SPECTROSCOPIC AND SYNTHETIC STUDIES OF LICHEN METABOLITES

A thesis

submitted to the

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by

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TO

my father

ABSTRACT

Lichen metabolites from various *Nephroma* and *Pseudocyphellaria* species have been subjected to a number of spectroscopic and synthetic investigations. Hypoiodite reaction of 15α-acetoxyhopan-17(21)-en-24-ol yielded a complex mixture of products, while similar reaction of 7-oxo-hopan-22-ol resulted in cleavage of the side-chain. Preparation of an α-sulphenylated 15-ketone was achieved as the first step in a carbonyl transposition sequence. Complete assignment of the ^{13}C nmr spectra of four pentacyclic and two ring A-cleaved stictane derivatives provided evidence of the boat-ring B. The low frequency infrared spectra of a number of stictane and hopane triterpenoids yielded structurally significant correlations. Two depsidonal constituents of *N. australe* were demonstrated to be hypostictic acid and hyposalazinic acid. The reactions of mercuric acetate with unsaturated compounds are reviewed and anomalous products of Jones reagent oxidation investigated.

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ACKNOWLEDGEMENTS

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I am grateful to my sister Elisabeth and my parents, especially my father, for their assistance in checking.

Finally, it is my pleasure to thank Mrs M. V. Quilter for cheerfully enduring the frustrations of typing this thesis.

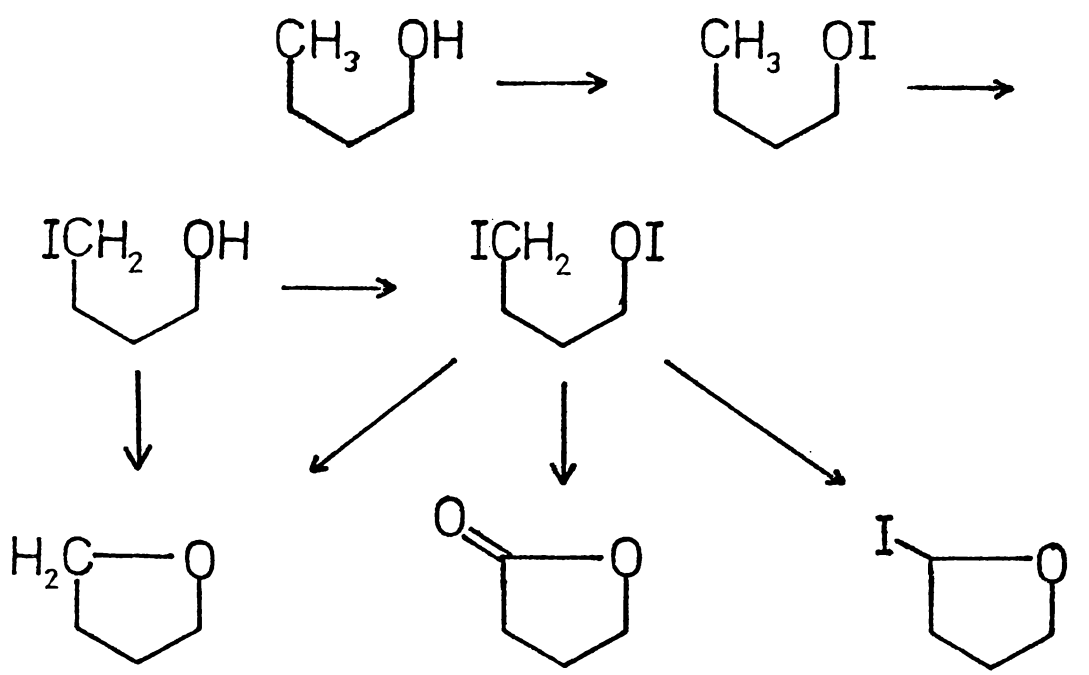
CHAPTER ONE

HYPOIODITE REACTIONS

1.1 Introduction

The mechanism of the hypoiodite reaction has been intensively studied by several workers[1-5], so need not be discussed in detail here. Suffice it to say that hypoiodite oxidation involves the homolytic cleavage of an alkyl hypoiodite, derived from an hydroxyl group. The resulting alkoxy radical abstracts an appropriately placed δ-hydrogen atom and intramolecular substitution ensues (Figure 1).

FIGURE 1

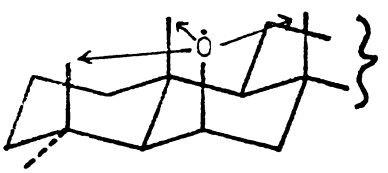


The alkyl hypoiodites, being transient in nature, are prepared *in situ* from the alcohol by the action of N-iodosuccinimide or a metal [Ag(I), Hg(II) or Pb(IV)] acetate and iodine.

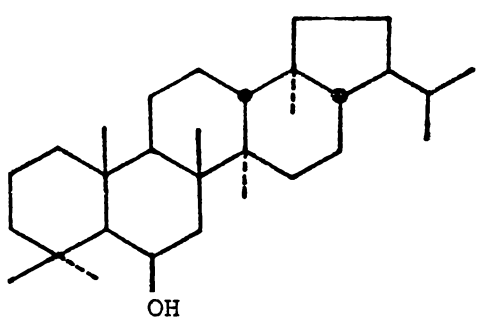
In the course of an investigation of the reactions of a variety

of triterpenoidal alcohols under the conditions of the hypoiodite reaction, Cong[6] obtained two cyclic ethers in approximately equal proportions from 21αH-hopan-6β-ol (1). Predictions based on the course of previous hypoiodite reactions suggested that substitution could have occurred at any or all of three sites, as indicated in Figure 2.

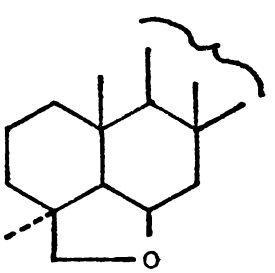
FIGURE 2



Initially the products were thought to be 6β,24-epoxy-21αH-hopane (2) and 6β,25-epoxy-21αH-hopane (3) but subsequent high resolution mass spectrometry, which was performed after the completion of the present investigation, has revealed that the former product was 6β,26-epoxy-21αH-hopane (4).



(1)



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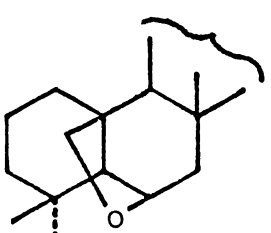

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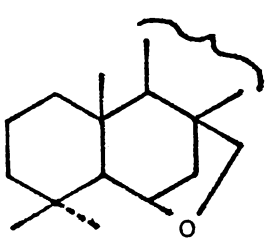
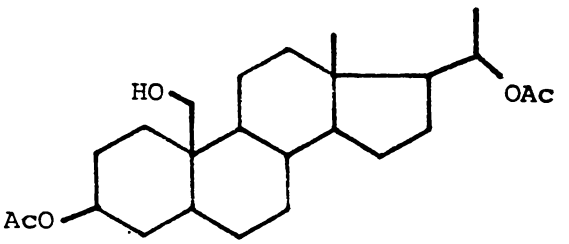


(4)

It appears that two factors would influence the product distribution in this instance, (a) the conformational rigidity of the non-activated carbon centre[5] and (b) the favourableness of the formation of a 6-membered transition state in chair conformation[2]. The results of this hypoiodite reaction suggested the former consideration to be the most important, since although only substitution on C-24 would permit a 6-membered transition state to adopt an unhindered chair conformation, yet there is no significant degree of substitution at this position. Instead, it was the more constrained methyl groups (10β- and 8β-), at bridgehead sites, which were substituted.

Recently[8,9] a new lichen metabolite, 15α-acetoxy-22-hydroxy-hopan-24-oic acid (5), has been isolated in these laboratories. Before the nature of the cyclic ether products obtained from (1) were established this metabolite (5), having the 4β-methyl group functionalised to a carboxyl group, was considered to provide a potential source of a 6β,24-epoxyhopane derivative by a reverse hypoiodite reaction on the 24-alcohol, obtained by reduction of the carboxyl group. Thus the original object of this investigation was to compare any cyclic ether products obtained from the hypoiodite reaction of the 24-hydroxyl group with the cyclic ethers obtained by Cong[6] from (1), in order to establish the structure of one or more of the products.

Most hypoiodite oxidations have been performed on secondary[2] hydroxyl groups attached to ring carbon atoms. However, there is an example[10] of intramolecular substitution by a primary hydroxymethyl group, in which the treatment of 3β,20β-diacetoxypregnan-19-ol (6) with lead tetraacetate and iodine yielded 3β,20β-diacetoxy-8β,19-epoxypregn-14(15)-ene (7) as the major product together with a small amount of 3β,20β-diacetoxy-11β,19-epoxypregnane (8).



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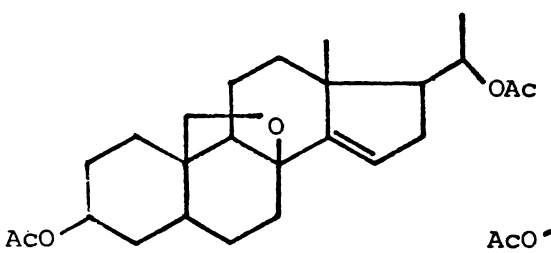

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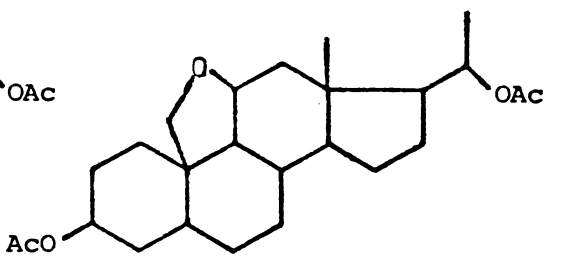


(8)

Whereas the hydroxyl group in (6) is on a bridgehead methyl group a 24-hydroxyl substituted hopane derivative, as employed in the present investigation, would possess an hydroxyl group on a less conformationally constrained position, which may reduce the likelihood of intramolecular substitution. However, a consideration of possible transition states suggested that substitution at the 6β-position would be favourable.

1.2 Preparation of 15α-Acetoxyhop-17(21)-en-24-ol (10)

The substrate for the present hypoiodite oxidation was required to contain a 24-hydroxyl group, but be otherwise unreactive under the conditions of the hypoiodite reaction. Thus reduction of the

carboxylic acid group of the lichen metabolite 15α-acetoxy-22-hydroxy-hopan-24-oic acid (5) to an hydroxymethyl group was a necessary preparatory step. Of the remaining functional groups acetoxyl functions are relatively inert to the hypoiodite conditions, while the removal or protection of the 22-hydroxyl group was demonstrated to be necessary by the observation[11] that it reacts to yield a large number of products with lead tetraacetate, the oxidising agent of choice for the initial reactions.

The most straight forward, highest yielding method of dealing with the 22-hydroxyl group was considered to be dehydration using sulphuric acid in glacial acetic acid, a method which has been demonstrated[12] to yield the 17(21)-ene isomer as the sole product. Accordingly (5), on dehydration, yielded more than 70% of 15α-acetoxyhop-17(21)-en-24-oic acid (9) (scheme 1) with thin layer chromatography (t.l.c.) on silica gel (50% ether-hexane) giving some indication of minor amounts of anhydride formation.

Since a cursory examination of the literature revealed that sodium borohydride reduces acid chlorides[13], but not esters it was decided to prepare the water-sensitive acid chloride of 15α-acetoxyhop-17(21)-en-24-oic acid (9) and reduce it *in situ*, without isolation. Accordingly the treatment of (9) with thionyl chloride, followed by sodium borohydride reduction returned only poor (< 10%) yields of the 24-alcohol (10). However subsequent preparations, using oxalyl chloride to replace the more vigorous thionyl chloride, yielded up to 60% 15α-acetoxyhop-17(21)-en-24-ol (10) (scheme 1) on reduction.

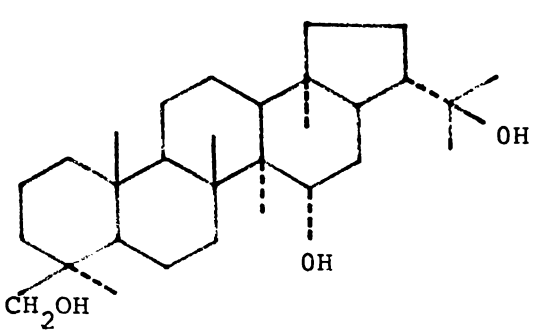
Although some difficulty arose from impure (9) and (10) decomposing to more polar products both were found to be quite stable once they were crystalline, yet neither would sublime.

TABLE 1

^{1}H nmr methyl group chemical shifts (δ ppm)

	4α-	10β-	8β-	14α-	18α-	21(2)
15α-Acetoxy-22-hydroxy-hopan-24-oic acid (5)[a]	1.21	0.74	1.06	1.09	0.79	1.16(av)
Δ for 22-OH → 17(21)ene[b]			-0.02	+0.10	+0.06	-0.25
Calc. for 15α-acetoxy-hop-17(21)-en-24-oic acid (9)	1.21	0.74	1.04	1.19	0.85	0.91(av)
Obs. for 15α-acetoxy-hop-17(21)-en-24-oic acid (9)	1.23	0.76	1.04	1.19	0.88	0.86,0.95[c]
Hopane-15α,22,24-triol (11)[a]	0.94	0.81	1.03	0.99	0.76	1.18(av)
Δ for 22-OH → 17(21)-ene[b]			-0.02	+0.10	+0.06	-0.25
Δ for 15α-OH → 15α-OAc[b]				+0.10	+0.05	-0.03
Calc. for 15α-acetoxy-hop-17(21)-en-24-ol (10)	0.94	0.81	1.01	1.19	0.87	0.90(av)
Obs. for 15α-acetoxy-hop 17(21)-en-24-ol (10)	0.98	0.82	1.02	1.18	0.88	0.85,0.96[c]

[a]Refs. 8 and 9. [b]Calculated from data in ref.14. [c]Doublet, J 6Hz.



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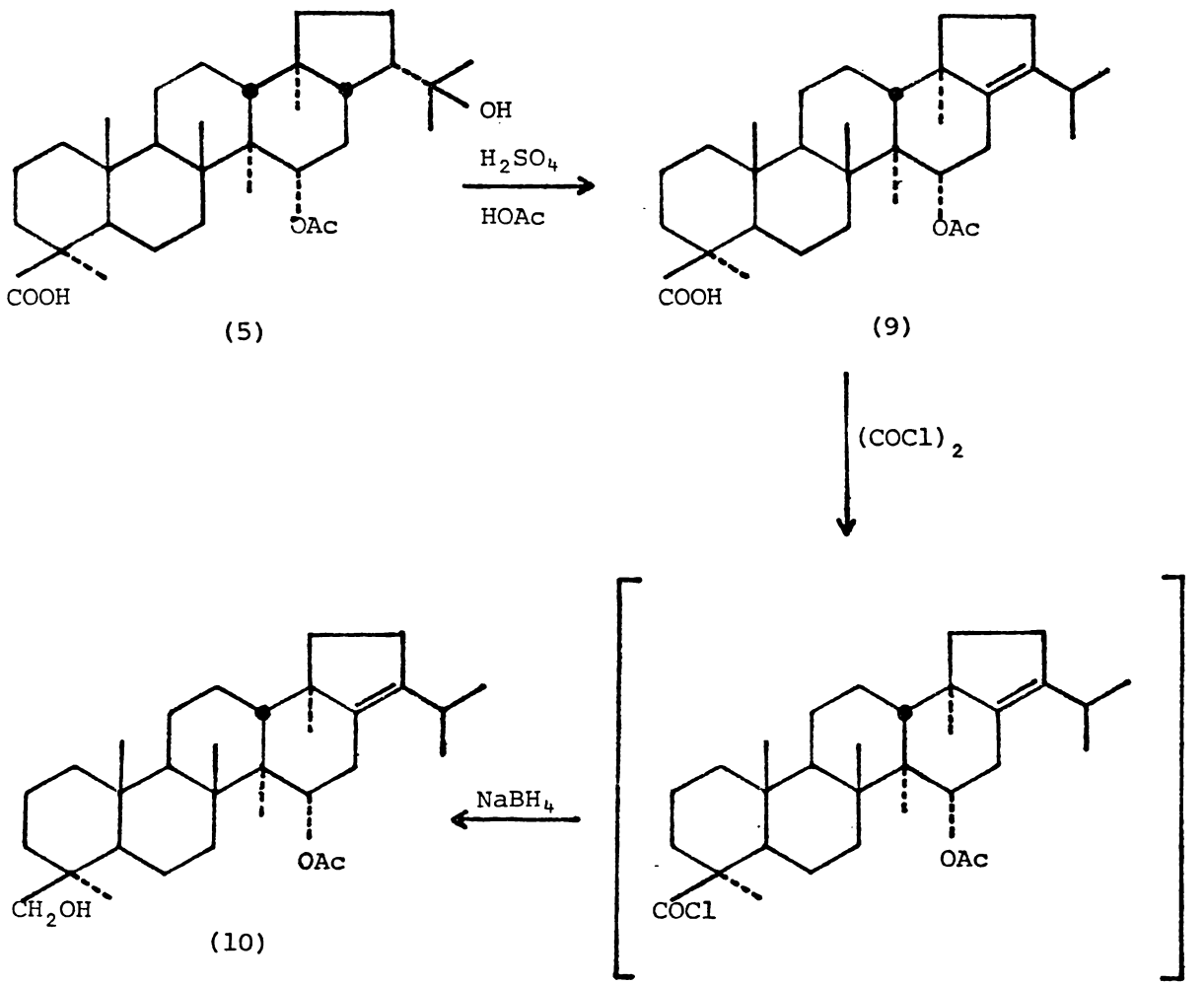
SCHEME 1.



