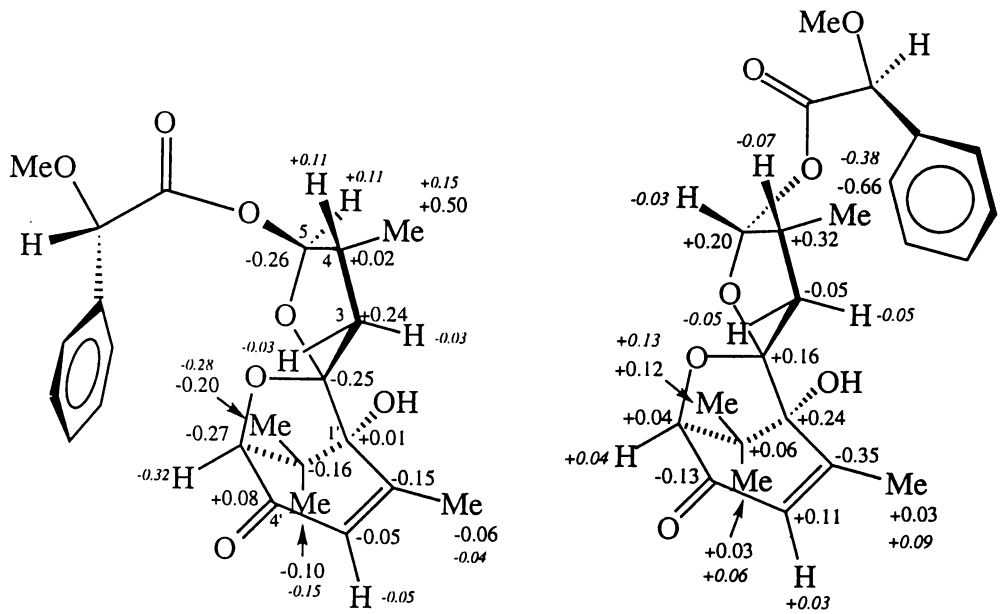


Figure 5.6. Comparison of ^{13}C and ^{1}H (*italics*) $\Delta\delta_{SR}$ values observed for (a) **159c/160c**, and (b) **159a/160a**.

It was assumed that concentration and temperature had negligible effects on ^{13}C chemical shifts of the two diastereoisomers. The differential ^{13}C chemical shifts observed for **159c/160c** and **159a/160a**, suggested that the diamagnetic effect of the phenyl group may not be clearly separated from other shielding or deshielding contributions. The discrepancies in $\Delta\delta$ values of C-5/H-5 in **159c/160c** and C-4/H-4,4-Me in **159a/160a** may be attributed to the anisotropic effects associated with the carboxyl and carbonyl oxygen atoms of the ester moiety (Chapter Three). Pehk *et al.* (1993) also observed inconsistencies in $\Delta\delta$ values of carbon nuclei in close proximity to the ester moiety.

Intermolecular diastereoisomeric interactions may account for the inconsistencies in $\Delta\delta$ data observed for carbon nuclei of the enone system, and the carbon attached to the hydroxyl group. (e.g. C-1' and C-4' in **159c/160c**; C-2' and C-4' in **159a/160a**). These interactions would be more noticeable for carbon nuclei distant from the acid moiety, where phenyl ring currents are weak. Irregularities in ^{13}C differential chemical shifts of the enone side-chain, in MPA esters of ionone **22** (Section 3.2.4), were attributed to this type of interaction.

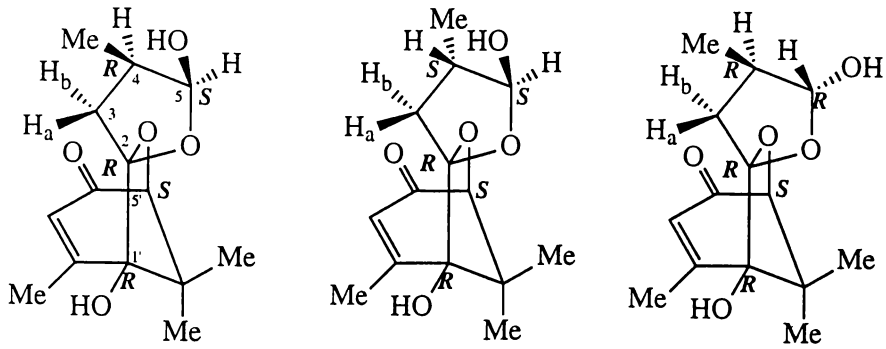
Results of the present study indicated that ^{13}C chemical shifts may not reflect trends observed for ^{1}H nuclei. This conclusion is in contrast to that claimed

by Pehk *et al.* (1993) (Chapter Three). Further work is required to validate the use of ^{13}C differential shifts to aid configurational assignment.

Assignment of Absolute Stereochemistry

The systematic arrangement of ^{1}H $\Delta\delta$ values observed for each diastereoisomeric MPA pair **159a/160a-159c/160c**, demonstrated a consistency in the region of the molecule effectively shielded. The arrangement of $\Delta\delta$ values observed either side of the plane formed from H-5 and the ester carbonyl exhibited a relationship between magnitude of shielding ($\Delta\delta$) and distance from the phenyl substituent. Those observations were an indication that the MPA moiety was attaining a conformation similar to that observed for other sterically unhindered secondary alcohols (Chapter Three). Prediction of the same enantiomer for each **25a-25c** was additional evidence which supported the use of Mosher-Trost method in the present study.

The esterified carbon of **159c** was assigned the *S* configuration, according to the IUPAC rules of nomenclature. The order of priority of groups is reversed when considering the ester derivative and parent hemiacetal; thus the configuration of the parent hemiacetal at C-5 was assigned *R*. This stereochemical information, coupled with the established relative configuration of the remaining asymmetric centres (Chapter Four), determined the absolute configuration of **25c** as 1'*R*,2*R*,4*R*,5*R*,5'*S*. Similarly, the absolute configurations of kamahines **25a** and **25b** were determined to be 1'*R*,2*R*,4*R*,5*S*,5'*S* and 1'*R*,2*R*,4*S*,5*S*,5'*S*, respectively. The absolute stereochemistries of kamahines **25a-25c**, determined in the present study, are presented in Figure 5.7.



(25a) 1'*R*,2*R*,4*R*,5*S*,5'*S*

(25b) 1'*R*,2*R*,4*S*,5*S*,5'*S*

(25c) 1'*R*,2*R*,4*R*,5*R*,5'*S*

Figure 5.7. Absolute stereochemistries of kamahines **25a**, **25b** and **25c**.

The absolute configuration of the kamahines was not corroborated by an X-ray crystallographic study. Although attempts to induce crystallisation of MPA derivatives were unsuccessful in the present study, initial investigations indicated that *p*-bromobenzoate derivatives could be crystallised.

5.2.4. Conformation of Kamahine MPA Esters

Conformational Studies of MTPA and MPA Esters

The configuration correlation models for α-phenylacetic acids were originally devised on an empirical basis (Dale and Mosher, 1973). Knowledge of the conformational behaviour of those esters was essential if the methodology was to be established on theoretical grounds. ORD and CD studies of mandelate, MPA and MTPA esters were interpreted in terms of their conformations, in which the α-hydroxy, α-methoxy, or α-CF_3 groups eclipse the carbonyl group, respectively (Barth *et al.*, 1970). The same conformations occur in the solid state as shown by X-ray structures of MPA esters (Trost *et al.*, 1986; Siegel and Thornton, 1988) and MTPA esters (Doesburg *et al.*, 1982; Oh *et al.*, 1989) of secondary alcohols. The band profile analysis of the IR adsorptions on the (*R*)-MTPA esters of several cyclohexanols has shown that the Mosher conformation of the MTPA moiety is much more preferable (7:3) than the conformer with the CF_3 group *anti* to the ester carbonyl (Merckx *et al.*, 1983).

In a number of more recent studies, the conformational properties of MTPA and arylmethoxyacetic acids (AMAAs) have been investigated using molecular mechanics. Interest in MPA derives from its use as a chiral auxillary in Diels-Alder reactions. Molecular modeling and theoretical molecular orbital calculations have shown the conformational properties of the MPA chiral auxillary are important in determining diastereofacial selectivity of Diels-Alder reactions (Tucker *et al.*, 1990).

AMAAs are of interest due to their increasing use as reagents for chiral analysis by NMR. Conformational analysis has proved useful for the rational design of new anisotropic reagents such as AMAAs (Seco *et al.*, 1994; Latypov *et al.*, 1995), in accounting for the smaller differential shielding observed for MTPA, in comparison to MPA esters (Latypov *et al.*, 1996), and identification of more suitable conditions for NMR experiments (Seco *et al.*, 1994; 1995). Izumi *et al.* (1994) used both molecular mechanics and NOE experiments as a basis for extension of the Mosher method to tertiary alcohols. The absolute stereochemistries of tertiary alcohols had not previously been determined using the Mosher method due to difficulties in predicting the conformation of the ester.