1.3 Identification of 15α-Acetoxyhop-17(21)-en-24-oic acid (9) and 15α-Acetoxyhop-17(21)-en-24-ol (10)

Comparison of the methyl group chemical shifts obtained for the acid (9) and the alcohol (10) (table 1) with those calculated from the methyl group resonances of 15α-acetoxy-22-hydroxyhopan-24-oic acid (5) and hopane-15α,22,24-triol (11)[8,9] together with the appropriate substituent effect parameters both enabled assignment of the methyl group signals and indicated that the nature of the products was as

desired.

Further demonstrations of the structures of (9) and (10) were provided by the presence in both spectra of an acetoxyl methyl group absorption (δ 2.01) together with a multiplet (δ 4.96) corresponding to the C*H*OAc proton. In addition the spectrum of the acid (9) contained a broad absorption (δ 9.90) assignable to the proton of the carboxylic acid function while an AB quartet, centred at δ 3.59, in the spectrum of the alcohol (10) indicated the presence of a hydroxymethyl group (-C*H*$_2$OH).

The appearance of the C-21 methyl group signals as an AB_6 doublet and the absence of a molecular ion from the mass spectrum of both compounds was consistent with the presence of a 17(21)-double bond. A 17(21)-double bond often appears to enhance mass spectral loss of the side chain (- 43 m.u.) and β-directed cleavage of the 18α-methyl group. Thus the mass spectral peaks observed from both (9) and (10) arose from combinations of one or other of these fragmentations together with loss of acetic acid and in addition peaks from the well documented[12,15] cleavage through ring C were also present.

1.4 Initial Hypoiodite Reaction

The first hypoiodite reaction of 15α-acetoxyhop-17(21)-en-24-ol (10) was performed using lead tetraacetate and iodine in refluxing benzene, in an atmosphere of nitrogen and under U.V. irradiation. Chromatography of the product mixture by preparative thin layer chromatography (p.l.c.) on silica gel (10% ether-hexane) yielded four fractions, all of which were less polar than the starting material. The upper three fractions, A, B and C (*ca* 2% each), being single spots by t.l.c. while the lower fraction D (*ca* 60%) consisted of a continuum

of compounds (as seen on t.l.c.).

Gas liquid chromatography (g.l.c.) of the crude product gave evidence of at least ten components and the p.l.c. fractions were little better. Both fractions B and C possessed a major peak, amounting to not more than 60% of the total material. The methylene units of these components (table 2) are very low for triterpenoidal acetates, compared with the values for the 15α-acetoxyhopane derivatives given. Indeed even the methylene unit value for the hydrocarbon (hopane) is significantly higher than that for either product, suggesting that

TABLE 2

Methylene unit values

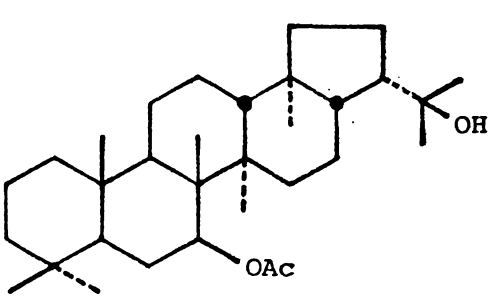
	SE 30[a] (*ca.*1.5%)	T	OV 17 (*ca.*1%)	T
Fraction B	25.4 ± 0.2	230°	31.4 ± 0.1	230°
Fraction C	30.6 ± 0.3	230°	33.6 ± 0.1	230°
15α-Acetoxyhop-21-ene[b]	33.8	250°		
15α-Acetoxy-22-tri-methyl-siloxyhopane[b]	35.5	240°	38.4	230°
Hopane[c]	32.2	250°		

[a]Equivalent to OV 101, [b]Ref.16, [c]Ref.17.

some degree of degradation has occurred. However, due to the product complexity no further characterisation was attempted, instead the possibility of using mercuric acetate in place of lead tetraacetate was investigated.

1.5 Reaction of Mercuric Acetate-Iodine with the Isopropanol Group of Hopane Derivatives

Although Kalvoda and Heusler[1] have proposed that mercuric acetate is more prone to produce side reactions during hypoiodite oxidation, Cong[18] has found that it gave better yields of major products in the hopane series. (The use of mercuric acetate is reviewed in appendix 1). However prior to the commencement of an investigation of the mercuric acetate-hypoiodite reaction of 15α-acetoxyhop-17(21)-en-24-ol (10), it was considered to be desirable to determine the effect of mercuric acetate, under the conditions of the hypoiodite reaction, on the 22-hydroxyl group. If the latter proved to be inert, a possibility suggested by the lower reduction potential of mercuric acetate compared with lead tetraacetate, the necessity of removing it preparatory to reacting the 24-hydroxyl group under the conditions of the hypoiodite reaction would be obviated. It was hoped in this way to overcome the problem of the unaccountable instability of crude 15α-acetoxyhop-17(21)-en-24-ol (10).



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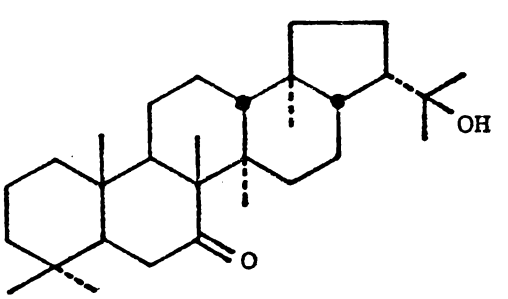


(13)

TABLE 3

^{1}H nmr methyl group chemical shifts (δppm.)[a]

	4α-	4β-	10β-	8β-	14α-	18α-
hop-17(21)-ene[b]	0.84	0.79	0.84	0.94	1.05	0.84
+ Δ 7-oxo[b]	+0.00	+0.01	+0.18	+0.22	+0.09	0.00
Calc. for 7-oxo-22,29,30-trisnorhop-17(21)-ene (14)	0.84	0.80	1.02	1.16	1.14	0.84
Obs. for 7-oxo-22,29,30-trisnorhop-17(21)-ene (14)	0.85	0.82	1.01	1.19	1.13	0.88
7-oxohopane[b]	0.86	0.82	1.01	1.15	1.06	0.70
+ Δ 21-oxo[c]				0.05	0.03	0.03
Calc. for 7,21-dioxo-22,29,30-trisnorhopane (17)	0.86	0.82	1.01	1.20	1.09	0.73
7-oxo-17αH-hopane[d]	0.84	0.81	1.00	1.20	1.11	0.89
+ Δ 21-oxo[e]				-0.14	0.04	0.22
Calc. for 7,21-dioxo-22,29,30-trisnor-17αH-hopane (18)	0.84	0.81	1.00	1.06	1.15	1.11
Obs. for 7,21-dioxo-22,29,30-trisnor-17αH-hopane (18)	0.85	0.82	1.01	1.11	1.16	1.14

[a]The revision after Ageta *et al.* (Ref. 19) (the 10β-methyl group of hopane resonates at a higher field than the 4α-methyl group) has been incorporated into the table (see also Refs. 8 and 9). [b]Ref. 14.

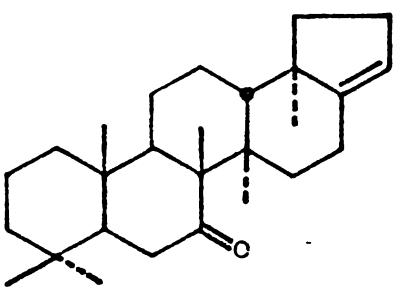
[c]Calculated by comparison of the spectra of 21-oxo-22,29,30-trisnorhopan-7β-ol (Ref. 15) and hopan-7β-ol (Ref. 14).

[d]Ref. 20.

[e]Calculated by comparison of the spectra of 21-oxo-22,29,30-trisnor-17αH-hopan-7β-ol and 22,29,30-trisnor-17αH-hopan-7β-ol (Ref. 15).

Accordingly 7β-acetoxyhopan-22-ol (12), in a pilot scale reaction, was unchanged after several hours refluxing in benzene or toluene with mercuric acetate, but addition of iodine and calcium carbonate produced complete conversion to products (t.l.c.) within 2.5 hours.

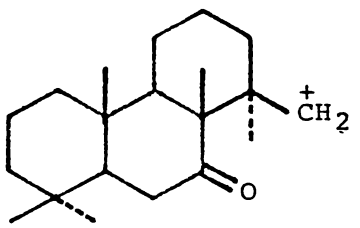
Subsequent reactions were performed on 7-oxohopan-22-ol (13) and produced one major product (10-20%), which possessed a mass spectral molecular ion of *m/e* 382 and evidence of only six methyl group signals in the ^{1}H nmr spectrum. The structure 7-oxo-22,29,30-trisnorhop-17(21)-ene (14) was proposed and addition of the substituent effect of a 7-ketone to the methyl group resonances of the angular



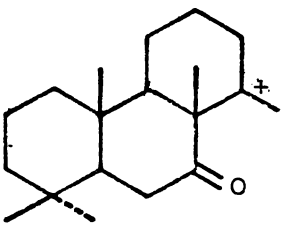
(14)

methyl groups of hop-17(21)-ene produced a series of δ-values (table 3) which are in excellent agreement with those obtained from the reaction product. Further support for this formulation (14) came from the presence of a single proton absorption at δ 5.18 (assignable to the C-21 olefinic proton), and mass spectral peaks arising from cleavage through ring C [*m/e* 205 (ring A/B) and 147 (ring D/E)], β-directed cleavage [*m/e* 367 ($M^{+}\cdot - \cdot CH_3$), 289 (ion i) and 275 (ion ii)] and the McLafferty rearrangement-based cleavages (*m/e* 233, 220 and 207). (Structures are

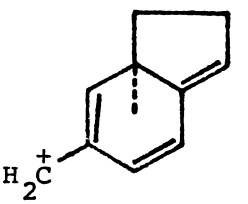
assigned to the latter in appendix 2.)



ion i : *m/e* 289

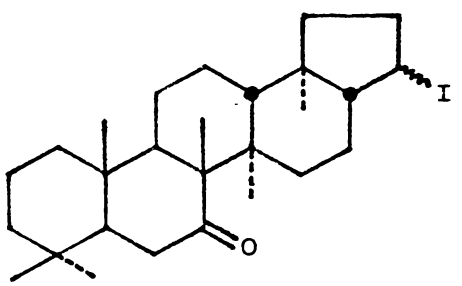


ion ii : *m/e* 275



ion iii : *m/e* 145

In addition there was mass spectral evidence for two iodinated species, (15) and (16), having molecular ions at *m/e* 510 and 508 respectively, each with a base peak corresponding to loss of an iodine atom (*m/e* 383 and 381 respectively).



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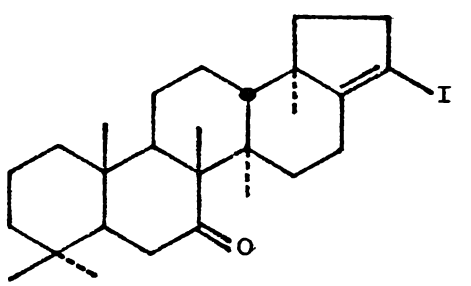
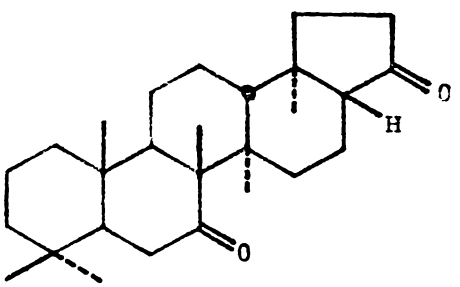


(16)

Increasing the proportion of mercuric acetate in the reaction mixture *[*from 2:4:1, mercuric acetate - iodine - 7-oxohopan-22-ol (13) to 4:4:1*]* resulted in a decrease in the yield of the unsaturated ketone (14), while the proportion of various more polar products was increased. Of these products only one (contained in fraction I) was obtained in sufficient quantity to isolate. The latter had a mass spectral molecular ion (*m/e* 398), which suggested that the isopropanol side chain had been replaced by a doubly bonded oxygen. Ions appearing at *m/e* 233, 220, 207 and 205 (base peak) confirmed (see appendix 2) that the ring

A/B portion, including the 7-ketone had remained unchanged and intense infrared absorptions at ν_{max}^{KBr} 1724 and 1690 cm^{-1} corresponded to the carbonyl stretching bands of ketones in 5- and 6-membered rings respectively.

Table 3 sets out the calculation of the methyl group resonances of both 7,21-dioxo-22,29,30-trisnorhopane (17) and its 17αH-epimer, 7,21-



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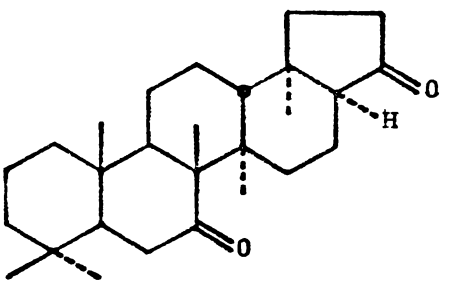
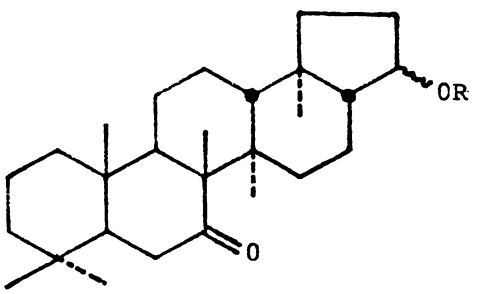


(18)

dioxo-22,29,30-trisnor-17αH-hopane (18). It is evident (see table 3) that a characteristic of (17) would be a ^1H methyl group resonance (18α-methyl group) at *ca.* δ 0.73. The absence of such an absorption in the spectrum of the reaction product suggested that as a result of a keto-enol equilibration 7,21-dioxo-22,29,30-trisnor-17αH-hopane (18) had formed and indeed this was confirmed by the agreement between the calculated and observed spectra of the latter (table 3).

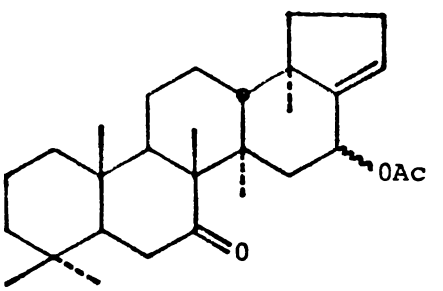
Although another product fraction (H) had two acetoxyl methyl group signals in the ^1H nmr spectrum even multiple run t.l.c. failed to produce any resolution. However, hydrolysis yielded a product mixture, which evidently consisted of two components (t.l.c.) whose t.l.c. R_F-values were consistent with their being ketols rather than diketones (*i.e.* the parent compounds were not enol acetates). Although, obviously still impure the original fraction, after

recrystallisation, possessed only one acetoxyl methyl group signal (δ 2.10), while a mass spectral peak of the same crystals at *m/e* 442, which appeared to be the molecular ion, suggested the structure (19). Correspondingly the more polar of the hydrolysed fractions possessed a molecular ion at *m/e* 400 with accompanying evidence of structure (20) *[m/e* 382 ($M^{+}\cdot-H_2O$) and 367 (382-Me•)*]*. Although the basis for these structural assignments *[*(19 and 20)*]* is rather tenuous, yet it is



(19) R = Ac

(20) R = H



(21)

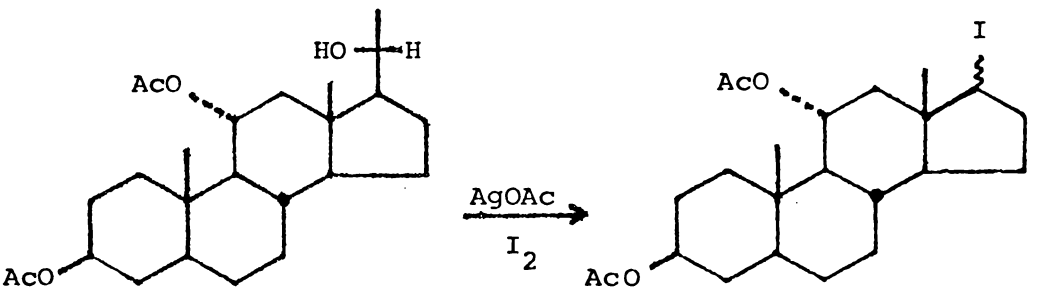
supported by parallels in the literature (section 1.6).

The mass spectrum of hypoiodite fraction G *[m/e* 440 ($M^{+}\cdot$), 425 ($M^{+}\cdot-Me^{\bullet}$), 380 ($M^{+}\cdot-HOAc$), 145 (100%, ion iii)*]* indicated the possibility that functionalisation of C-16 had occurred to form (21).

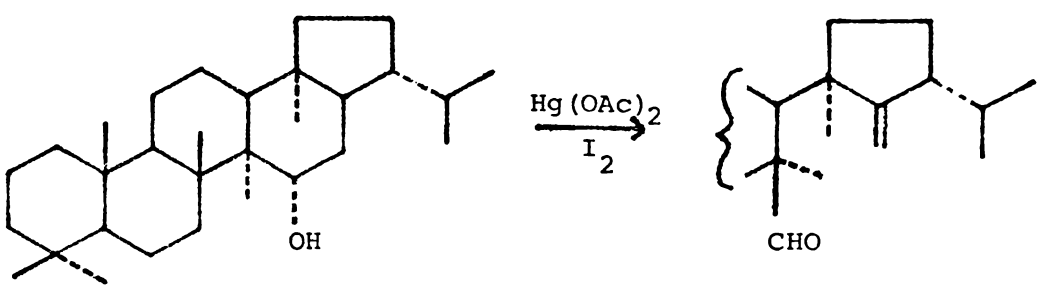
In addition all of the hypoiodite products of 7-oxohopan-22-ol (13) possessed ring A/B fragments (*m/e* 233, 220, 207 and 205) whose structures are discussed in appendix 2.

1.6 Mechanistic Discussion and Literature Comparison

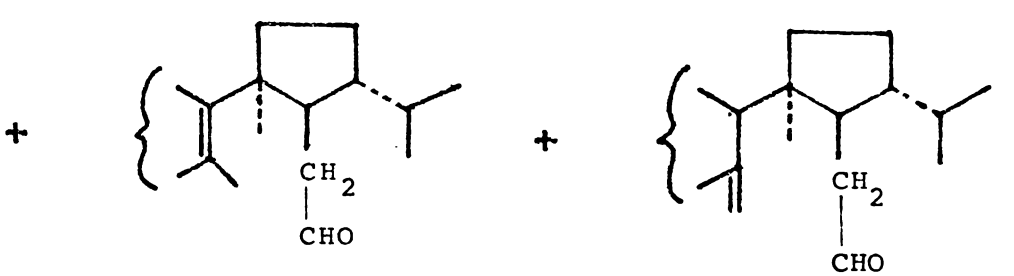
Such cleavage products as those obtained in the present study have been produced previously both from the hypoiodite reaction and from reactions utilising lead tetraacetate as the sole reagent.



(22) (23)



(24)

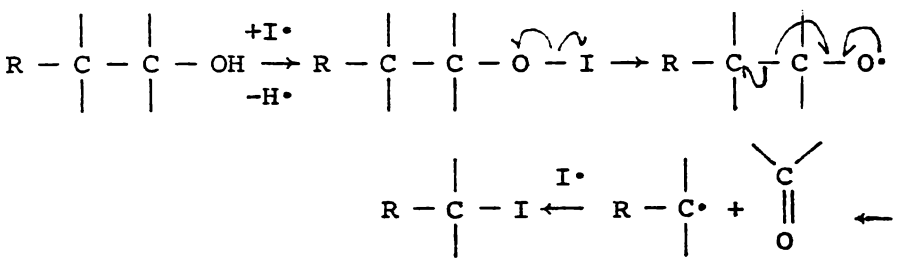


(25) (26)

Apparently cleavage occurs where, for steric or configurational reasons, intramolecular hydrogen abstraction is inhibited. Both a radical[21] and, quite recently, a concerted ionic mechanism[22] have been proposed to account for these products.

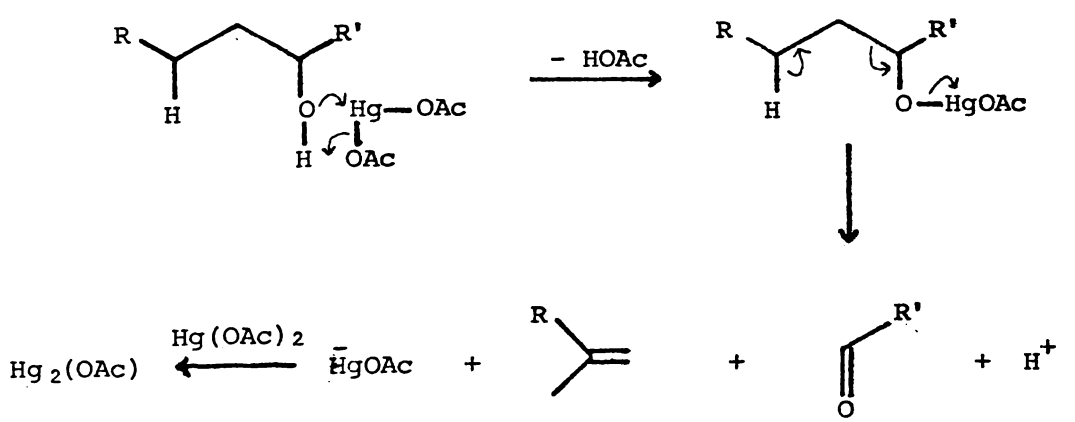
The radical mechanism (presented in modified form in figure 3) was used to explain the formation of the iodo-cleavage product (23)

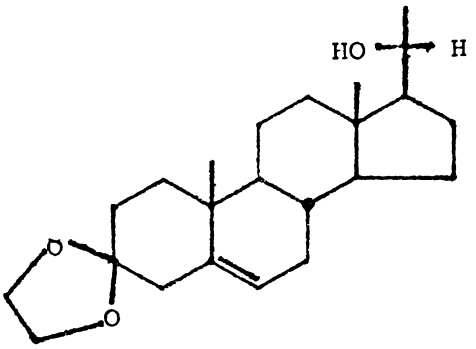
FIGURE 3



from the hypoiodite reaction of (22). On the other hand the ionic mechanism proposed by Cong[22] (in generalised form in figure 4) neatly accounts for products which are unsaturated at the point of cleavage *[e.g.* (24)-(26)*]*. The hypoiodite reaction is conventionally regarded

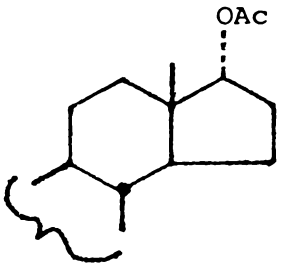
FIGURE 4





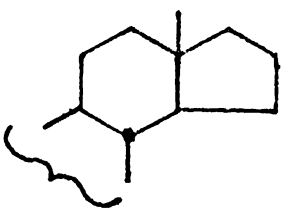
(27)

$Pb(OAc)_4$



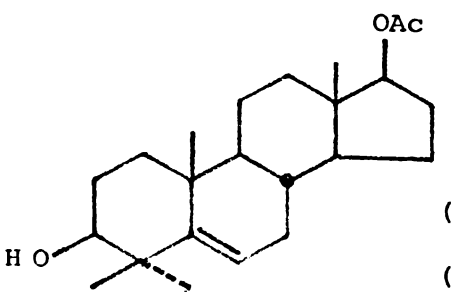
(28)

+



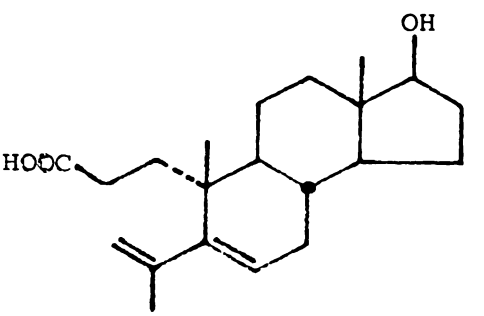
(29)

+ Other non-cleavage products

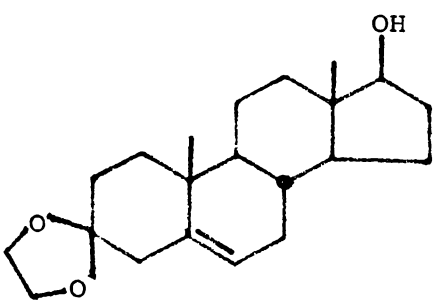


(i) $Pb(OAc)_4$

(ii) NaOH

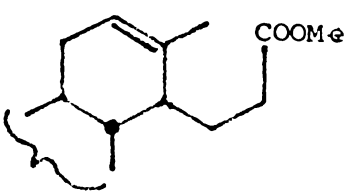


(30)



(i) $Pb(OAc)_4$

(ii) CH_2N_2



(31)

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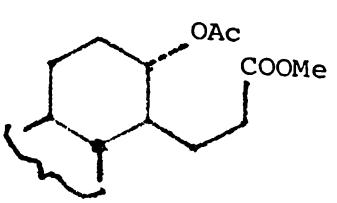

(32)

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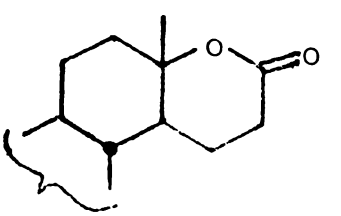
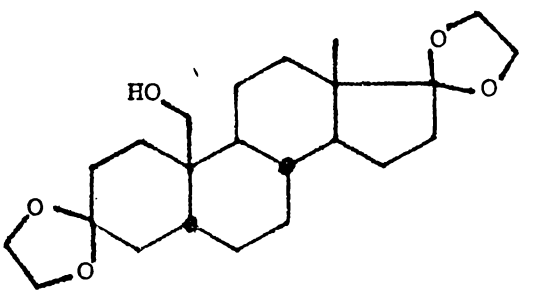
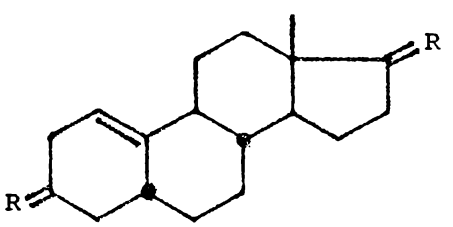




(37)

$Pb(OAc)_4$



(33)

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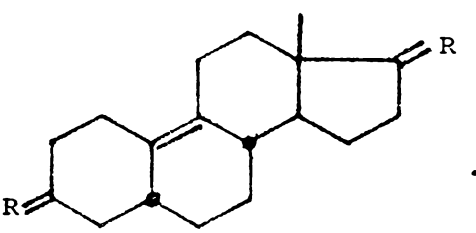

(34)

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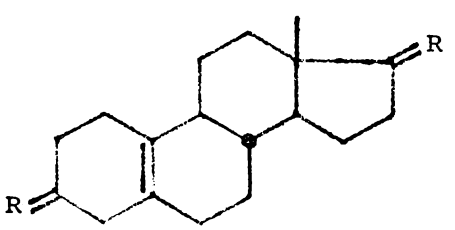

(35)

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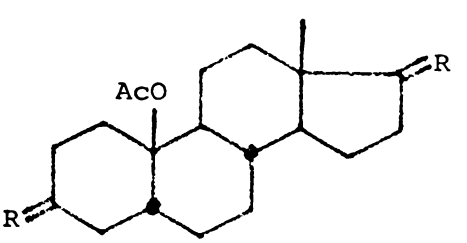


(36)

as occurring by a radical mechanism but some ionic processes may be concurrently proceeding as in figure 4.

The products (28) and (29) of the lead tetraacetate reaction[23] (performed in the absence of calcium carbonate) of (27) suggested that radical and ionic cleavage may be co-occurring, with (29) being formed by radical cleavage followed by addition of a hydrogen radical and (28) by concerted ionic cleavage accompanied by attack of an acetate ion. Further cleavage products such as (30)-(32)[24] and (33)-(36)[10] have been produced from other lead tetraacetate reactions. [Lead tetraacetate, being a stronger oxidising agent than mercuric acetate, oxidises the first-formed aldehyde to an acid - *cf.* (24)-(26)]. These products have been accounted for in terms of radical mechanisms, but Cong's mechanism also provides an adequate explanation. The reaction site of (37)[10] closely parallels that of 7-oxohopan-22-ol (13) the difference being in the replacement of the methyl groups of the isopropanol group by hydrogen atoms.

However the ionic mechanism is unable to account for the observation, made in the present study, that iodine is necessary for any reaction to occur, even at elevated temperatures. Thus to apply Cong's mechanism, where mercuric acetate is the oxidising agent, it is necessary to modify it to include the initial formation of an alkyl hypoiodite. The hypoiodite will react more readily with mercuric acetate than the hydroxyl group since an O-I bond has approximately half the strength of an O-H bond[25]. Once thus initiated the reaction follows the mechanism proposed in figure 4. The formation of 7-oxo-22, 29,30-trisnorhop-17(21)-ene (14) may then be envisaged to occur as depicted in either figure 5 or 6.