Conformational Searching Using Molecular Mechanics

Over the past 20 years, molecular mechanics (or force field calculations) has developed into a powerful standard method for studying molecular structure and related properties. In particular, molecular mechanics (MM) using recently developed conformational search techniques, is an invaluable method for the calculation of conformational geometries.

Molecular mechanics is often the method of choice for studying molecular geometry and related properties due to its speed, accuracy, and ease of understanding. Quantum mechanics (usually a molecular orbital approach) may be used; however this approach requires a large amount of computer time, and semiempirical methods have been developed to reduce computational time. The latter two methods are not suitable for conformational searching.

Molecular mechanics treats molecules as a collection of atoms held together by simple harmonic or elastic forces. Calculations are based on an empirical set of equations for the potential energy of molecules which include terms for vibrational

bond stretching, bond angle bending, twisting about bonds, and other interactions between atoms in a molecule :

$$E = E_{stretch} + E_{bend} + E_{torsion} + E_{VDW}$$

(E_{VDW} sums up the interactions (van der Waals) between atoms of a molecule that are not bonded to each other).

The set of functions, called the force field, contains adjustable parameters that are optimised to get the best fit of known properties of molecules. Parameterisation relies on experimental or theoretical molecular orbital (*ab initio*) data obtained from small simple molecules and the assumption is made that corresponding parameters and force field constants can be used in calculations for larger or more complicated ones. Using MM implemented force fields, optimisation occurs to refine the starting structure toward one of lower energy, converging when a minimum energy structure is obtained.

Force fields have been parametised to give excellent geometries and relative conformational energies of most organic molecules; among them, MM2 (Allinger, 1977), MM3 (Allinger *et al.*, 1989), AMBER (Weiner *et al.*, 1984), and CV (Dauber-Osguthorpe *et al.*, 1988) force fields. These force fields are implemented in various computer programs such as MacroModel (Mohamadi *et al.*, 1990) or Chem3D (Cambridge Scientific Computing). Force fields differ in the number and types of potential energy functions used although these differences are usually subtle. A recent comparison of several MM methods demonstrated that MM2* or MM3* force fields were the most accurate for a wide range of organic molecules (Gundertofte *et al.*, 1996). A general description of MM, including the underlying concepts and its limitations, is presented in two reviews (Engler *et al.*, 1973; Boyd and Lipkowitz, 1982)

The conformational geometries and energies of molecules are calculated using MM implemented force fields and conformational search techniques. The general approach is to search for the set of all minimum energy conformers (or at least a representative subset) within given energetic bounds (Mohamadi *et al.*, 1990). Typically, a search begins with the creation of a set of three-dimensional structures distributed throughout conformational space which serve as starting geometies for energy minimisation to nearby minimum energy conformers. The

resulting energy-minimised structures are collected and any duplicate conformers eliminated to give a set of unique low-energy minima. Convergence criteria such as the minimum duplication rate, or establishing that multiple, nonidentical searches (i.e. using different starting geometries) yield the same set of conformers, are often taken as evidence of search convergence (Chang *et al.*, 1989).

A variety of search methods have been proposed and used for searching the conformational space of small molecules. These methods vary by the means used to generate such starting geometries. Torsion angles (internal coordinates), cartesian coordinates (external coordinates), and matrices of internuclear distances (distance geometry) are coordinate systems commonly used to describe molecular geometries. The chosen coordinate system is varied either systematically or randomly to generate the starting geometries distributed throughout conformational space.

The effectiveness of the various methods for searching the conformational space of highly flexible molecules is discussed by Saunders *et al.* (1990). Internal coordinates and torsion angle searches in particular are efficient methods as the available space is reduced. Exploring torsion angle space has been approached by a systematic and random approach. The strength of the former approach is that it rapidly generates starting geometries which are sampled uniformly from all regions of torsion angle space. Saunders (1987) noted however, that one must decide which dihedral angles to vary and by what amount and therefore it must be "skillfully customised for each structure examined". Moreover, a large number of starting structures are generated when the number of possible values for each torsion angle is large.

Currently, the most efficient conformational search technique for small organic molecules is the Monte Carlo torsion angle search method. The approach utilises a random element in exploring conformational space with generation of starting structures by random variation of various predetermined torsion angles. This method has been described as being an efficient procedure which locates all the accessible minimum conformations (Saunders, 1990; Mohamadi *et al.*, 1990).

Conformational Analysis of MPA esters 159a-159c

The origin of consistent trends in ^{1}H differential shielding of **159a-159c** and **160a-160c** was examined, particularly since it was evident from model

building that MPA derivatives of kamahine could give diamagnetic shielding results sensitive to small conformational changes in the ester moiety. Conformational searches were performed on the (*S*)-MPA esters of kamahines **25a-25c** to obtain a set of energetic minima for each, which would provide some insight into the most significant conformation of the ester moiety on the NMR timescale.

The Monte Carlo torsion angle search is particularly well implemented in MacroModel. Torsion angle searches were initiated using an arbitrarily chosen MM2*-minimised conformation as the starting geometry. Initial structural minimisation was performed using the MacroModel-implemented MM2* force field with GB/SA chloroform solvent continuum included. The randomly varied torsion angles of the MPA moiety of **159a-159c** are shown in Figure 5.8. The torsion angles, between H-5 and carbonyl, and methoxyl oxygen and carbonyl, were defined as ϕ and ψ, respectively. Simulations were performed both *in vacuo* and with GB/SA solvent, for which essentially the same results were obtained

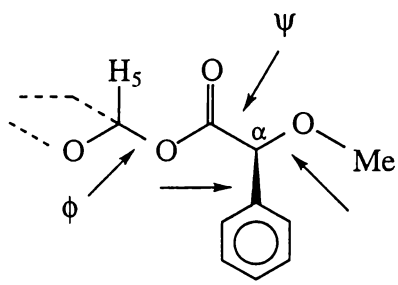
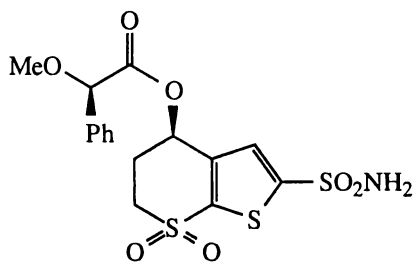


Figure 5.8. Variable torsion angles used to generate starting geometries: ϕ = H-5–C-5–O–C=O, ψ = O=C–C$_\alpha$-O (arrows point to rotatable bonds).

The suitability of MM for calculation of MPA geometries was investigated, particularly as the MacroModel-implementation of MM2 (MM2*), indicated some low quality torsional parameters for the ester moiety. The use of low quality parameters (parameters considered reasonable, but are generally untested) indicates an energy calculation and minimised geometry which is qualitative at best (Mohamadi *et al.*, 1990). Tucker *et al.* (1990) and Latypov *et al.* (1995) also noted that MM2 and CV force fields, respectively, lacked accurate torsional parameters for certain bond rotations of α-alkoxy esters. Those authors achieved quantitative relative energies of conformers *via* theoretical molecular orbital (*ab initio*) calculations, or the semiempirical AM1 approach (Dewar *et al.*, 1985).

A Monte Carlo torsion angle search was performed on compound **161**, which has been used to correlate solution and solid-state conformations of MPA esters. Trost *et al.* (1986) showed that the absolute stereochemistry of **161**, determined from the NMR correlative model, was consistent with that established by X-ray crystallography. The crystallographically-determined value of ψ was 29°, in good agreement with the predictive model. The ϕ torsion angle was not mentioned; a QUEST[1] search and GSTAT[2] analysis of the Cambridge Scientific Database (CSD) (Broom *et al.*, 1994), revealed ϕ for **161** was 26.05°.



(161)

The conformational search of compound **161** showed similar results to those obtained crystallographically: conformers within 4 kJ/mol of the global minimum exhibited average torsion angles of ψ = 9.6° and ϕ = 39.9°. The stereo view shown in Figure 5.9 could thus depict a time-averaged representation of the conformational space covered by the freely rotating MPA group.

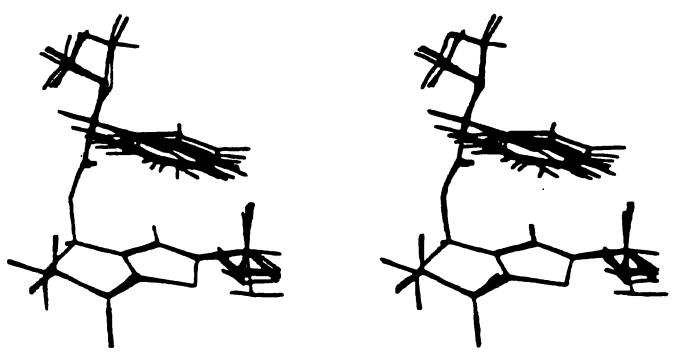


Figure 5.9. Stereo view of overlayed conformations within 4 kJ/mol of the MM2* global minimum of **161**.