The formation of the other products follows from these

FIGURE 5

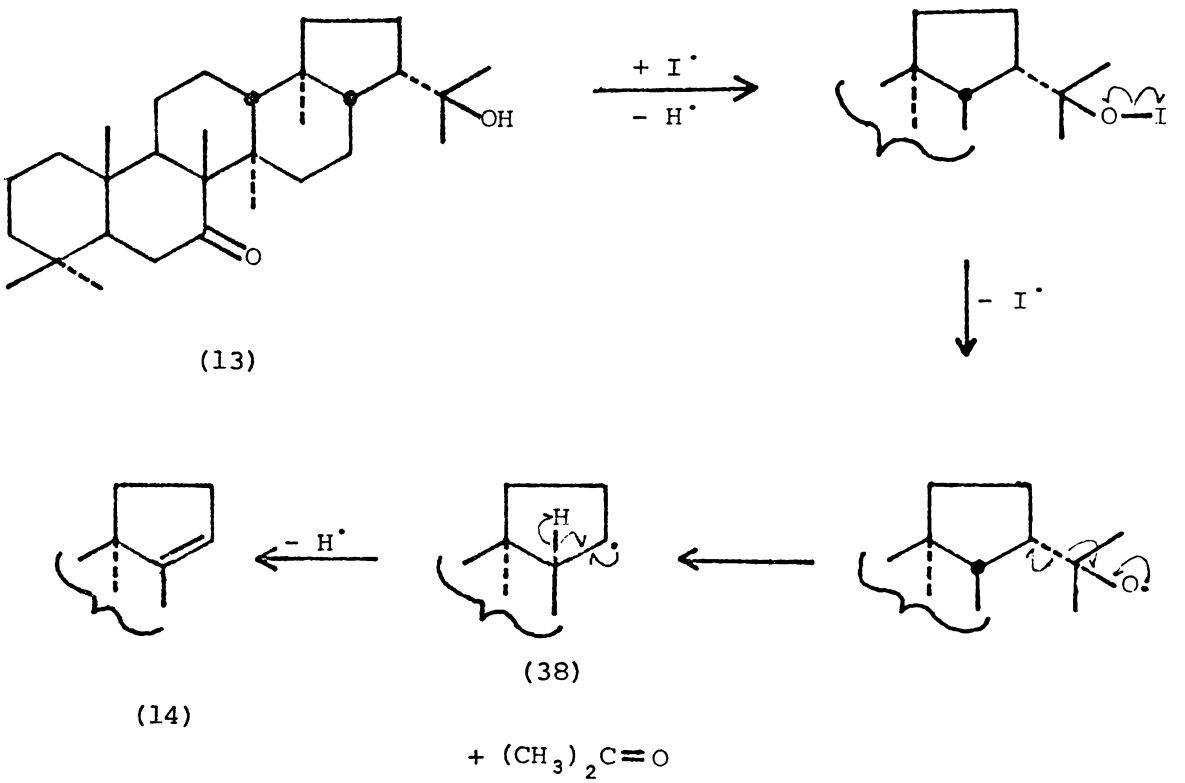
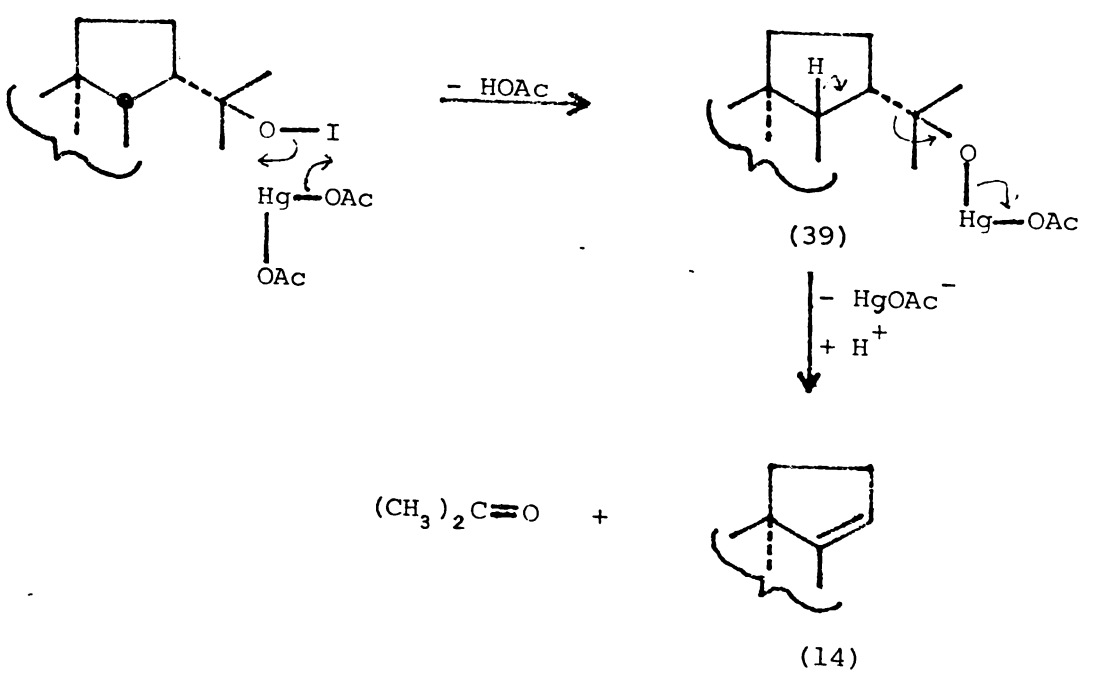


FIGURE 6

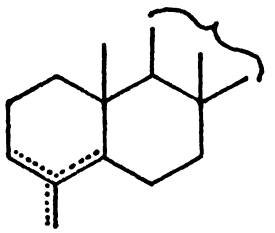


mechanisms. Either addition of an iodine radical to (38) or an iodide ion to (39) would yield the iodoketone (15). In accordance with the accepted scheme of the hypoiodite intramolecular cyclisation, where the iodoepoxide intermediate undergoes further oxidation[4], the diketone (18) is probably formed by oxidation of the iodoketone (15), hence the greater abundance of the former in the presence of excess oxidising agent (mercuric acetate). Indeed the observation that the yield of 7-oxo-22,29,30-trisnorhop-17(21)-ene (14) is reduced by an increase in the proportion of mercuric acetate suggests the alternative that the latter may also be formed *via* the iodoketone (15) some of which remains as such, some is oxidised to the diketone (18) while the remainder undergoes a radical decomposition to (14). The production of the unsaturated iodoketone (16) is more difficult to envisage. Possibly it occurred by iodine radical attack on the unsaturated ketone (14).

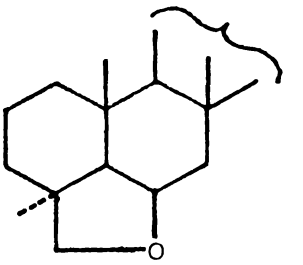
Products such as (29)[23] would appear to reflect the acid conditions in the absence of calcium carbonate so would not be expected in the present situation. However, acetoxyl substitution as in (28)[24], (32)[24] and (36)[10] might be anticipated hence the suggestion that 21ξ-acetoxy-22,29,30-trisnorhopan-7-one (19) was one of the products in spite of the scanty spectral evidence.

1.7 The Hypoiodite Reaction on 15α-Acetoxyhop-17(21)-en-24-ol (10) Using Mercuric Acetate

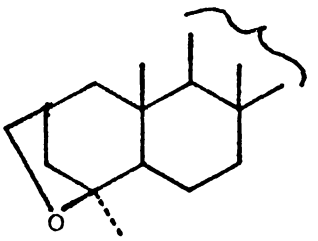
Having established that the tertiary 22-hydroxyl group does indeed react under the conditions of the hypoiodite reaction, when mercuric acetate is the oxidising agent, attention was once again turned to the hypoiodite reaction of 15α-acetoxyhop-17(21)-en-24-ol (10).



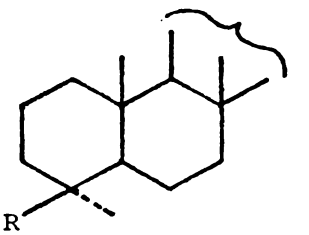
(40)



(41)



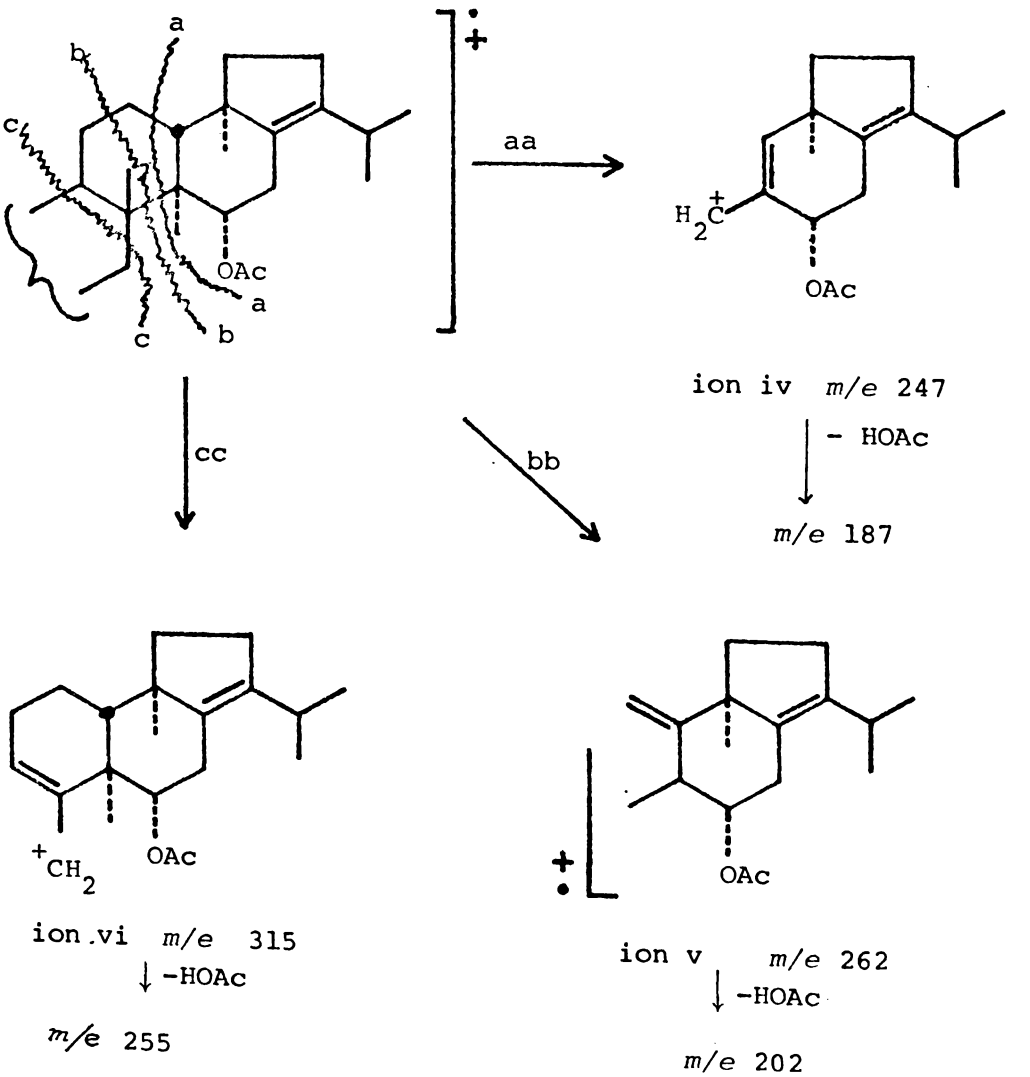
(42)



(43) R = OAc

(44) R = CH_2OAc

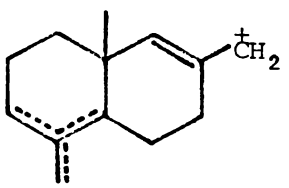
FIGURE 7



T.l.c. of a pilot scale hypoiodite reaction using mercuric acetate indicated the presence of one major product and small amounts of a few minor components, all of which had higher R_F-values than the starting material. In spite of this promising result each of two subsequent hypoiodite reactions produced a different series of products (by t.l.c.) which in turn were different from those produced in the preliminary reaction. Chromatography by p.l.c. yielded only three relatively clean fractions (one discrete spot on t.l.c., < 2% yield in total).

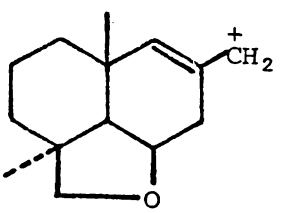
Mass spectrometry of these fractions produced a large number of mostly low intensity peaks, with no peaks corresponding to the molecular ions of any of the products considered to be possible, (40)-(44). However, the latter eventuality was not unexpected since the parent compound (10) gave no molecular ion. Nevertheless, there was evidence that rings D and E had remained intact since peaks arising from the cleavages indicated in figure 7 were present. Evidence that the presence of these peaks was not merely coincidental was implied by the cluster of ions around ion v (*m/e* 262 and 202), which was observed for an equivalent ion in the stictane series by Holland and Wilkins[26].

There was in addition evidence of ions corresponding to ring A/B fragments (ions vii - xi, where in each case the maximum intensity over the three spectra is quoted) of each of the possible derivatives. A peak at *m/e* 151 (ion xii)[22] is also indicative of the presence of a 6,24-epoxide. Ion vii would also result if acetic acid were lost from ion x, but the independent origin of the former is demonstrated by the presence of a peak at *m/e* 175 when there is no peak at *m/e* 235.

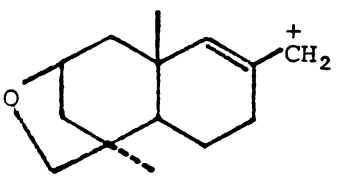


ion vii

m/e 175, 72%

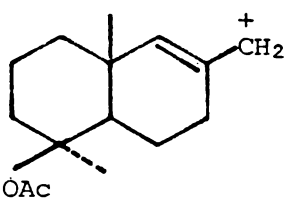


ion viii



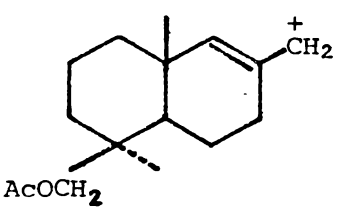
ion ix

m/e 205, 35%



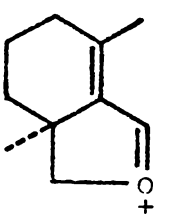
ion x

m/e 235, 29%



ion xi

m/e 249, 25%



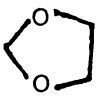
ion xii

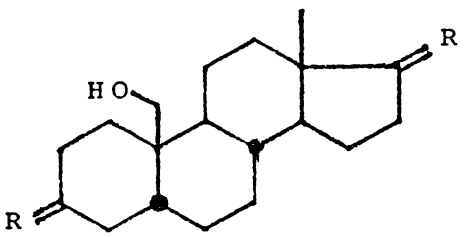
m/e 151, 64%

1.8 Discussion and Conclusion

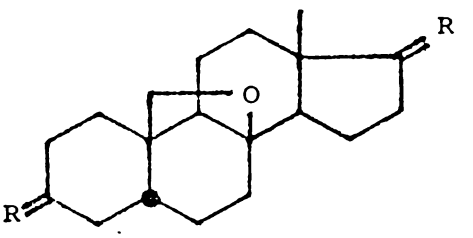
A radical and an ionic mechanism were found to account equally well for the nature of the cleavage products of the reaction of 7-oxohopan-22-ol (13) under the conditions of the hypoiodite reaction, while literature parallels further confirmed the structural assignments (section 1.6).

The results for 15α-acetoxyhop-17(21)-en-24-ol (10) were rather disappointing, though it is not uncommon for hypoiodite reactions to yield ill-defined mixtures of products. Kalvoda and Heusler[4] have associated such problems with the use of benzene as the solvent but other workers[18,27] have experienced equal success with both benzene and cyclohexane.

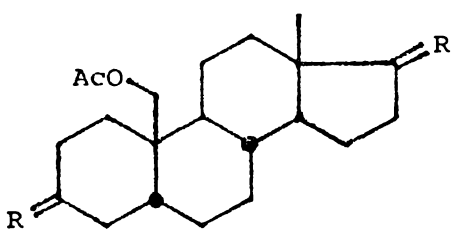
(37), (45) - (49) R = 



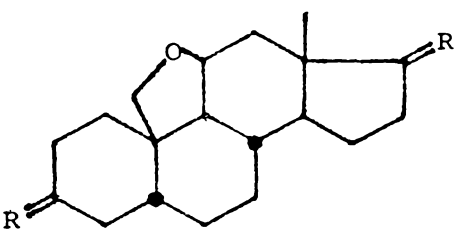
(37)



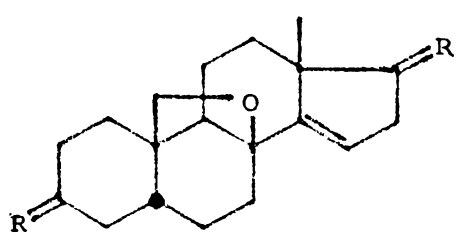
(45)



(46)



(47)



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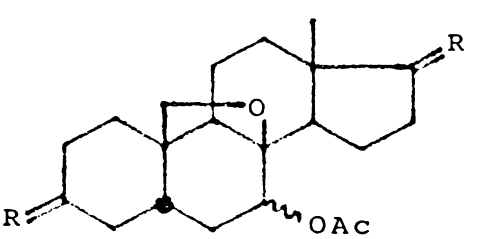

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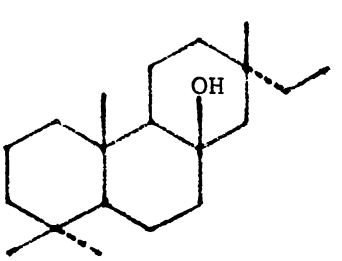
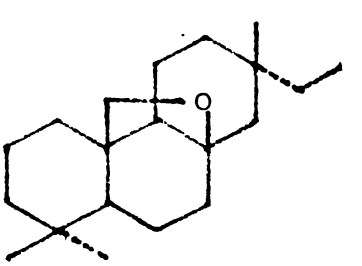


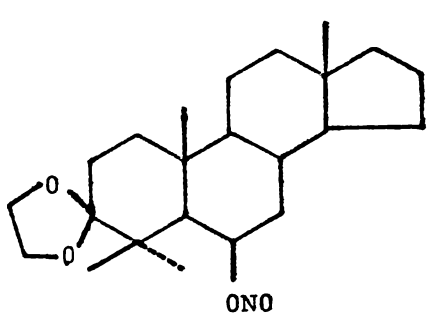
(50)



(51)

Such an hydroxymethyl function as in 15α-acetoxyhop-17 (21)-en-24-ol (10) would possess relative rotational freedom compared with the restraint imposed on the secondary alcohols usually used in hypoiodite reactions. Hence the often lower proportion of products of intramolecular substitution from compounds where the alcohol function is once removed from a ring and the tendency to form cleavage products instead (section 1.6). The reaction of 3,17-diethylenedioxy-5βH-androstan-19-ol (37) with lead tetraacetate,[10] in particular, serves to illustrate the complexity of products [(33)-(36) and (45)-(49)] obtainable under such circumstances. Indeed some of these products are analogous to those for which some evidence exists in the mass spectra of products of the hypoiodite reaction of 15α-acetoxyhop-17(21)-en-24-ol (10) (section 1.7).

Terminal rings often adopt a flattened chair conformation and are less conformationally constrained than other rings within a polycyclic structure. The consequences of this consideration are illustrated by (a) the hypoiodite oxidation of 21αH-hopan-6β-ol (1)[6] (section 1.1), (b) the hypoiodite oxidation of isopimaran-8β-ol (50)[27,28], which yielded 8β,20-epoxyisopimarine (51) as the sole epoxide product and (c) the photolysis of 3-ethylenedioxy-4,4-dimethyl-5αH-androstan-6β-yl nitrite (52)[29],



(52)

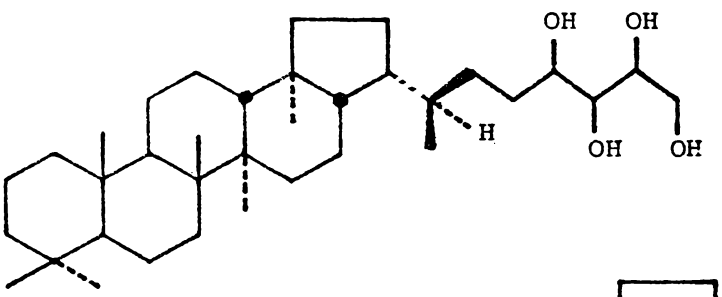
which produced products of 10β-methyl and 4β-methyl functionalisation in a ratio of 4.5:1. In the present case, with a functionalised methyl group in a terminal ring, a complimentary argument would suggest that intramolecular substitution would be less likely than in (37) and possibly a greater complexity of products would be produced.

CHAPTER TWO

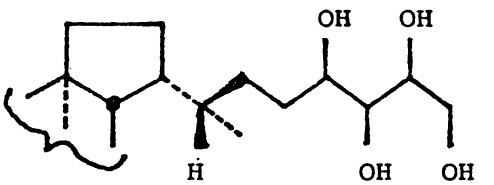
1,2-CARBONYL TRANSPOSITION

2.1 Introduction

Recently a C_{35} hopane derivative, bacteriohopanetetrol has been isolated from the bacterium *Acetobacter xylinum*[30] and from organic material in sedimentary rock.[31] The presence of both the (22R)- and (22S)-



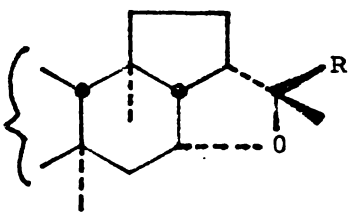
(53)



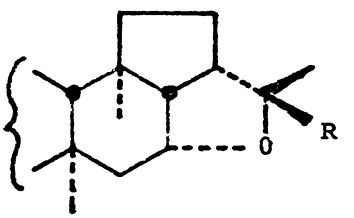
(54)

epimers, (53) and (54), has been demonstrated.[31] These two epimers were tentatively identified by relating a difference in the chemical shift of the C-22 methyl group to a proposed difference in the steric environment of the group.

However, the rotational freedom of C-22 limited the reliability of this approach. Unequivocal identification would be achieved by eliminating this rotational freedom, for example, by forming the 16α,22-epoxides (55) and (56). These compounds could be distinguished by ^{13}C nmr in a manner analogous to that by which the signals of axial and



(55)

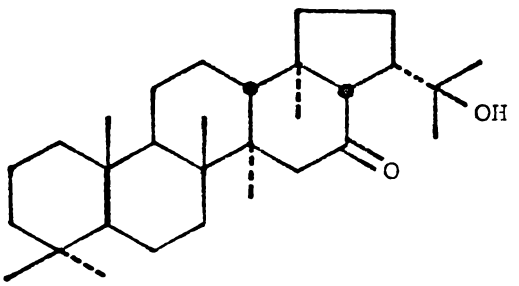


(56)

equatorial *[section 3.4(i)]* and endo and exo[32] carbon-containing substituents can be assigned. Thus the C-29 methyl group signal of the derivative of the (22R)-diastereoisomer (55) would be expected upfield of that of the alternative epoxide (56).

Such epoxides could be synthesised by dehydration between a 16α, 22-dihydroxyl system, for example, which necessitates a source of significant quantities of a 16α,22-dihydroxyhopane derivative. By synthetic modifications of the side chain of a hopane metabolite (having an isopropanol side chain) prior to formation of the epoxide, any pair of C-22 substituents could be acquired, while stereoselective cleavage of each of the epoxides would yield a known diastereoisomer.

Hence the object of this preliminary investigation was to achieve a high-yielding synthesis of 16-oxohopan-22-ol (57) by 1,2-carbonyl transposition of 15-oxohopan-22-ol (58).



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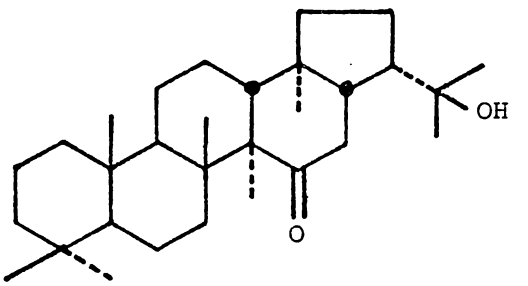

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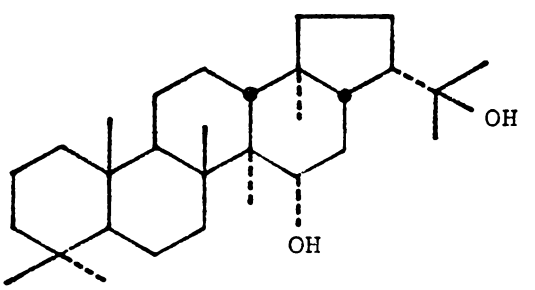
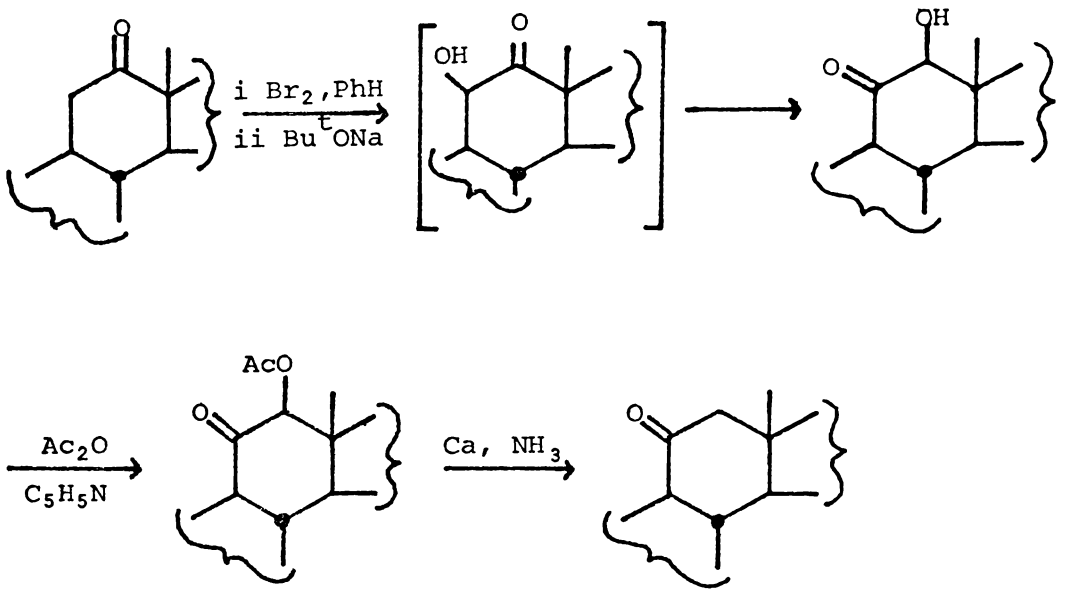


(59)

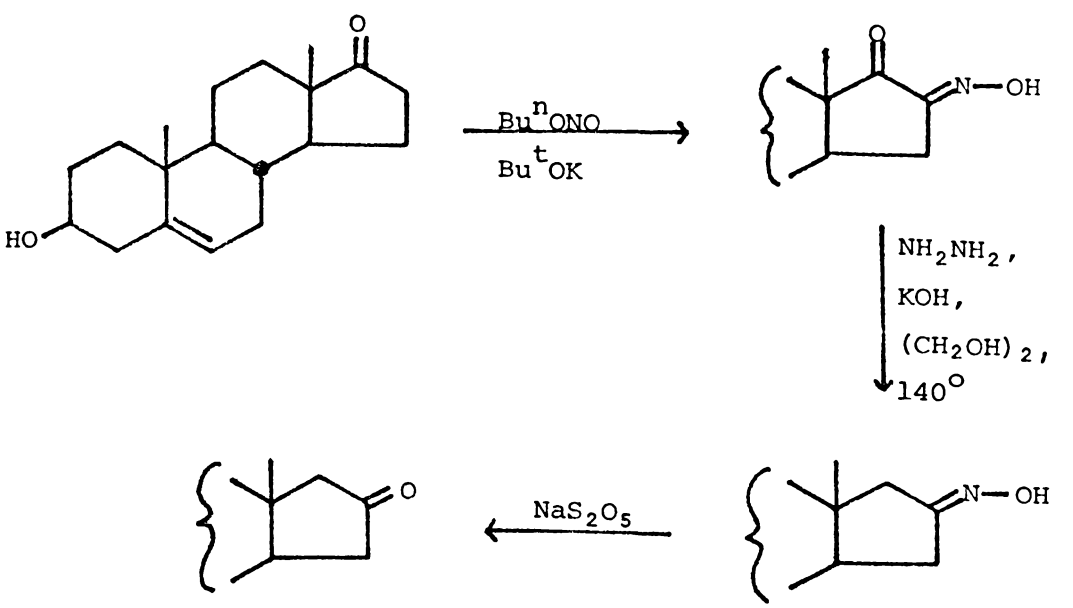
The latter is easily prepared from the readily available lichen metabolite hopane-15α,22-diol (59).

Recently, a 7-oxohopane derivative has been successfully transposed[33] to a 6-oxohopane analogue, but all procedures,[33] so far attempted for transposing the 15-ketone have been very low-yielding. The major difficulty is the steric congestion at both of these sites (C-6/7 and C-15/16).

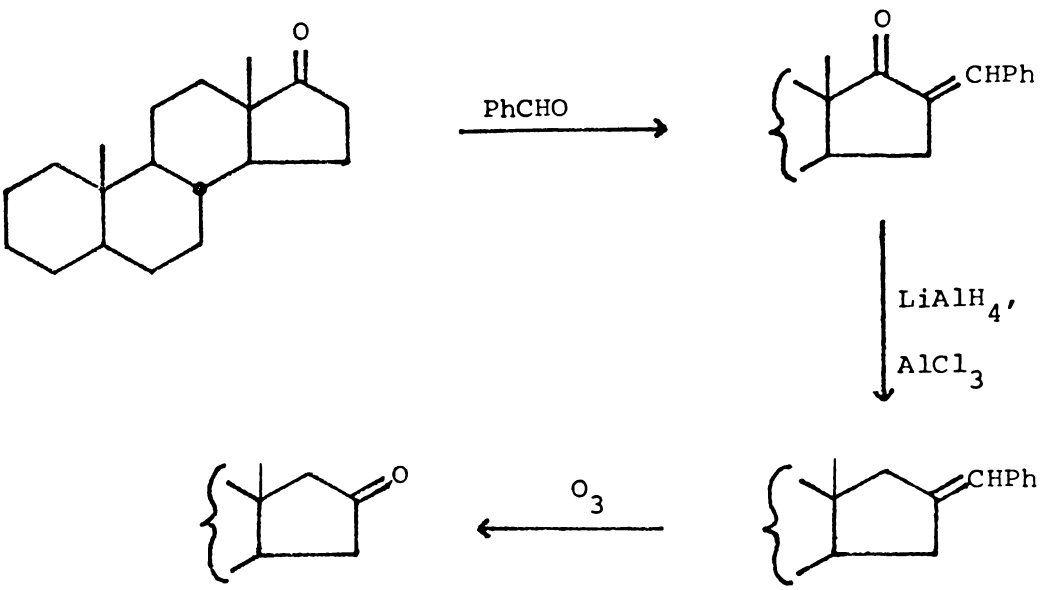
SCHEME 2



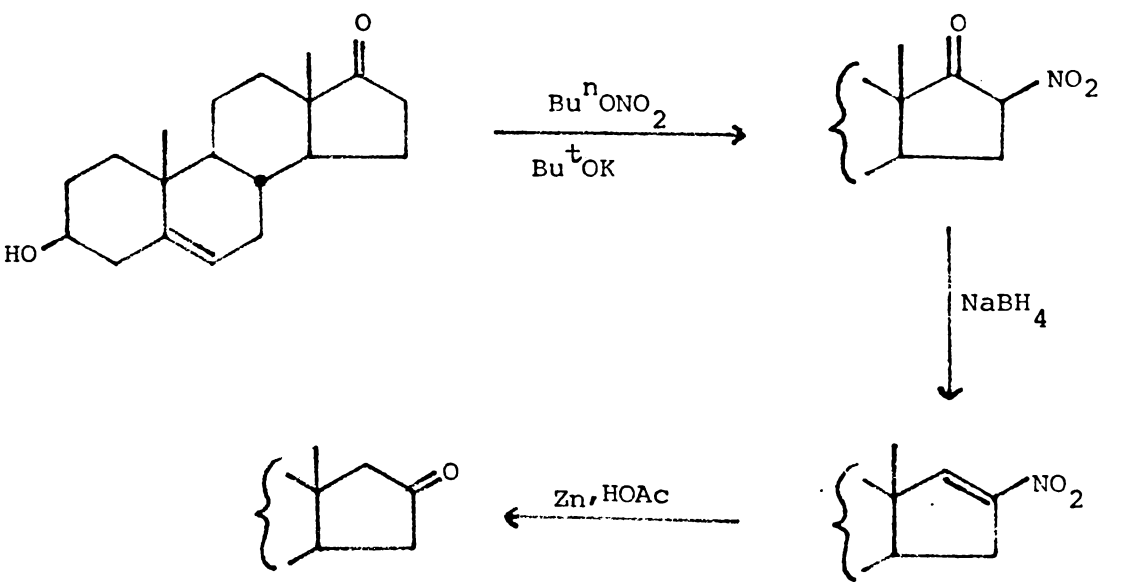
SCHEME 3



SCHEME 4



SCHEME 5



2.2 Review of 1,2-Carbonyl Transposition Methods

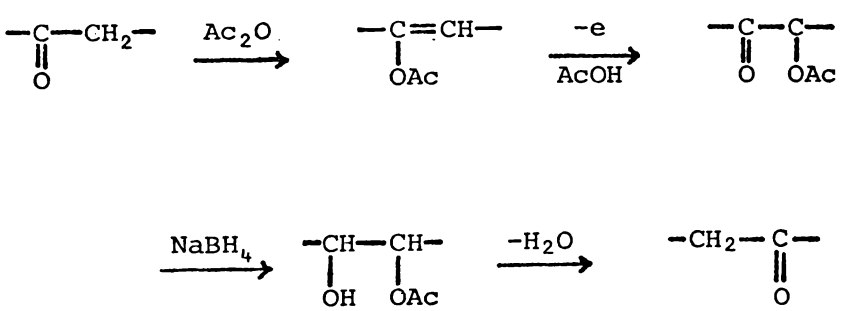
Methods of 1,2-carbonyl transposition have been thoroughly and extensively reviewed by Ramaiah,[34] so a detailed review need not be embarked on here but merely an overview presented, including more recent developments.

Routes which have been employed include those which rely heavily on the superior thermodynamic stability of those intermediates giving rise to a carbonyl at the new position. Scheme 2[35,36] gives an example, for which the steroidal 5-membered ring D is the driving force.

Functionalisation of the α-carbon, in more directive methods, has been achieved by oximation,[37] aldol condensation,[38,39] and nitration.[40] While the transposition was effected by subsequent reduction as outlined in schemes 3 - 5.