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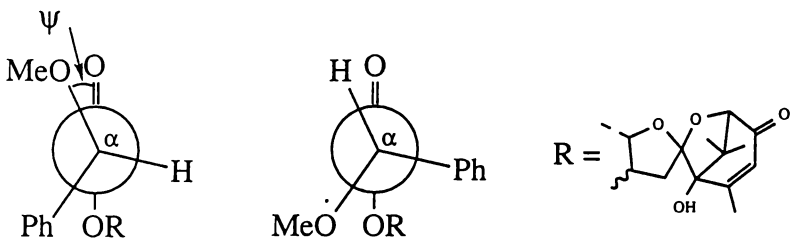
[1] The CSD QUEST package allows the user to search for a defined structural fragment within the CSD and save the search results in the FDAT format.

[2] The GSTAT or VISTA package takes an input from the QUEST search and allows the user to search for defined structural parameters (for example, a defined torsion angle).

Torsion angle searches of **159a-159c** showed two preferred orientations for rotation about the C_α-CO bond; one in which the C=O and C_α-OMe bonds were *synperiplanar* (*sp*) ($\psi = \pm 2\text{-}10°$), and another higher in energy for which those bonds were *antiperiplanar* (*ap*) ($\psi = \pm 160\text{-}175°$) (Figure 5.10). The overall preference for approximately coplanar arrangements of the C_α-OMe and C=O bonds was consistent with MM and *ab initio* calculations of hydroxyacetic acid (Hanson and White, 1988) and methoxyacetic acid (Tucker *et al.*, 1990), and an MM calculation of the MPA ester of acrolein (Tucker *et al.*, 1990).



(a) (b)

Figure 5.10. Energy-minimised conformations of the MPA acid moiety of kamahine esters **159a-159c**. Newman projections viewed along the C_α-CO bond show a) the *synperiplanar* ($\psi = 2\text{-}10°$) and b) the *antiperiplanar* ($\psi = 160\text{-}175°$) orientations of C_α-OMe and C=O bonds)

The preference of the phenyl group to lie approximately perpendicular to the carbonyl group (i.e. a Ph-C_α–C=O dihedral angle near 90°) in the favoured *sp* conformation, is coincident with that proposed for the transition state of Diels-Alder reactions employing MPA as a chiral auxillary (Siegel and Thornton, 1988; Tucker *et al.*, 1990; Tripathy *et al.*, 1991). With reference to the greater stability of the *sp* arrangement, Tucker *et al.* (1990) commented that the distance between methoxyl and carbonyl oxygen atoms in the *sp* form was greater (2.81Å) than that of the methoxyl and carboxyl oxygen atom (2.65Å) of the *ap* form.

Subsequent studies detailing MM (CV force field) and semiempirical AM1 calculations on MPA and AMAA esters of (-)-menthol confirm these esters exist in solution essentially as mixtures of two rotamers, in which the carbonyl and α-methoxy groups are either *sp* (ϕ = 15-30°) or *ap* (ϕ = 130-140°) (Seco *et al.*, 1994; Latypov *et al.*, 1995; 1996). Those authors presented experimental evidence

based on low temperature ^{1}H and ^{13}C NMR spectroscopy in support of proposed solution equilibria.

Seco *et al.* (1995) showed proton differential shielding ($\Delta\delta$) increased with decreasing NMR probe temperature and attributed that observation to a bias of the main equilibrium (*sp/ap*) towards the more stable shielded *sp* rotamer. The dynamics of AMAA esters were studied by Latypov *et al.* (1995) who demonstrated that resonances of separate conformers could be resolved at low temperatures (153 K). In that study, ring current shielding increments (Johnson and Bovey, 1958) of the shielded conformers compared well with experimental values.

The phenyl ring of MPA derivatives **159a-159c** was planar with the C_{α}-H bond, consistent with recent MM calculations of AMAAs (Latypov *et al.*, 1995; 1996). In contrast, MTPA esters show no clear preference for any particular ring orientation (Latypov *et al.*, 1996). This unfavourable conformational characteristic leads to shielding and deshielding effects that partially cancel each other, leading to diminished differential chemical shifts (Latypov *et al.*, 1996).

The methoxyl methyl group was *gauche* to the carbonyl and *anti* to the phenyl ring (Ph-C_{α}–O-Me dihedral angle of *ca* 180°) (Figure 5.11). This conformation minimises both unfavourable steric interactions between the methyl and phenyl, and repulsive electrostatic interactions between the methoxyl lone pairs and the carbonyl oxygen (Tucker *et al.*, 1990). The two alternative *gauche* arrangements of the methyl and phenyl groups were also observed, consistent with low barriers to rotation around the C_{α}-OMe bond (*ca* 4 kJ/mol) reported by Tucker *et al.* (1990) and Latypov *et al.* (1995).

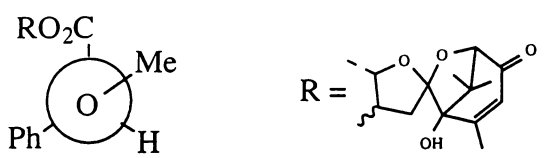


Figure 5.11. Newman projection along the C_{α}-OMe bond showing the preferred conformation of the methoxy methyl group.

The preferred conformation of C-5–H and C=O bonds was *gauche* (i.e. -60° $< \phi <$ +60°) (Figure 5.12), with a magnitude average torsion angle (ϕ) of approximately 40°. Other MM studies of mandelate and AMAA esters of (-)-

menthol also report a *gauche* preference (Ivanov, 1986; Latypov *et al.*, 1995; 1996). This preference is in accord with experimental and theoretical studies which indicate a strong preference for this arrangement in esters of both aliphatic and unsaturated alcohols (Mathieson, 1965; Culvenor, 1966; Pirkle and Hauske, 1976; Wiberg and Laidig, 1987; Mark and Noe, 1989 (and cited references)). The favoured (*Z*) conformation of acids and esters is generally attributed to a stereoelectronic effect related to the anomeric effect (Kirby, 1983; Eliel *et al.*, 1994). Various other factors may be responsible for the conformational preference, including dipole-dipole interactions (Wiberg and Laidig, 1987), and aromaticity (Mark and Noe, 1989).

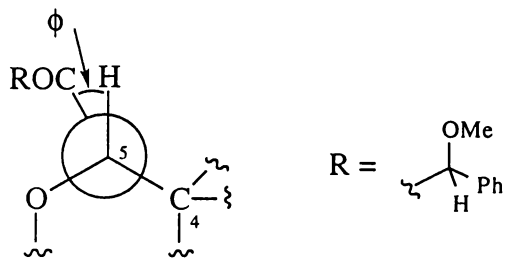


Figure 5.12. Newman projection viewed along the C-5–O bond. The dihedral angle between CO–O and C-5–H bonds was in the range $-60° < \phi < +60°$.

A QUEST search and GSTAT analysis for MTPA and MPA esters in the CSD revealed that for the eight MTPA and two MPA esters in the CSD, the methine proton lay *gauche* to the carbonyl oxygen, with an average magnitude torsion angle between methine and carbonyl group (ϕ_{ave}) of 24° (Broom *et al.*, 1994). This search was updated to include all MPA esters in the database as at March 1998 (VISTA[1] software was used for this torsion angle analysis). Nine MPA esters were found, with a ϕ_{ave} of 29°. A general search of the CSD revealed that for esterified 2-hydroxytetrahydrofurans $|\phi_{ave}|$ was 38°. Twenty-three of the twenty-five entries in the CSD had torsion angles with the carbonyl group rotated towards the tetrahydrofuran oxygen (as in acetate **149c**), with $|\phi_{ave}| = +36.2°$.

Crystallographic evidence suggested the preferred conformation of 2-hydroxytetrahydrofuran esters was that in which the ester carbonyl group was rotated towards the tetrahydrofuran oxygen (i.e. positive ϕ). The significance of conformational conclusions drawn from X-ray data may be questioned, particularly

[1] VISTA is a more recent package which carries out the same analysis as the GSTAT software.

when dealing with groups which involve free rotation. However, it may be argued that a consistent conformational pattern of groups in a range of crystals involving different packing arrangements (i.e. different intermolecular forces), indicates intramolecular stereoelectronic effects associated with the ester structures are of more significance. The crystal structure of acetate **149c** gave a ϕ angle of +36°. In comparison, a torsion angle search of the same compound gave a global minimum structure with ϕ = +43°, indicating good correlation between ϕ (crystal structure) and ϕ (MM2*) in this instance.