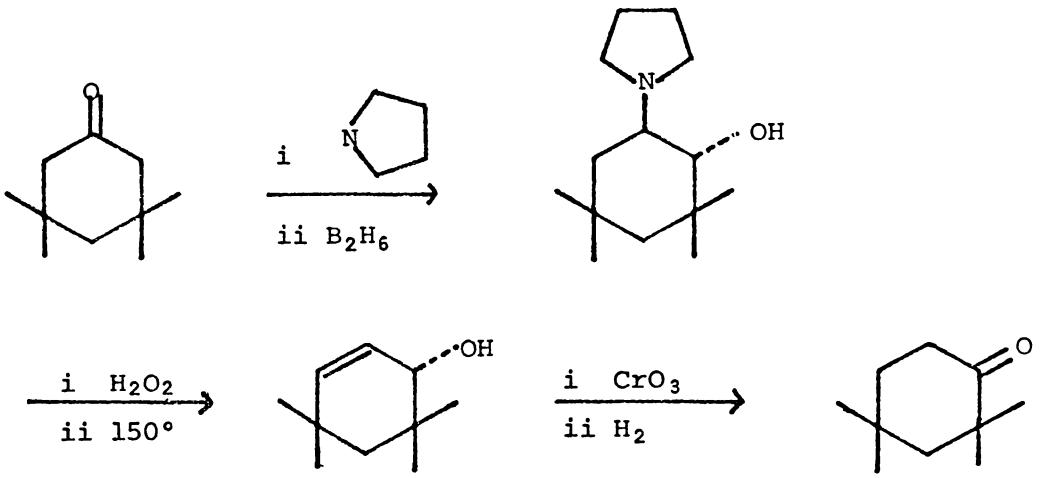
Recently some Japanese workers[41] have published a method involving anodic oxidation of the enol acetate derivative of the ketone followed by reduction (or addition of a Grignard reagent, if alkylation is desired) and acid catalysed dehydration and hydrolysis in one step (Scheme 6).

SCHEME 6

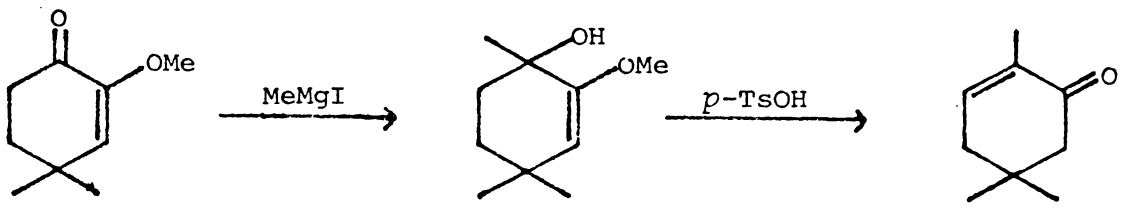


Using one of the various hydroboration procedures[42,43] an enol-type (*e.g.* enol methyl ether, enamine *etc.*) or olefinic derivative of the ketone is hydroborated, followed by treatment with basic hydrogen

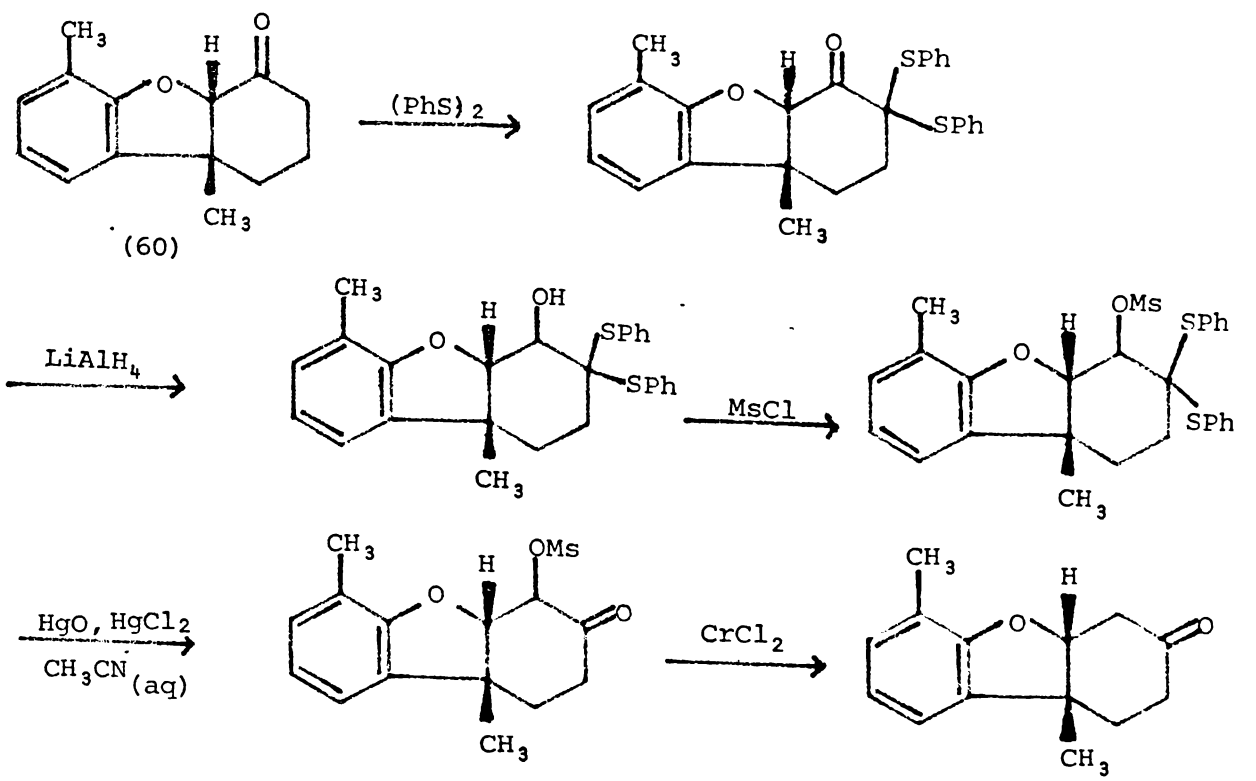
SCHEME 7



SCHEME 8



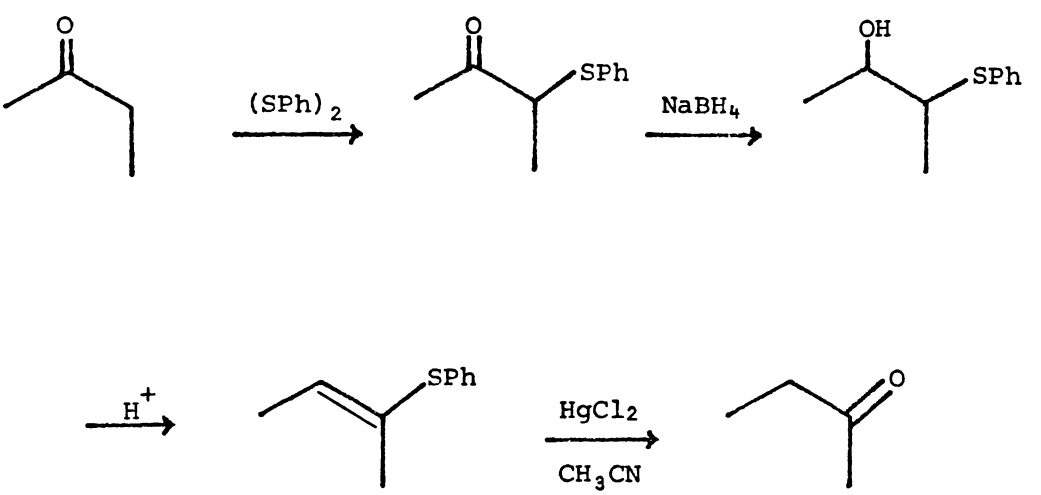
SCHEME 10



peroxide during the work-up procedure. The result is overall addition of water, where the hydroxyl group is on the carbon adjacent to the position of the original carbonyl group. On removal of the enol protecting group and oxidation, the transposed ketone was realised (*e.g.* scheme 7). A method somewhat akin to this has been described by Lange *et al.*[44] (scheme 8). The presence of an α-methoxyl substituent obviated the necessity for hydroboration.

Trost *et al.*[45] have developed a carbonyl transposition procedure employing α-sulphenylated esters and ketones, prepared from the reaction of diphenyldisulphide with the lithium enolate. Sequential reduction, dehydration and hydrolysis yielded the desired ketone. This method, (in a generalised form in scheme 9) has been used subsequently in a modified form by other workers.[46]

SCHEME 9

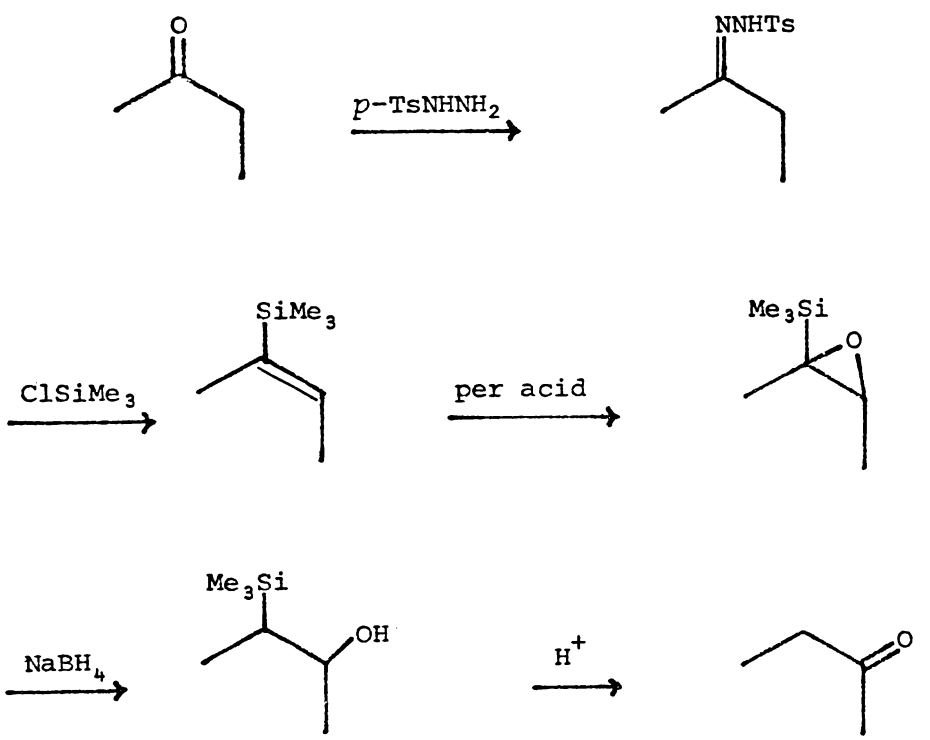


Yee and Schultz,[47] working on the pre-alkaloid system (60), found the hydroboration procedures unsatisfactory, problems being encountered in attempts to remove the substituent α- to the new ketone.

Their use of Trost's method also failed, since sulphenylation yielded the bisulphenylated ketone. However, use was made of the latter to prepare the thioketal mesylate, from which, on hydrolysis and reductive cleavage, the desired product, was realised (scheme 10).

In accord with the recently discovered manifold uses of organosilicon compounds in organic synthesis, a ketone transposition employing vinyl silanes has been developed.[48]

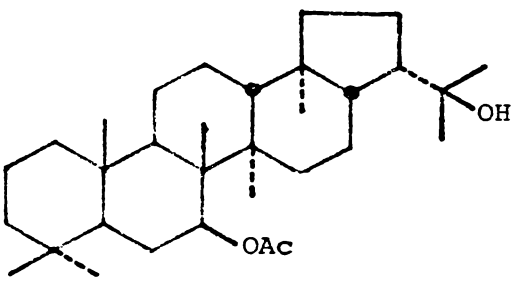
SCHEME 11



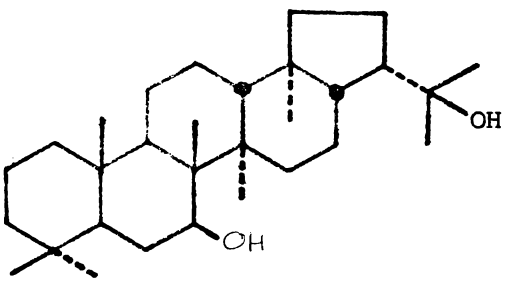
The vinyl silane was prepared from a hydrazone derivative of the parent ketone. Peroxidation of the vinyl silane yielded an epoxide, which, on reduction, regiospecifically produced the β-hydroxysilane. Acid catalysed desilylation effected the transposed ketone (scheme 11 outlines a generalised form).

2.3 Preparation of 1,2-carbonyl transposition substrates

Since it was intended to perform 1,2-carbonyl transpositions on both 7-oxohopan-22-ol (13) and 15-oxohopan-22-ol (58) it was necessary to prepare these from the readily available lichen metabolites 7β-acetoxyhopan-22-ol (12) and hopane-15α,22-diol (59) respectively.



(12)



(62)

Initial hydrolysis of (12) yielded hopane-7β,22-diol (62), but since no success was achieved using the acidic Jones reagent to oxidise the diols (62) and (59) (see Appendix 2), oxidation using the basic chromium trioxide and pyridine was attempted and gave the desired products, 7-oxohopan-22-ol (13) and 15-oxohopan-22-ol (58) respectively.

2.4 α-Sulphenylation of the ketones

Because it is both novel and widely applicable, Trost's method,[45] employing α-sulphenylated ketone intermediates was the means chosen to effect these carbonyl transpositions.

This procedure involves, initially, the preparation of the α-sulphenylated derivative of the ketone, which is synthesised by sulphenylating the lithium enolate. Two methods of obtaining lithium enolates have been employed in this context. One of these[49] produced the enolate

on the addition of methyllithium to the silylenolether derivative, while by the alternative procedure,[49,50] the ketone was treated directly with a strong lithium amide (Bronsted-Lowry) base, generated *in situ* from n-butyllithium and the appropriate amine. Although there are indications[49] that the former procedure is higher-yielding, the latter is the more commonly used, presumably because it is a "one pot" process.

Examples of the lithium amide bases, which have been utilised in sulphenylation reactions are lithium diisopropylamide,[51] lithium cyclohexylisopropylamide,[50] lithium dicyclohexylamide[52] and lithium 2,2,6,6-tetramethylpiperidide.[47] Olofson and Dougherty[53] by evaluating the efficiency, in reaction, of a number of these selective proton abstractors, demonstrated that the base strengths of the compounds listed here increase in the order given. Thus, although lithium cyclohexylisopropylamide has been most frequently employed, lithium dicyclohexylamide is superior in base strength and its amine precursor is much cheaper. Hence the latter, being favoured above lithium 2,2,6,6-tetramethylpiperidide because of the ready local availability of dicyclohexylamine, was chosen for these experiments.

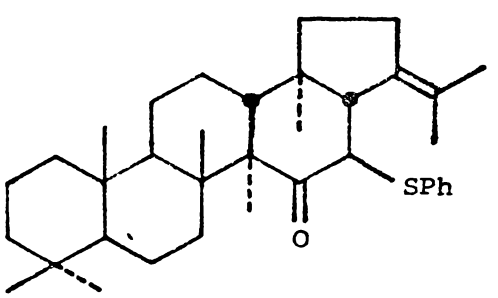
Previously sulphenylation has been achieved using elemental sulphur, disulphides (CH_3,C_6H_5) and sulphenyl chlorides (CH_3,C_6H_5).[54] Elemental sulphur is useful for only the most reactive enolates, while, although sulphenyl chlorides react immediately with lithium enolates at $-100^{\circ}C$, separation problems are created by their reactivity towards the lithium amide bases. Since the sulphur-sulphur bond strength of diphenyldisulphide is approximately one-third[50] that of simple dialkylsulphides it is a more effective sulphenylating agent. Because of the

highly hindered nature of the adjacent sites (C-6 and C-16) it was considered to be necessary to achieve optimum sulphenylation conditions by using diphenyldisulphide.

On occasions[45,49,51] tetrahydrofuran (THF) has been used as the sole solvent for the enolisation/sulphenylation sequence, but addition of hexamethylphosphoramide (HMPA) with the sulphenylating agent has produced both enhanced rates of sulphenylation and improved yields of the product.[50] HMPA is a polar, aprotic solvent, which dissolves inorganic salts such as alkali metal halides[55] and increases the reactivity of the conjugate bases of weak acids by stabilising them. In addition, and most importantly in this instance, it is remarkably stable to nucleophiles and bases.[56]

In accord with the foregoing discussion an initial sulphenylation reaction was attempted using an excess of both the lithium amide base and the disulphide, the latter of which was added in HMPA, to the solution of the lithium enolate in THF. However, since this first attempt resulted in the recovery of unchanged 15-oxohopan-22-ol (58), cyclohexanone was selected as an unhindered ketone substrate, on which to test the efficacy of the reagents and the manipulation techniques. The mass spectra of the products of the sulphenylation of cyclohexanone provided clear evidence that sulphenylation had occurred. The major fraction (p.l.c.) had molecular ions at *m/e* 208 and 206, which corresponded to 2-phenylthiocyclohexanol and 2-phenylthiocyclohexanone respectively. These ions were accompanied by losses of the thiophenyl radical and the thiophenol molecule, which produced peaks at *m/e* 99, 98, 97 and 96. 2,6-Diphenylthiocyclohexanol appeared to be the major constituent of the minor fraction, since its mass spectrum exhibited a base peak at *m/e* 316.

This successful sulphenylation of cyclohexanone indicated that the hindered nature of the 16-position of 15-oxohopan-22-ol (58) prevented substitution α- to the ketone and necessitated improvements to the reaction conditions. Hence a modified sulphenylation of 15-oxohopan-22-ol (58) was attempted, using freshly distilled dicyclohexylamine and HMPA (both from potassium hydroxide), while the recommended[50] ratio of 1:2:2, ketone to base to disulphide was employed. In addition since it was considered that HMPA may aid the formation of the lithium enolate the ketone-substrate (58) was added to the THF solution of the lithium amide in THF-HMPA. Heating at 80°C for an hour, after the addition of the diphenyldisulphide was intended to further increase the yield of the α-sulphenylated ketone. This procedure achieved the desired sulphenylation in 33% yield, but concurrently dehydrated the 22-hydroxyl group to produce 15-oxo-16β-phenylthiohop-21-ene (63). In addition, mass



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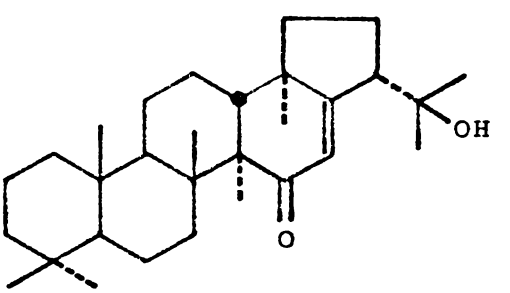


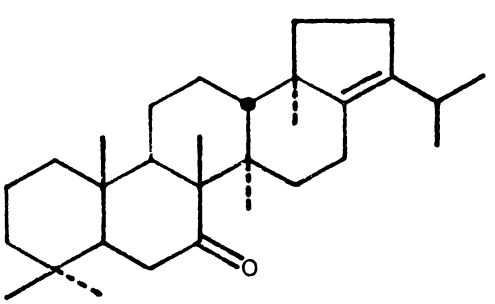
(64)

spectral evidence suggested that 15-oxohop-16-en-22-ol (64) was a minor product (section 2.5).

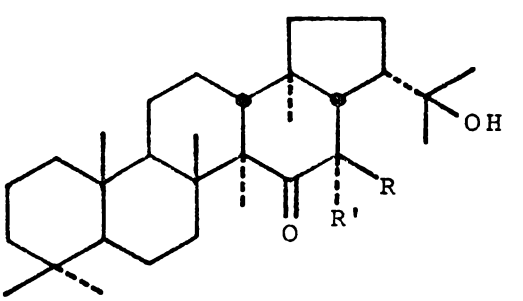
A concurrent sulphenylation reaction was performed on 7-oxohopan-22-ol (13) under identical conditions, except that a three-fold excess of diphenyldisulphide was employed. This adjustment was made because it is known[50] that the lithium amide base competes with the lithium enolate for diphenyldisulphide.

The result was a complex mixture of products, the mass spectra of which possessed what appeared to be molecular ions at *m/e* 548, 546, 532 and 530. Attempts to purify the material (p.l.c. and recrystallisation) were unsuccessful, so identification of the components was not pursued.

Because it was considered that the 22-hydroxyl group may be a complicating factor 7-oxohop-17(21)-ene (65) was used as the substrate for the next sulphenylation attempt. On the basis of the observation made by Seebach and Teschner,[54] that heating may reduce the yield of the α-sulphenylated ketone adduct, the reaction mixture was maintained at room temperature after the addition of diphenyldisulphide. However, again a complex mixture of products was obtained and no evidence of the desired material was apparent by mass spectrometry but recovery of 7-oxohop-17(21)-ene (65) amounted to 24% of the original material.



(65)



(66) R = SPh, R' = H

(67) R = H, R' = SPh

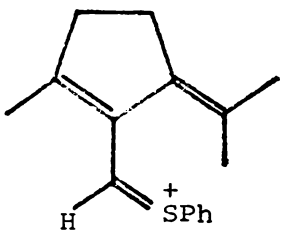
Since α-sulphenylation of the 15-ketone was proving to be more fruitful, both in the number and the nature of the products, attention was turned once more to 15-oxohopan-22-ol (58). A repeat sulphenylation of this material, utilising a substrate to base to disulphide ratio of 1:2:3 and maintaining a temperature of 50°C (30°C lower than that suspected of producing dehydration) for two hours subsequent to the addition of the diphenyldisulphide produced 54% yield of 15-oxo-16β-phenylthiohopan-22-ol (66) (section 2.5), while 30% of unchanged starting material was recovered. A further, larger scale, sulphenylation using freshly redistilled HMPA and dicyclohexylamine produced the α-sulphenylated ketone (66) in 81% yield while 17% of 15-oxohopan-22-ol (58) was recovered. In addition a small amount of material, which appeared to be 15-oxo-16α-phenylthiohopan-22-ol (67), possessing a slightly higher t.l.c. R_F-value than the major product (66) and a mass spectral molecular ion identical to the latter (*m/e* 550), was isolated in a crude state but not further investigated.

2.5 Characterisation of the Sulphenylation Products

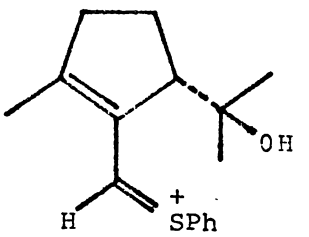
(i) Mass spectra

The mass spectrum of 15-oxo-16β-phenylthiohopan-22-ol (66) possessed the appropriate molecular ion (*m/e* 550), which was preceeded by the expected losses of water (*m/e* 532) and water plus either the thiophenyl radical (·SPh) (*m/e* 423) or the thiophenol molecule (HSPh) (*m/e* 422). The base peak, at *m/e* 243, corresponded to the sulphonium ion (ion xiii), while the next most intense peak (*m/e* 261, 81%) was apparently the 22-hydroxyl analogue of ion xxiii(ion xiv). Peaks at *m/e* 227 and 203 corresponded to the ring D/E fragments ions xv and xvi, respectively,

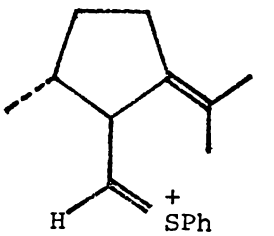
where the latter appears to have undergone a McLafferty rearrangement.



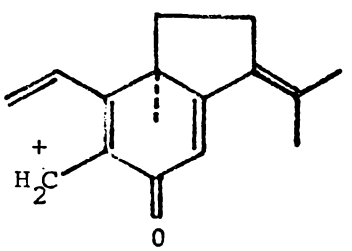
ion xiii : *m/e* 243



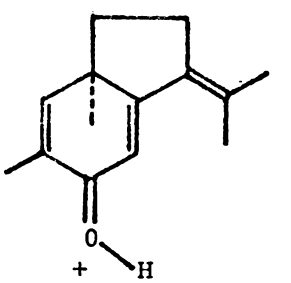
ion xiv : *m/e* 261



ion xvii : *m/e* 245



ion xv : *m/e* 227

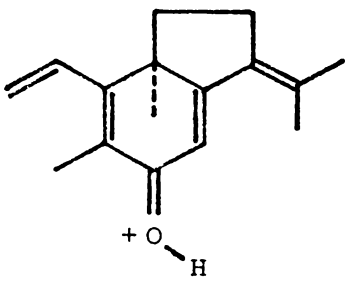


ion xvi : *m/e* 203

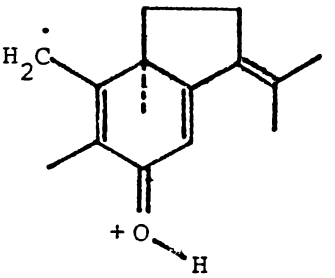
The mass spectrum of the sample suspected of containing 15-oxo-16α-phenylthiohopan-22-ol (67) possessed a base peak at *m/e* 245 (ion xvii) *[*ions xiii and xiv are much less intense than in the spectrum of (66)*]*, suggesting that the stereochemistry of the alternative epimer favoured acquisition of a hydrogen radical over loss of the same.

The nature of the product of an earlier reaction, 15-oxo-16β-phenylthiohop-21-ene (63), was indicated by the presence of a molecular ion at *m/e* 532, while the position of the double bond was suggested by the presence of a base peak at *m/e* 422, corresponding to loss of thiophenol. Loss of the latter would be facilitated by the presence of a 21-ene, since the resultant species would be a conjugated dienone. A peak at

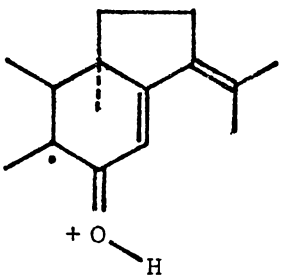
m/e 243 was assignable to the sulphonium ion, ion xiii, while McLafferty rearrangement-based cleavages produced ions xvi, xviii, xix and xx. Cleavage through ring C yielded either the ring A/B fragment (ion xxi) or the ring D/E fragment (ion xxii).



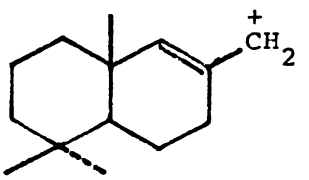
ion xviii : *m/e* 229



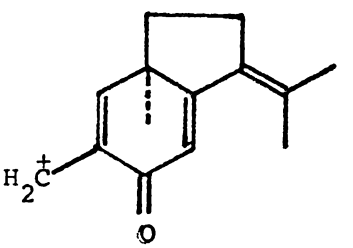
ion xix : *m/e* 216



ion xx : *m/e* 218



ion xxi : *m/e* 191



ion xxii : *m/e* 201

Although the mass spectrum of what was thought to be 15-oxohop-16-en-22-ol (64) possessed an insignificant (5%) peak at *m/e* 440 ($M^{+\cdot}$) its assignment was confirmed by the presence of the aforementioned mass spectral peaks *[m/e* 422 ($M^{+\cdot}-H_2O$), 229 (ion xviii), 218 (ion xx), 216 (ion xix), 203 (ion xvi), 201 (ion xxii) and 191 (ion xxi)*]* and by its t.l.c. R_F being similar to that of 15-oxohopan-22-ol (58).

(ii) ^{1}H nmr spectra

The ^{1}H nmr spectra of both 15-oxo-16β-phenylthiohopan-22-ol (66) and 15-oxo-16β-phenylthiohop-21-ene (63) are characterised by a multiplet around δ 7.4, which was readily assigned to the aromatic protons of the phenylthiol group.

A doublet at δ 4.15 (*J* 14Hz) in the spectrum of 15-oxo-16β-phenylthiohopan-22-ol (66) corresponded to the C*H*SPh proton, the coupling constant of which indicated that the phenylthiol group is equatorial (*i.e.* β-face) such that between the axial C*H*SPh proton and the axial C-17 proton a dihedral angle of approximately 180^o obtains. In contrast the spectrum of the dehydrated sulphenylated ketone (63), which would be expected to have the phenylthiol group in the same configuration, possessed a broad multiplet at δ 4.23, indicating a reduction in the coupling constant. This reduction would be a consequence of the now closer proximity of the side chain (due to the sp^2 hybridisation of C-21) to the phenylthiol group, which necessitates some distortion of ring D, thus reducing the dihedral angle between the two protons (C-16 and -17).

The three most shielded methyl group signals in the spectra of both of the α-sulphenylated ketones (63) and (66) were assigned to the methyl groups disposed about ring A, while two signals at δ 1.24 in the spectrum of 15-oxo-16β-phenylthiohopan-22-ol (66) are possibly from the C-21 methyl groups (table 4). The allylic (C-21) methyl group signals of hop-21-ene[14] occur at δ 1.56 and 1.72, so signals at δ 1.85 and 2.02 in the spectrum of the unsaturated α-sulphenylated ketone (63) were assigned to the C-22 methyl groups, where the additional deshielding is attributed to the proximity of the phenyl group. The only other methyl group resonance significantly affected by the unsaturation of hop-21-ene

was the 18β-methyl group, which experienced a shielding effect of 0.21 ppm [14] (relative to hopan-22-ol). Since, between the two α-sulphenylated ketones (63) and (66), the most shifted of the remaining signals was that at δ 1.02 in the spectrum of the unsaturated compound, it was assigned to the 18α-methyl group to which the signal at δ 1.21 in the spectrum of the 22-hydroxy-derivative (66) was also attributed (overall substituent effect 0.19 ppm). Due to the distortion of ring D evidenced by the change in the coupling constant of the *CH*SPh proton, the 14α-methyl group would be expected to experience some small shift in resonance frequency,

TABLE 4

^{1}H nmr methyl group chemical shifts (δ ppm)

	4α-	4β-	10β-	8β-	14α-	18α-	21(2)
15-Oxo-16β-phenylthio-hop-21-ene (63)	0.83	0.83	0.90	0.96	1.30	1.02	1.85, 2.02
15-Oxo-16β-phenylthio-hopan-22-ol (66)	0.83	0.83	0.91	0.96	1.24*	1.21*	1.24*, 1.24

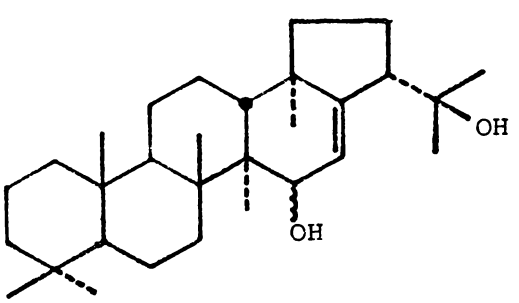
* May need interchanging.

not observed in hop-21-ene. Hence, the signals at δ 1.24 [from (66) and 1.30 from (63)] were assigned to the C-14 methyl group. The assignment of the remaining pair of signals (at δ 0.96 in the spectra of both compounds) to the 8β-methyl group follows.

2.6 Reduction of 15-Oxo-16β-phenylthiohopan-22-ol (66)

Trost *et al.*[45] found sodium borohydride or lithium aluminium hydride at room temperature sufficient to effect reduction of the α-sulphenylated ketones to the corresponding α-sulphenylated alcohols in almost quantitative yield. However, it was anticipated that 15-oxo-16β-phenylthiohopan-22-ol (66) would require more rigorous conditions because of its exceptionally hindered nature. Accordingly, a reduction attempt using sodium borohydride in ethanol, involving a total of 2.5 hours at reflux and 3 days at room temperature yielded only desulphenylation products.

The mass spectrum of these sodium borohydride reduction products possessed two molecular ions (*m/e* 442 and 440, each accompanied by a peak corresponding to a loss of water), which indicated the presence of hop-16-ene-15ξ,22-diol (68) and 15-oxohop-16-en-22-ol (69). A ketone would not be expected to survive sodium borohydride reduction, so (69) was probably generated during the work-up procedure.