The preference for a positive value of ϕ in cyclic hemiacetal esters may reflect a tendency to minimise steric interactions between the carbonyl group and substituents of the ring. In cyclic hemiacetal esters, rotation of the carbonyl group towards the acetal oxygen may be indicative of some stabilising interaction between the two oxygen atoms. Verification of this conformational preference by theoretical quantum mechanics was beyond the scope of the present study. Electronic effects (e.g. orbital interactions) may be more accurately predicted using this approach.

The conformational properties of **159c** are shown in Figure 5.13, which shows the structures and relative steric energies of four clusters of fully optimised conformational minima using the MM2* force field. The clusters exhibit conformers within 3–4 kJ/mol of the equilibrium conformer. Geometrically similar conformations were located from the list of conformers, as this provides an understanding of relationships between the many conformations obtained. This approach is analogous to sampling the various minimum potential energy troughs. The nature of large ensembles of molecular conformations obtained from conformational searches may be analysed in an efficient manner using recently developed cluster analysis software (Shenkin and McDonald, 1994).

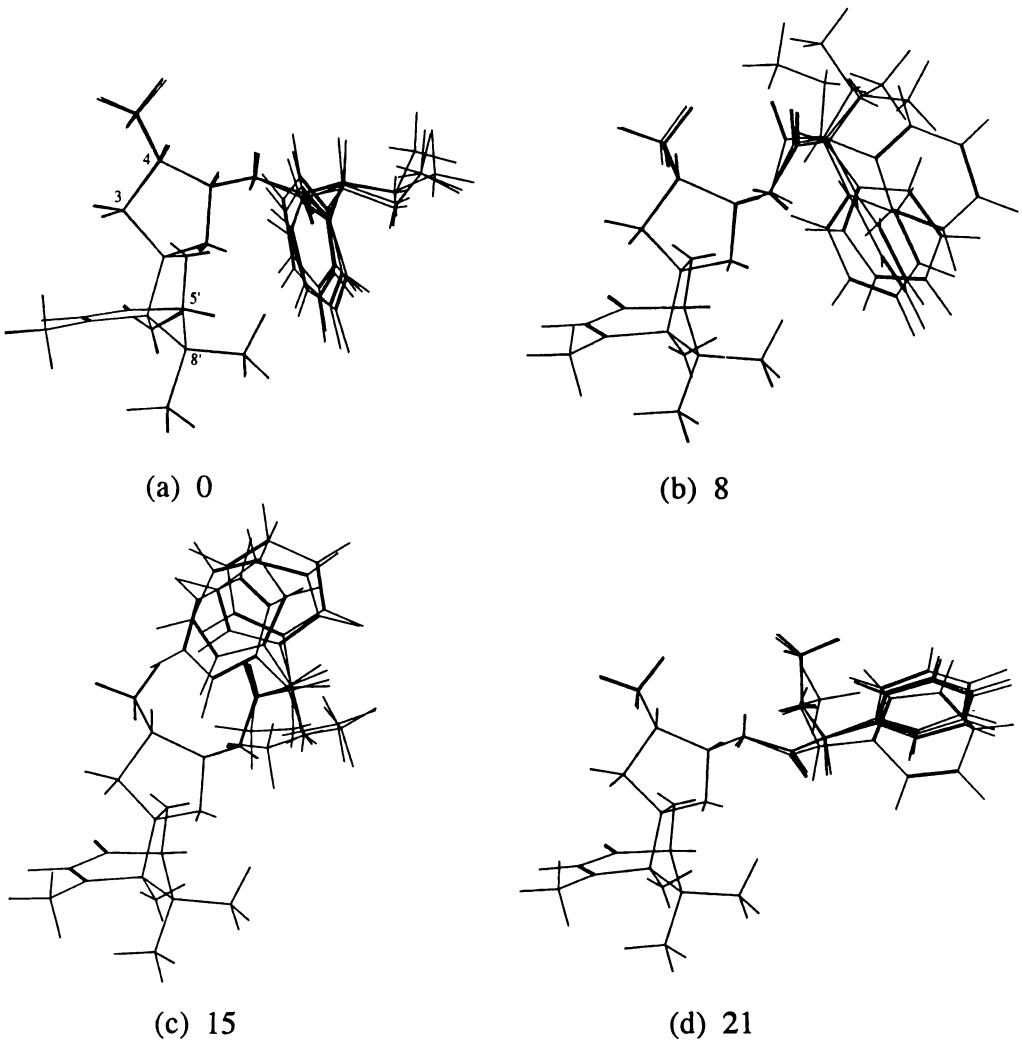


Figure 5.13. Minimum energy conformations of **159c** and their relative steric energies (kJ/mol). Clusters of four conformers within 4 kJ/mol of the equilibrium conformer are shown.

All conformers of **159c** within 8 kJ/mol of the global minimum (Figure 5.13(a)) exhibited the methoxyl oxygen and the ester carbonyl group *sp* (torsion angle ψ of $\pm 10°$), and the carbonyl group rotated towards O-1 of the tetrahydrofuran ring ($+35° < \phi < +60°$, $\phi_{ave} = +40°$). The global minimum exhibited $\phi = +48°$, which compared well with that of the global minimum ($\phi = 43°$) and X-ray crystal structure ($\phi = 36°$) of the corresponding acetate **149c**. The orientation of the phenyl group planar with the C_α-H bond, meant H-5' and 8'-Me were within the positively shielded region of the phenyl ring.

Comparison of the shielding effects predicted by the Mosher-Trost model and MM2*-calculated global minimum conformation of **159c** revealed that the latter structure more closely represented the time-averaged preferred conformation of the MPA group. In the idealised conformation, the phenyl substituent was in a position which predominantly shielded 8'-Me_{eq}, and H-5' to a lesser extent. ^1H NMR chemical shifts however, indicated that the greater shielding effect was experienced at H-5' ($\Delta\delta$ = -0.32 as opposed to $\Delta\delta$ = -0.28 for 8'-Me_{eq}). This observation was better explained by the ester conformation exhibited in the global minimum, where the phenyl group was closer in proximity to H-5'. This effect is illustrated for compound **159c** where the global minimum (ϕ = +48°) and the Mosher-Trost model structure (ϕ = 0°) are overlayed (Figure 5.14).

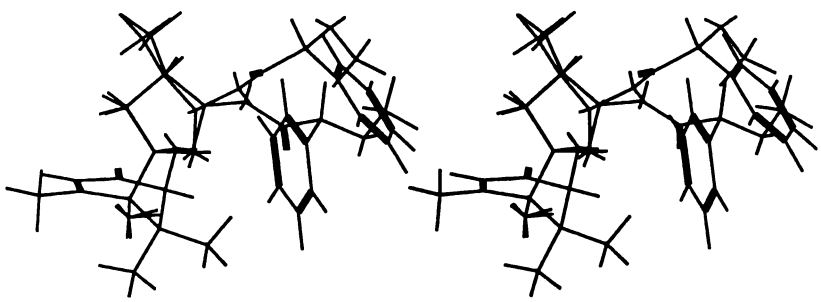


Figure 5.14. Stereo view of model structure (ϕ = 0°) and global minimum (ϕ = +48°) of **159c** overlayed.

The higher relative energy (8 kJ/mol) of *sp* conformer (b) ($-35° < \phi < -60°$) may reflect the increased steric interaction of the carbonyl group with substituents at C-4. Rotating the CO–COMe array to the *ap* arrangement (conformers (c) and (d)) is expected to be much more energetically demanding. The *ap* conformers predicted shielding effects which were much reduced (conformer (d)), or inconsistent with that observed experimentally (conformer (c)).

Results of the fully optimised conformational searches of **159a** showed four conformers, with respect to rotation about the C-5–O bond and C_α-CO bonds in accordance with that observed for **159c**. Clusters of the four geometrically similar conformers of **159a** are shown in Figure 5.15, along with the relative steric energies of the corresponding local minimum conformers. The energy differences between the minima conformations of **159a** and **159c** were significantly different. In contrast, the four local minima observed for **159c**, were

separated by relatively large energies (6-8 kJ/mol), the three local minima of **159a** had energies within 3 kJ/mol. The similarities in *sp/ap* conformer energies may be due to destablilising interactions between the MPA and kamahine moieties. Similar results were obtained in simulations of **159b** (Broom *et al.*, 1994).

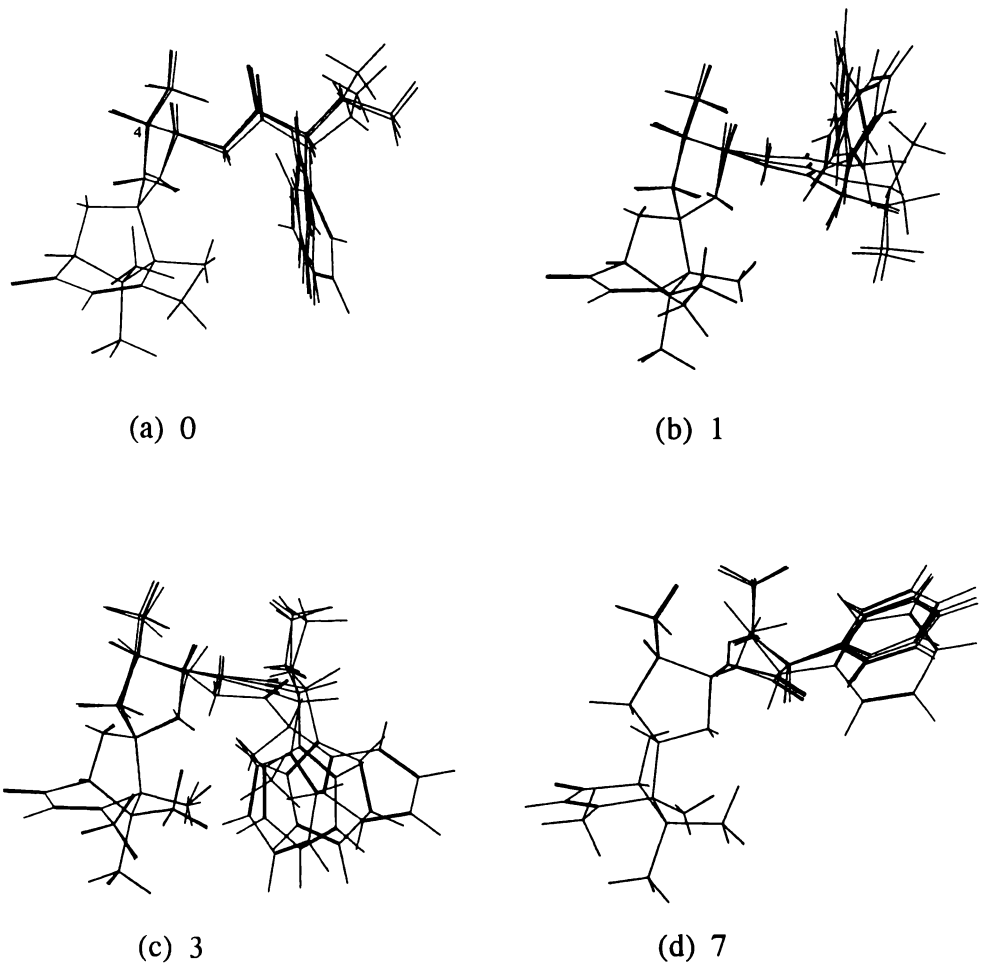


Figure 5.15. Minimum energy conformations of **159a** and their relative energies (kJ/mol). Clusters within 4 kJ/mol of the equilibrium conformer are shown.

The experimentally observed shielding of **159a** could be rationalised in terms of conformational preference. The *sp* conformer (b), exhibiting a ϕ angle of +44°, was consistent with the observed shielding data of **159a**. This result was in accord with cystallographic evidence which indicated a preference for positive ϕ for hemiacetal esters. The global minimum *sp* conformer with ϕ = -45° led to significant changes in the diamagnetic shielding effects to those actually observed.

These variations are significant for compounds such as the kamahines, where small changes in ϕ could result in significant changes in the magnitude of the observed $\Delta\delta$ values. Shown in Figure 5.16 is an overlay of the two lowest energy conformers for **159a**.

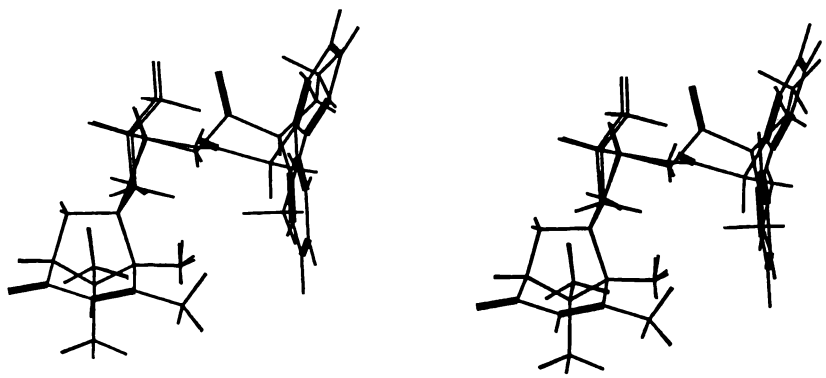


Figure 5.16. Stereo views of an overlay of two lowest minima for **159a**. The conformer with $\phi = +44°$ most closely correlates to the observed $\Delta\delta$ values.

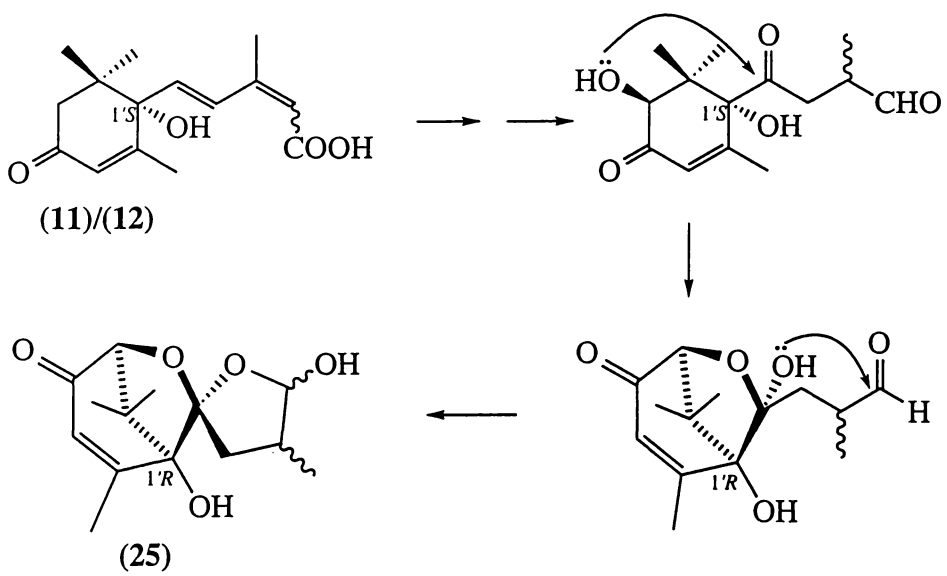
The differences in shielding predicted for **159a** in particular, suggested that Mosher-Trost rules should be applied with caution, particularly in cases where the compounds under investigation have structures which may give diamagnetic shielding results sensitive to small conformational changes in the MPA moiety. Since this study was reported (Broom *et al.*, 1994), a MM study was used to assess the origin of $\Delta\delta$ trends in MTPA esters of sesquiterpene hemiacetals (Guella *et al.*, 1996).

NOE-difference experiments were performed on MPA esters **159a** and **159c** in an attempt to corroborate the global minimum conformations. Various other investigators have sought to verify the conformational models by NOE studies (Adamczeski, 1990; Izumi *et al.*, 1994; Fukushi *et al.*, 1994b) These studies have demonstrated that stereochemically significant NOEs can be observed in MPA and MTPA esters, although the correlations are weak (internuclear distances of *ca* 3 Å are reported in one study). NOE-difference spectra obtained by irradiation of the methine proton of the acid moiety in **159a** and **159c**, showed poor signal to noise. The NOEs were weak as distances between the observed protons were greater than 4 Å. It was not possible to conclude the dominant conformer in solution based on those spectra.

The contribution of different conformers (a)-(d) to the NMR exchange mixture in **159a** and **159c**, could be confirmed by dynamic NMR (DNMR) studies, as has been recently reported by Seco *et al.* (1995) and Latypov *et al.* (1995). DNMR evidence could be used to support the crystallographic and MM2* data, which suggest the most significant conformation of the hemiacetal MPA ester on the NMR time scale is represented by a ϕ angle of *ca* +25-50° and a ψ angle of ±10°.

5.2.5. Biosynthetic Origin of Kamahine (25) – Stereochemical Considerations

ABA is a likely precursor of kamahine based on the structural similarities of the two compounds (Chapter Four). Formation of kamahine from ABA requires cyclisation of the side chain, a step in which C-1' does not undergo transformation. Naturally occurring ABA, which has the 1'*S* configuration (Koreeda *et al.*, 1973; Harada *et al.*, 1973), gives rise to enantiomeric kamahines with the 1'*R* configuration (owing to changes in IUPAC priorities at this position) (Scheme 5.1). Application of Mosher-Trost protocol established that all three diastereoisomeric kamahines have the 1'*R* configuration.



Scheme 5.1. Stereochemical aspects of the transformation of (*S*)-(+)-ABA **(11)/(12)** to kamahine **(25)**.

In plants, regioselective attack of 3-hydroxy-5,6-epoxy carotenoids affords xanthoxin **(124)**, a precursor to ABA. The 1'*S* absolute configuration of ABA is

consistent with both the established absolute configuration of these carotenoids (3*S*,5*R*,6*S*) (Bartlett *et al.*, 1969), and the probable mechanism for epoxide opening of xanthoxin (retention of configuration at C-6). All 5,6-epoxy-β end-groups have been shown to have the 5*R*,6*S* configuration (Britton, 1989). Thus, no distinction can be made between ABA and 5,6-epoxy xanthophylls as potential precursors of kamahine. Stereochemical considerations indicated that known (3*S*,5*R*,6*R*)-trihydroxy xanthophylls (Chapter Three) were not precursors of kamahine. The probable pathway between these two compounds involves oxidation at C-3 and dehydration at C-4–C-5, giving rise to kamahine with the *S* configuration at C-1'.

5.3. Conclusions

(S)- and *(R)*-MPA derivatives of hemiacetals are readily prepared by DCC coupling using DMAP. ^{1}H and ^{13}C NMR of (*S*)- and (*R*)-MPA esters of the kamahines indicated the parent compounds **25a-25c** were single enantiomers. MPA derivatives were characterised by 2D DQFCOSY and XHCORR, and NOE-difference experiments.

The absolute stereochemistry of **25a-25c** was determined by analysis of the ^{1}H NMR nonequivalence of the corresponding (*S*)- and (*R*)-MPA esters using the extended Mosher-Trost configurational model. A systematic arrangement of $\Delta\delta$ values were observed for each diastereoisomeric pair, consistent with that established for MPA derivatives of secondary alcohols. The configuration of the ester-bearing carbon, coupled with the relative stereochemistries of **25a-25c** established in Chapter Four, indicated the kamahines had the absolute configurations (1'*R*,2*R*,4*R*,5*S*,5'*S*)-**25a**, (1'*R*,2*R*,4*S*,5*S*,5'*S*)-**25b** and (1'*R*,2*R*,4*R*,5*R*,5'*S*)-**25c**.

Monte Carlo torsion angle searches of (*S*)-MPA esters **159a-159c** indicated the preferred conformation of the ester moiety was that in which the methoxyl oxygen and the ester carbonyl group were approximately *synperiplanar* ($\psi = \pm 10°$). A *gauche* conformation was preferred between the proton at the ester carbon and carbonyl group ($-60° < \phi < +60°$). The crystallographic and MM2* data lead to the conclusion that the most significant conformation of the ester on the NMR timescale was probably best represented by a ϕ angle of *ca* +25-50° and a ψ angle of ±10° rather than the predictive model of $\phi = 0°$. The phenyl group of the

MPA moiety in hemiacetal esters **156a-159c** was in the same relative position to that observed for secondary alcohols, supporting extrapolation of the correlative model to hemiacetals.

Results of this study suggested that Mosher-Trost rules should be applied with caution, particularly in cases where the compounds under investigation have structures which may give diamagnetic shielding results sensitive to small conformational changes in the MPA moiety.

The absolute configuration of the kamahines was consistent with an ABA or 3-hydroxy-5,6-epoxy carotenoid precursor.

5.4. Experimental

All spectral data detailed in this Section were acquired as described in the Experimental Section of Chapter Two.

Molecular modeling was performed on a Silicon Graphics IRIS Indigo XS24 workstation running MacroModel V3.5x (Department of Chemistry, Columbia University). Monte Carlo torsion angle searches were performed *in vacuo* (essentially the same results were obtained with GB/SA chloroform solvent included). Each search consisted of the generation and minimisation of 300 structures, with a conformer being kept if it fell within 50 kJ/mol of the current global minimum and did not duplicate any previously stored conformer. Typically, each simulation would generate about 120 unique conformers. This process was repeated twice, each time starting from a conformer chosen arbitrarily from the most recent coordinate set to test for convergence of the simulation. A full search would typically require about 6 h of CPU time.

QUEST searches and VISTA analyses were carried out using CSD software (April 1998 release).

(*S*)- and (*R*)-MPA derivatives of **25a-25c** (10 mg) were prepared using the general esterification procedure outlined in the Experimental Section of Chapter Three. The crude products were purified by PLC using 1:1 Et_2O:PE and the resulting bands extracted with Et_2O. Each MPA derivative was obtained as a

colourless oil. (*S*)-MPA derivatives **159a-159c** eluted as three well-resolved bands:-

*(S)-MPA ester **159c*** (R_f = 0.53), (4 mg, 26%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.83 (3H, s, 8'-Me_{ax}), 0.89 (3H, s, 8'-Me_{eq}), 1.10 (3H, d, $J_{4\text{-}Me,4}$ = 6.9 Hz, 4-Me), 1.58 (1H, dd, $J_{3a,3b}$ = 12.9, $J_{3a,4}$ = 9.5 Hz, H-3_a), 2.01 (3H, d, $J_{2'\text{-}Me,3'}$ = 1.5 Hz, 2'-Me), 2.06 (1H, dd, $J_{3b,3a}$ = 12.9, $J_{3b,4}$ = 7.1 Hz, H-3_b), 2.55 (1H, m, H-4), 2.71 (1H, s(br.), OH), 3.43 (3H, s, O<u>Me</u>), 3.60 (1H, d, $J_{5',3'}$ = 2.2 Hz, H-5'), 4.80 (1H, s, -C<u>H</u>Ph(OMe)), 5.85 (1H, dq, $J_{3',5'}$ = 2.2, $J_{3',2'\text{-}Me}$ = 1.5 Hz, H-3'), 5.96 (1H, d, $J_{5,4}$ = 4.1 Hz, H-5), 7.34–7.48 (5H, m, H-Ar). ^{13}C NMR (75.5 MHz) δ: 16.65 (q, 4-Me), 18.00 (q, 8'-Me_{ax}), 20.20 (q, 2'-Me), 21.52 (q, 8'-Me_{eq}), 36.89 (d, C-4), 40.62 (t, C-3), 51.84 (s, C-8'), 57.39 (s, O<u>Me</u>), 82.27 (d, OCO<u>C</u>HPh(OMe)), 85.00 (s, C-1'), 89.64 (d, C-5'), 103.83 (d, C-5), 114.67 (s, C-7'), 125.36 (d, C-3'), 127.39–128.74 (d, 4 x C-Ar), 135.89 (s, C-Ar), 164.52 (s, C-2'), 170.38 (s, -O<u>C</u>OCHPh(OMe)), 194.72 (s, C-4'). IR: υ 3466 (OH), 2922 (CH), 1753 (C=O, ester), 1680 cm^{-1} (C=O, α,β-unsaturated ketone). UV (EtOH): λ_{max} 214 nm (log ε 3.3). EIMS data for **159a-159c** and **160a-160c**: *m/z* 251 (30%, $M^{+\cdot}$-165 (-OCOCHPh(OMe)), 152 (20), 121 (100), 99 (25), 77 (25), 43 (60).

*(S)-MPA ester **159a*** (R_f = 0.59), (8 mg, 52%). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.54 (3H, d, $J_{4\text{-}Me,4}$ = 6.8 Hz, 4-Me), 1.00 (3H, s, 8'-Me_{ax}), 1.27 (3H, s, 8'-Me_{eq}), 1.55 (1H, t, $J_{3a,3b}$ = $J_{3a,4}$ = 13.0 Hz, H-3_a), 1.76 (1H, dd, $J_{3b,3a}$ = 13.0, $J_{3b,4}$ = 7.0 Hz, H-3_b), 2.10 (3H, d, $J_{2'\text{-}Me,3'}$ = 1.4 Hz, 2'-Me), 2.50 (1H, m, H-4), 2.60 (s(br.), OH), 3.45 (3H, s, O<u>Me</u>), 3.95 (1H, d, $J_{5',3'}$ = 2.2 Hz, H-5'), 4.76 (1H, s, OC<u>H</u>Ph(OMe)), 5.90 (1H, dq, $J_{3',5'}$ = 2.2, $J_{3',2'\text{-}Me}$ = 1.4 Hz, H-3'), 6.36 (1H, d, $J_{5,4}$ = 4.8 Hz, H-5), 7.35–7.48 (5H, m, H-Ar). ^{13}C NMR (75.5MHz) δ: 11.28 (q, 4-Me), 17.97 (q, 8'-Me_{ax}), 20.46 (q, 2'-Me), 21.68 (q, 8'-Me_{eq}), 36.35 (d, C-4), 38.21 (t, C-3), 51.86 (s, C-8'), 57.44 (s, O<u>Me</u>), 82.41 (d, -CO<u>C</u>HPh(OMe)), 84.53 (s, C-1'), 90.00 (d, C-5'), 100.35 (d, C-5), 116.02 (s, C-7'), 125.36 (d, C-3'), 127.33–129.02 (d, 4 x C-Ar), 136.13 (s, C-Ar), 165.15 (s, C-2'), 169.29 (s, O<u>C</u>OCHPh(OMe)), 194.36 (s, C-4'). IR: υ 3488 (OH), 2933 (CH and ArH), 1756 (C=O, ester), 1683 cm^{-1} (C=O, α,β-unsaturated ketone). UV (EtOH): λ_{max} 229 nm (log ε 3.4).

*(S)-MPA ester **159b*** (R_f = 0.65), (2 mg, 13%). ^{1}H NMR (300 MHz, $CDCl_3$), δ: 1.00 (3H, s, 8'-Me_{ax}), 1.10 (3H, d, $J_{4\text{-}Me,4}$ = 7.2 Hz, 4-Me), 1.28 (3H, s, 8'-Me_{eq}), 1.50 (1H, d, $J_{3b,3a}$ = 13.0 Hz, H-3_b), 1.95 (1H, d, $J_{4,3a}$ = 7.5 Hz, H-4), 1.97 (1H, dd $J_{3a,3b}$ = 13.0, $J_{3a,4}$ = 7.5 Hz, H-3_a), 2.00 (3H, d, $J_{2'}$-

$_{Me,3'}$ = 1.6 Hz, 2'-Me), 2.62 (s(br.), OH), 3.42 (3H, s, OMe), 3.96 (1H, d, $J_{5',3'}$ = 2.2 Hz, H-5'), 4.75 (1H, s, OCOCHPh(OMe)), 5.87 (1H, dq, $J_{3',5'}$ = 2.2, $J_{3',2'\text{-Me}}$ = 1.6 Hz, H-3'), 6.10 (1H, s, H-5), 7.35–7.43 (5H, m, H-Ar). IR: υ 3389 (OH), 2922 (CH), 2856 (ArH), 1744 (C=O, ester), 1672 cm^{-1} (C=O, α,β-unsaturated ketone). UV (EtOH): λ_{max} 217 nm (log ε 3.1).

(*R*)-MPA derivatives of **25a-25c** eluted as two well-resolved bands:-*(R)-MPA ester* ***160c*** (R_f = 0.48), (5 mg, 32%). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.95 (3H, d, $J_{4\text{-Me},4}$ = 6.9 Hz, 4-Me), 0.98 (3H, s, 8'-Me_{ax}), 1.17 (3H, s, 8'-Me_{eq}), 1.61 (1H, dd, $J_{3a,3b}$ = 13.1, $J_{3a,4}$ = 8.9 Hz, H-3_a), 2.05 (3H, d, $J_{2'\text{-Me},3'}$ = 1.5 Hz, 2'-Me), 2.09 (1H, dd, $J_{3b,3a}$ = 13.1, $J_{3b,4}$ = 7.5 Hz, H-3_b), 2.44 (1H, m, H-4), 2.76 (s(br.), OH), 3.42 (3H, s, OMe), 3.92 (1H, d, $J_{5',3'}$ = 2.1 Hz, H-5'), 4.83 (1H, s, OCOCHPh(OMe)), 5.85 (1H, d, $J_{5,4}$ = 4.2 Hz, H-5), 5.90 (1H, dq, $J_{3'5'}$ = 2.1, $J_{3',2'\text{-Me}}$ 1.5 Hz, H-3'), 7.32–7.48 (5H, m, H-Ar). ^{13}C NMR (75.5 MHz) δ: 16.15 (q, 4-Me), 18.10 (q, 8'-Me_{ax}), 20.26 (q, 2'-Me), 21.73 (q, 8'-Me_{eq}), 36.87 (d, C-4), 40.38 (t, C-3), 52.00 (s, C-8'), 57.39 (s, OMe), 82.49 (d, OCOCHPh(OMe)), 84.89 (s, C-1'), 89.91 (d, C-5'), 104.09 (d, C-5), 114.92 (s, C-7'), 125.41 (d, C-3'), 127.34–128.89 (d, 4 x C-Ar), 135.89 (s, C-Ar), 164.67 (s, C-2'), 170.46 (s, OCOCHPh(OMe)), 194.64 (s, C-4'). IR: υ 3464 (OH), 2932 (CH), 2855 (ArH), 1744 (C=O, ester), 1680 cm^{-1} (C=O, α,β-unsaturated ketone). UV (EtOH): λ_{max} 198 (log ε 6.0), 240 nm (log ε 3.4).

The remaining band (R_f = 0.55) contained a 2:1 mixture of (*R*)-MPA **160a** and **160b**. Multiple-elution PLC using 1:1 then 2:3 Et_2O:PE afforded a sample of **160a** (6 mg, 39%) and a sample containing both **160a** and **160b** (3 mg, 19%). ^{1}H and ^{13}C NMR data for **160b** was taken from the mixture **160a/160b**.

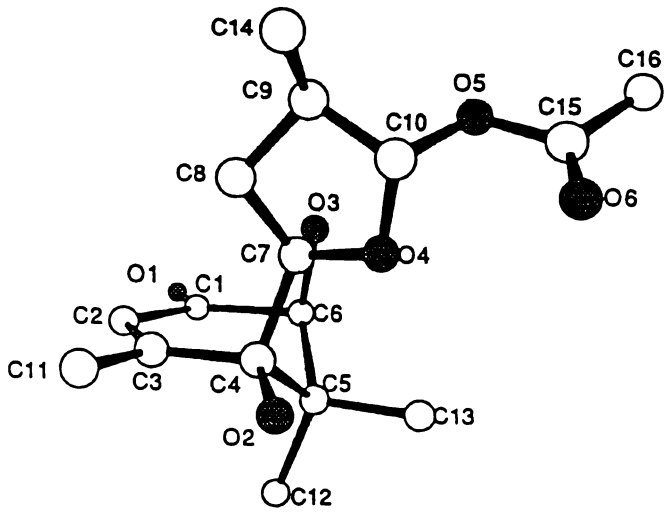
(R)-MPA ester ***160a***. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.92 (3H, d, $J_{4\text{-Me},4}$ = 6.8 Hz, 4-Me), 0.94 (3H, s, 8'-Me_{ax}), 1.14 (3H, s, 8-Me_{eq}), 1.60 (1H, t, $J_{3a,3b}$ = $J_{3a,4}$ = 12.6 Hz, H-3_a), 1.81 (1H, dd, $J_{3b,3a}$ = 12.6, $J_{3b,4}$ = 6.5 Hz, H-3_b), 2.01 (3H, d, $J_{2'\text{-Me},3'}$ = 1.6 Hz, 2'-Me), 2.57 (1H, m, H-4), 3.44 (3H, s, OMe), 3.91 (1H, d, $J_{5'3'}$ = 2.1 Hz, H-5'), 4.76 (1H, s, OCOCHPh(OMe)), 5.87 (1H, dq, $J_{3',5'}$ = 2.1, $J_{3',2'\text{-Me}}$ = 1.6 Hz, H-3'), 6.39 (1H, d, $J_{5,4}$ = 4.6 Hz, H-5), 7.35–7.47 (5H, m, H-Ar). ^{13}C NMR (75.5 MHz) δ: 11.94 (q, 4-Me), 17.94 (q, 8'-Me_{ax}), 20.43 (q, 2'-Me), 21.56 (q, 8'-Me_{eq}), 36.03 (d, C-4), 38.26 (t, C-3), 51.80 (s, C-8'), 57.47 (s, OMe), 82.63 (d, OCOCHPh(OMe)), 84.29 (s, C-1'), 89.96 (d, C-5'), 100.15 (d, C-5), 115.86 (s, C-7'), 125.25 (d, C-3'), 127.08–129.07 (d, 4 x C-Ar), 136.03 (s, C-Ar), 165.50 (s, C-2'), 169.47 (s,

OCOCHPh(OMe)), 194.49 (s, C-4'). IR (mixture **160a/160b**): υ 3625 (OH), 2934 (CH), 1750 (C=O, ester), 1680 cm^{-1} (C=O, α,β–unsaturated ketone). UV (EtOH) (mixture **160a/160b**): λ_{max} 216 (log ε 3.4), 250 nm (log ε 3.5).

*(R)-MPA ester **160b***. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.95 (3H, s, 8'-Me_{ax}), 1.15 (3H, d, $J_{4\text{-}Me,4}$ = 6.8 Hz, 4-Me), 1.15 (3H, s, 8'-Me_{eq}), 1.65 (1H, d, $J_{3b,3a}$ = 12.5 Hz, H-3_b), 2.00 (3H, d, $J_{2'\text{-}Me,3'}$ = 1.4 Hz, 2'-Me), 2.15 (1H, d, $J_{4,3a}$ = 7.5 Hz, H-4), 2.16 (1H, dd, $J_{3a,3b}$ = 12.6, $J_{3a,4}$ = 7.5 Hz, H-3_a), 3.44 (3H, s, OMe), 3.92 (1H, d, $J_{5',3'}$ = 1.9 Hz, H-5'), 5.23 (1H, s, OCOCHPh(OMe)), 5.87 (1H, dq, $J_{3',5'}$ = 1.9, $J_{3',2'\text{-}Me}$ = 1.4 Hz, H-3'), 6.14 (1H, s, H-5), 7.35–7.47 (5H, m, H-Ar). ^{13}C NMR (75.5 MHz) δ: 16.66 (q, 4-Me), 18.00 (q, 8'-Me_{ax}), 20.51 (q, 2'-Me), 21.58 (q, 8'-Me_{eq}), 37.57 (t, C-3), 38.16 (d, C-4), 51.61 (s, C-8'), 57.45 (s, OMe), 82.70 (d, OCOCHPh(OMe)), 84.88 (s, C-1'), 90.10 (d, C-5'), 104.86 (d, C-5), 117.50 (s, C-7'), 125.24 (d, C-3'), 127.16–129.10 (d, 4 x C-Ar), 135.90 (s, C-Ar), 165.44 (s, C-2'), 169.50 (s, OCOCHPh(OMe)), 194.73 (s, C-4').

Appendix One

X-ray Crystallographic Data



I. X-ray crystal structure of acetyl-kamahine C (**149c**) showing crystallographic numbering.

II. Final positional and thermal parameters determined for **149c**.

Atom	x	y	z	U
O-1	0.0751(7)	0.5190(5)	-0.2353(5)	0.032(2)
O-2	0.2355(7)	0.5770(5)	0.1442(4)	0.024(2)
O-3	0.0119(7)	0.4217(5)	-0.0176(4)	0.024(2)
O-4	0.0383(7)	0.4177(5)	0.1531(4)	0.021(2)
O-5	-0.1275(7)	0.2822(4)	0.1675(5)	0.023(2)
O-6	-0.1885(7)	0.3487(5)	0.3136(5)	0.034(2)
C-1	0.117(1)	0.5178(7)	-0.1478(7)	0.026(3)
C-2	0.277(1)	0.5175(7)	-0.1184(7)	0.027(3)
C-3	0.312(1)	0.5299(7)	-0.0229(7)	0.025(3)
C-4	0.186(1)	0.5359(7)	0.0537(7)	0.020(2)
C-5	0.045(1)	0.5935(7)	0.0124(7)	0.025(3)
C-6	-0.001(1)	0.5173(6)	-0.0652(7)	0.021(2)
C-7	0.118(1)	0.4290(6)	0.0628(7)	0.017(2)
C-8	0.227(1)	0.3411(6)	0.0627(7)	0.020(2)
C-9	0.126(1)	0.2584(7)	0.1037(7)	0.022(2)
C-10	0.029(1)	0.3151(7)	0.1767(7)	0.026(2)
C-11	0.477(1)	0.5337(7)	0.0134(7)	0.034(3)
C-12	0.086(1)	0.6984(7)	-0.0273(8)	0.033(3)
C-13	-0.086(1)	0.6067(7)	0.0877(7)	0.032(3)
C-14	0.212(1)	0.1723(8)	0.1489(8)	0.040(3)
C-15	-0.229(1)	0.3030(7)	0.2414(7)	0.017(2)
C-16	-0.386(1)	0.2634(8)	0.2204(8)	0.034(3)

III. Final positional and thermal parameters of calculated hydrogen atoms of **149c**.

Atom	x	y	z	U
H-2	0.359(1)	0.5083(7)	-0.1671(7)	0.044
H-6	-0.104(1)	0.5307(6)	-0.0908(7)	0.044
H-81	0.260(1)	0.3258(6)	-0.0041(7)	0.051
H-82	0.317(1)	0.3531(6)	0.1041(7)	0.051
H-9	0.069(1)	0.2232(7)	0.0528(7)	0.044
H-101	0.066(1)	0.3047(7)	0.2439(7)	0.044
H-111	0.485(1)	0.5402(7)	0.0846(7)	0.070
H-112	0.529(1)	0.5895(7)	-0.0183(7)	0.070
H-113	0.526(1)	0.4720(7)	-0.0074(7)	0.070
H-121	0.171(1)	0.6930(7)	-0.0745(8)	0.070
H-122	0.122(1)	0.7327(7)	0.0315(8)	0.070
H-123	0.001(1)	0.7356(7)	-0.0566(8)	0.070
H-131	-0.116(1)	0.5433(7)	0.1168(7)	0.070
H-132	-0.178(1)	0.6387(7)	0.0604(7)	0.070
H-133	-0.041(1)	0.6491(7)	0.1381(7)	0.070
H-141	0.279(1)	0.1423(8)	0.0991(8)	0.070
H-142	0.136(1)	0.1237(8)	0.1713(8)	0.070
H-143	0.275(1)	0.1939(8)	0.2045(8)	0.070
H-161	-0.466(1)	0.2856(8)	0.2659(8)	0.070
H-162	-0.371(1)	0.1921(8)	0.2279(8)	0.070
H-163	-0.418(1)	0.2779(8)	0.1531(8)	0.070

IV. Bond lengths determined for **149c**.

Bond Lengths (Å).			
O-1 – C-1	1.25(1)	C-3 – C-4	1.51(1)
O-2 – C-4	1.42(1)	C-3 – C-11	1.51(1)
O-3 – C-6	1.45(1)	C-4 – C-5	1.56(1)
O-3 – C-7	1.44(1)	C-4 – C-7	1.57(1)
O-4 – C-7	1.42(1)	C-5 – C-6	1.53(1)
O-4 – C-10	1.43(1)	C-5 – C-12	1.56(1)
O-5 – C-10	1.43(1)	C-5 – C-13	1.54(1)
O-5 – C-15	1.37(1)	C-7 – C-8	1.51(1)
O-6 – C-15	1.22(1)	C-8 – C-9	1.52(1)
C-1 – C-2	1.45(1)	C-9 – C-10	1.51(1)
C-1 – C-6	1.52(1)	C-9 – C-14	1.51(1)
C-2 – C-3	1.35(1)	C-15 – C-16	1.49(1)

V. Bond angles determined for **149c**.

Bond Angles (°).			
C-6 – O-3 – C-7	109.2(7)	C-12 – C-5 – C-13	107.1(8)
C-7 – O-4 – C-10	109.1(7)	O-3 – C-6 – C-1	106.6(7)
C-10 – O-5 – C-15	118.6(7)	O-3 – C-6 – C-5	105.8(7)
O-1 – C-1 – C-2	122.8(9)	C-1 – C-6 – C-5	109.8(8)
O-1 – C-1 – C-6	121.3(9)	O-3 – C-7 – O-4	110.0(7)
C-2 – C-1 – C-6	115.9(8)	O-3 – C-7 – C-4	104.1(7)
C-1 – C-2 – C-3	118.9(9)	O-3 – C-7 – C-8	110.0(7)
C-2 – C-3 – C-4	120.8(9)	O-4 – C-7 – C-4	110.5(7)
C-2 – C-3 – C-11	122.2(9)	O-4 – C-7 – C-8	102.5(7)
C-4 – C-3 – C-11	116.9(8)	C-4 – C-7 – C-8	119.7(7)
O-2 – C-4 – C-3	113.9(7)	C-7 – C-8 – C-9	103.1(7)
O-2 – C-4 – C-5	110.7(7)	C-8 – C-9 – C-10	100.7(7)
O-2 – C-4 – C-7	113.9(7)	C-8 – C-9 – C-14	116.0(8)
C-3 – C-4 – C-5	110.1(7)	C-10 – C-9 – C-14	113.2(8)
C-3 – C-4 – C-7	106.0(8)	O-4 – C-10 – O-5	109.6(7)
C-5 – C-4 – C-7	101.5(7)	O-4 – C-10 – C-9	108.3(8)
C-4 – C-5 – C-6	96.6(7)	O-5 – C-10 – C-9	108.1(8)
C-4 – C-5 – C-12	113.8(8)	O-5 – C-15 – O-6	121.5(8)
C-4 – C-5 – C-13	113.3(7)	O-5 – C-15 – C-16	111.7(8)
C-6 – C-5 – C-12	115.6(8)	O-6 – C-15 – C-16	126.9(9)
C-6 – C-5 – C-13	110.4(8)		

Appendix Two
Published Papers

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