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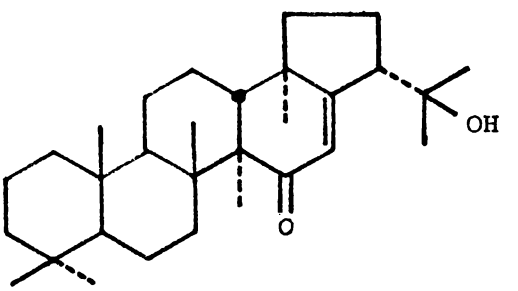
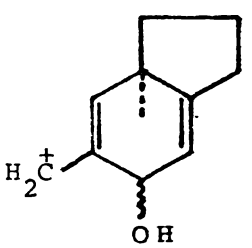
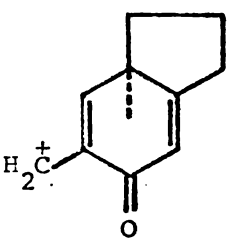


(69)

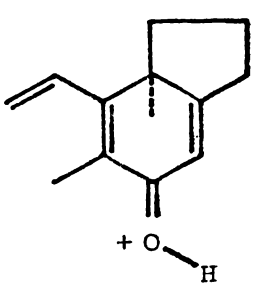
These designations were supported by t.l.c. of the product mixture, which revealed the presence of a major component of similar R_F to 15-oxohopan-22-ol(58) and a slower moving component with an R_F-value intermediate between those of the latter and hopane-15α,22-diol(59). A mass spectral peak at *m/e* 382, originating from the ketone derivative (69), corresponded to the loss of the side chain with hydrogen radical transfer[15] and had apparently lost a methyl radical to produce a peak at *m/e* 367. Similarly, the other major fragments have lost the side chain such that ions at *m/e* 163 and 161 can be assigned the structures ion xxiii *[from (68)]* and ion xxiv *[from (69)]* and ions at *m/e* 189 and 176, possibly arising as a result of the McLafferty rearrangement, could correspond to ions xxv and xxvi respectively.



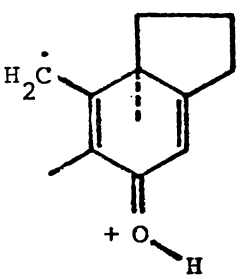
ion xxiii : *m/e* 163



ion xxiv : *m/e* 161



ion xxv : *m/e* 189

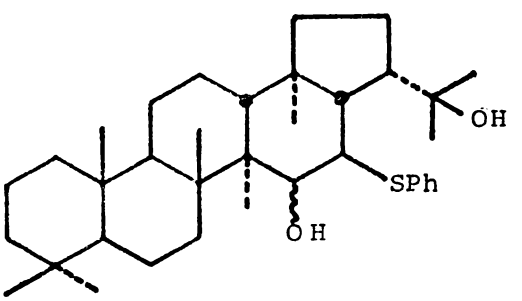


ion xxvi: *m/e* 176

Reduction using lithium aluminium hydride, both under reflux and at room temperature, also effected desulphenylation to give hopane-15α,22-diol(59) as the major product (<20% yield). Since hopane-15β,22-diol is the major product of the lithium aluminium hydride reduction of 15-oxohopan-22-ol(58),[12] it appears that the ketone group was reduced prior to desulphenylation, the steric requirements of the phenylthiol group causing the preferential formation of the 15α-alcohol.

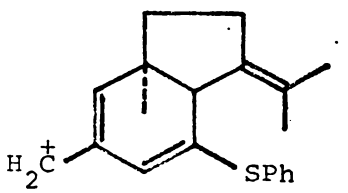
Because of the failure to effect reduction without desulphenylation, attention was turned to the much-praised[57-59] lithium triethylborohydride ("super hydride") as a possible solution. This compound is a very strong reducing agent,[57,59] and nucleophile,[56] capable of reducing even the highly hindered 2,2,4,4-tetramethylpentan-3-one[59] within 30 minutes, yet sulphides such as methyl *p*-tolyl sulphide[59] are almost inert towards it. However, lithium triethylborohydride was not available to these laboratories and all attempts to prepare it failed at the stage of the preparation of the triethylborane precursor.

A subsequent reduction using sodium and isopropanol[60] produced an almost quantitative yield of hopane-15α,22-diol(59) (identified by ^{1}H nmr). By contrast aluminium hydride[61] yielded a complex mixture of products, the mass spectra of which revealed some evidence of a small amount of 16β-phenylthiohopane-15ξ,22-diol(70).

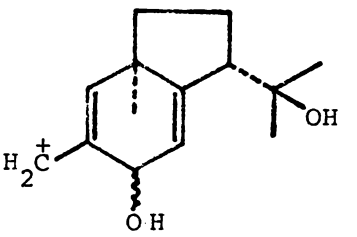


(70)

A small peak at *m/e* 552 (2%) corresponded to the molecular ion, while loss of thiophenyl radical gave an ion at *m/e* 443. Two other peaks at *m/e* 295 and 221 were possibly the ring D/E fragment ions xxvii and xxviii.



ion xxvii : *m/e* 295



ion xxxviii : *m/e* 221

However, due to the indifferent yield, this method was not pursued and carbonyl transposition by this procedure was abandoned at this point.

2.7 Further steps intended

The remaining two steps of Trost's 1,2-carbonyl transposition method[45] were dehydration followed by hydrolysis of the vinyl sulphide to yield the transposed ketone. Since any acid catalysed dehydration would remove the 22-hydroxyl group it was intended to attempt to dehydrate with the strong base, potassium *tert*-butoxide.[62] Using this method it was anticipated that electron donation to the d-orbitals of the sulphur of the phenylthiol group would assist dehydration.

Hydrolysis of vinyl sulphides to ketones or aldehydes was traditionally achieved using dilute acid.[63,64] However, such treatment would also result in elimination of the 22-hydroxyl group. Trost[45] and other workers[46,65-68] have effected the hydrolysis with aqueous solutions (with or without acetonitrile) of mercuric chloride or titanium tetrachloride.

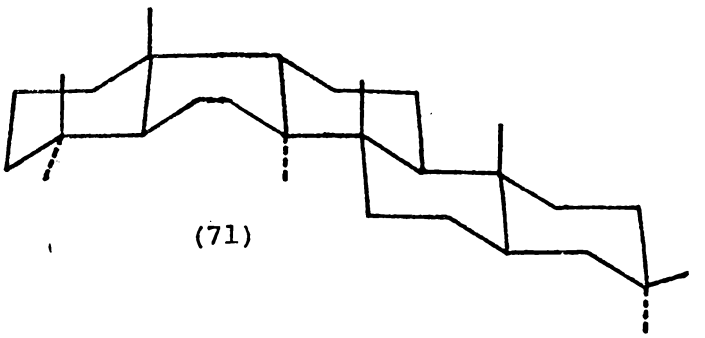
2.8 Attempted preparation of a Vinyl Silane

The vinyl silane method[48] of 1,2-carbonyl transposition also appeared to be a reasonably straight-forward method, but it failed at the first step using 7-oxohopan-22-ol (13) as the starting material. The vinyl silane is prepared *via* the benzenesulphonylhydrazone derivative of the ketone, by trapping the vinyl carbanion (formed by the action of an alkyl lithium reagent) with chloromethylsilane.[69,70] However, heating 7-oxohopan-22-ol (13) under reflux in toluene, with benzenesulphonylhydrazine for 44 hours failed to produce even a trace of the hydrazone.

It should be noted that the 7-oxo-position is extremely sterically hindered, such that it failed to form the 7-dithioketal after 20 hours reaction time.[71]

2.9 Conclusion

The failure encountered in previous attempts to effect 1,2-carbonyl transposition of 7-oxohopane and 15-oxohopane derivatives can, almost entirely, be accounted for in terms of the steric hindrance to substitutions at these sites. The present attempt is no exception to this generalisation. Indeed, of the carbonyl transpositions mentioned in the literature, these are the most hindered ketones, making it quite an achievement to have obtained both 15-oxo-16β-phenylthiohop-21-ene (63) and 15-oxo-16β-phenylthiohopan-22-ol (66).



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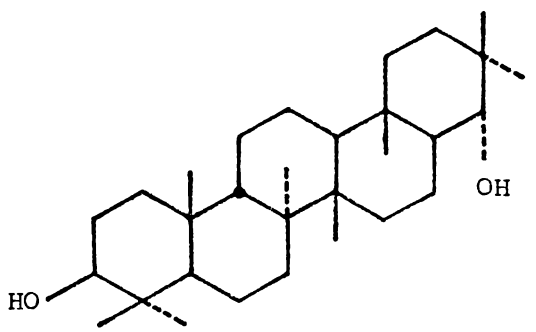

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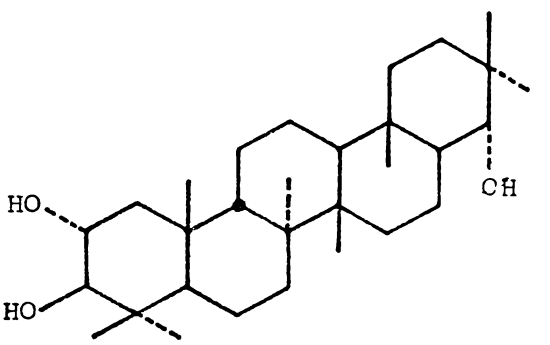

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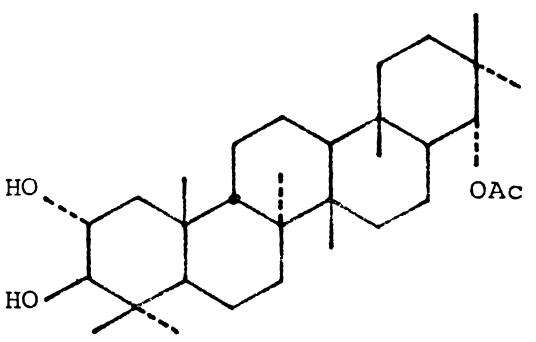

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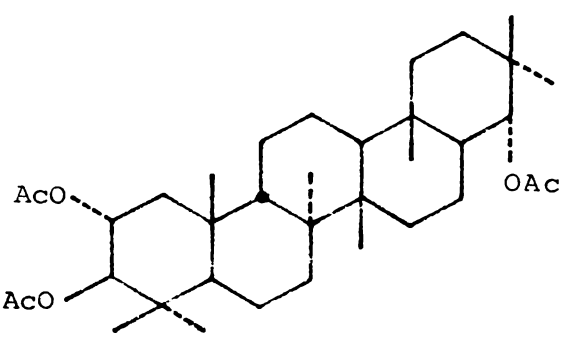

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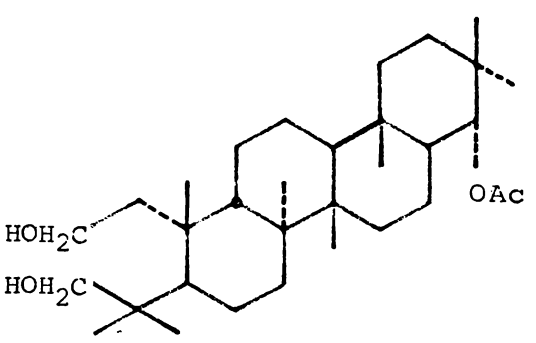

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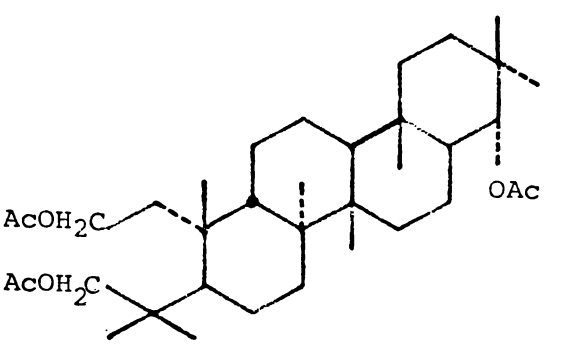


(77)

CHAPTER THREE

^{13}C NMR OF STICTANE DERIVATIVES

3.1 Introduction

Despite sensitivity problems ^{13}C nmr has been developed into an extremely useful diagnostic technique, due to the wide range of chemical shifts over which ^{13}C nuclei absorb (typically *ca.* 200 ppm for steroidal and triterpenoidal nuclei).

The object of the present investigation was, through complete assignment of the ^{13}C nmr spectra of a number of stictane (71) derivatives to obtain confirmatory evidence that ring B is in a boat conformation.[72-74] (Unequivocal support for structure (71) of stictane has been produced, since the commencement of this project by two independent crystallographic studies.[75,76]) With this stated aim in view the spectra of four pentacyclic stictane derivatives, stictane-3β,22α-diol (72), stictane-2α,3β,22α-triol (73), 22α-acetoxystictane-2α,3β-diol (74) and 2α,3β,22α-triacetoxystictane (75), together with two ring A-cleaved derivatives, 22α-acetoxy-2,3-secostictane-2,3-diol (76) and 2,3,22α-triacetoxy-2,3-secostictane (77), were investigated. Resolution in ^{13}C nmr is such that although each of these compounds possesses from 30 to 36 carbon atoms, yet the spectra contain remarkably few coincidences.

Preliminary allocation of these signals into categories based on the number of protons carried by the nucleus giving rise to that signal was made by comparing the proton noise decoupled spectrum with the corresponding single frequency off-resonance decoupled spectrum (SFORD). In the former type of spectrum each carbon signal appears as a singlet, due to irradiation across the proton region such that all proton coupling is

eliminated, while the latter spectrum is produced by reducing coupling to such a degree that only partial one-bond couplings are observed. However, under SFORD conditions methine and methylene carbons often produce ill-defined multiplets, as a consequence of the second order effect of protons on adjacent carbons having similar chemical shifts to the α-protons.[77] This allocation of signals was assisted by the observation that each spectrum could be divided into three sections. The highest field section (0-28 ppm) contained ten signals (six quartets and four triplets) plus the acetate methyl signals, thirteen signals (two quartets, five triplets and six singlets) occurred in the intermediate region (28-44 ppm), while further downfield were seven signals (four doublets and three triplets) plus the carbonyl carbon signals of the acetoxyl groups. The only exceptions to this generalisation occurred in the spectra of the diol (72) and the secostictane derivatives, (76) and (77). Since the diol (72) has one less oxygenated substituent, a carbinyl carbon absorbing in the lowest-field region was replaced by a methylene carbon absorbing in the intermediate section. Cleavage of ring A, yielding 2,3-secostictane derivatives, resulted in one methyl group signal moving from the intermediate region to the higher-field section.

The acetoxyl methyl group signals were located on the basis of their absence from the spectra of the diol (72) and the triol (73) and by comparison with the designated[78] location of similar methyl groups (table 5). The carbonyl carbon signals, being the lowest field absorptions, were readily assigned. The most deshielded of these is apparently derived from the 22α-acetoxyl group, while the carbonyl carbon from the 3β-acetoxyl group, having in common with the latter a *gem*-dimethyl system adjacent, possibly absorbs at 170.7 ppm, leaving

TABLE 5

The acetoxyl group signals (ppm from TMS)

	Me			C=O		
	2	3	22	2	3	22
Dihydroxyacetate (74)			20.9[a]			171.0[b]
Triacetate (75)	20.8	20.8	20.9	170.2	170.7	170.8
Secodihydroxy-acetate (76)			20.9			171.0
Secotriacetate (77)	20.8	20.8	20.9	170.6	170.6	170.6

[a] LIS additions: 1-4 Δ = 7Hz, 4-6 Δ = 41Hz

[b] LIS additions: 1-4 Δ = 6Hz, 4-6 Δ = 15Hz

the highest field signal to be assigned to the 2α-acetoxyl carbonyl carbon (table 5).

To assign the skeletal carbon and the angular methyl group carbon signals the additional techniques of lanthanide induced shift (LIS) and specific frequency decoupling experiments (on the methyl groups) were employed, while deductions were made from substituent effects. The assignments were completed by comparing the observed chemical shifts with those calculated from parameters derived for predictive purposes.

3.2 Discussion of Prediction Methods

Prediction methods with a theoretical basis[79,80] have so far proved largely unsatisfactory for steroidal systems. However, a number of more successful empirical methods have been introduced. The first of these, developed by Dalling and Grant, initially[81,82] attempted to quantify the effect of every possible relationship between nuclei within a

cyclohexane system. Subsequent papers[84,85] presented a more fundamental approach where a compromise between simplicity and accuracy was achieved. In this series of papers the shielding effects of γ-*gauche* hydrocarbon substituents were regarded as arising from steric compression.

Recently this view has been brought into question by the observation[85] that *syn*-1,3-diaxial methyl groups are mutually deshielding (*i.e.* the substituent effect is in the opposite direction from that predicted by the approach of Dalling and Grant). Moreover, microwave studies[86] reveal that the *gauche* conformation of propanol is the most stable, which implies that steric compression is absent in this form. In addition, a modified INDO (intermediate neglect of differential overlap) finite perturbation theory investigation[87] produced the surprising observation that the dependence of the methyl carbon chemical shifts in butane on the dihedral angle between the terminal carbon atoms depended solely on the conformation of the methyl group in question and was independent of the conformation of the other methyl group and hence of the so-called steric interactions involved.

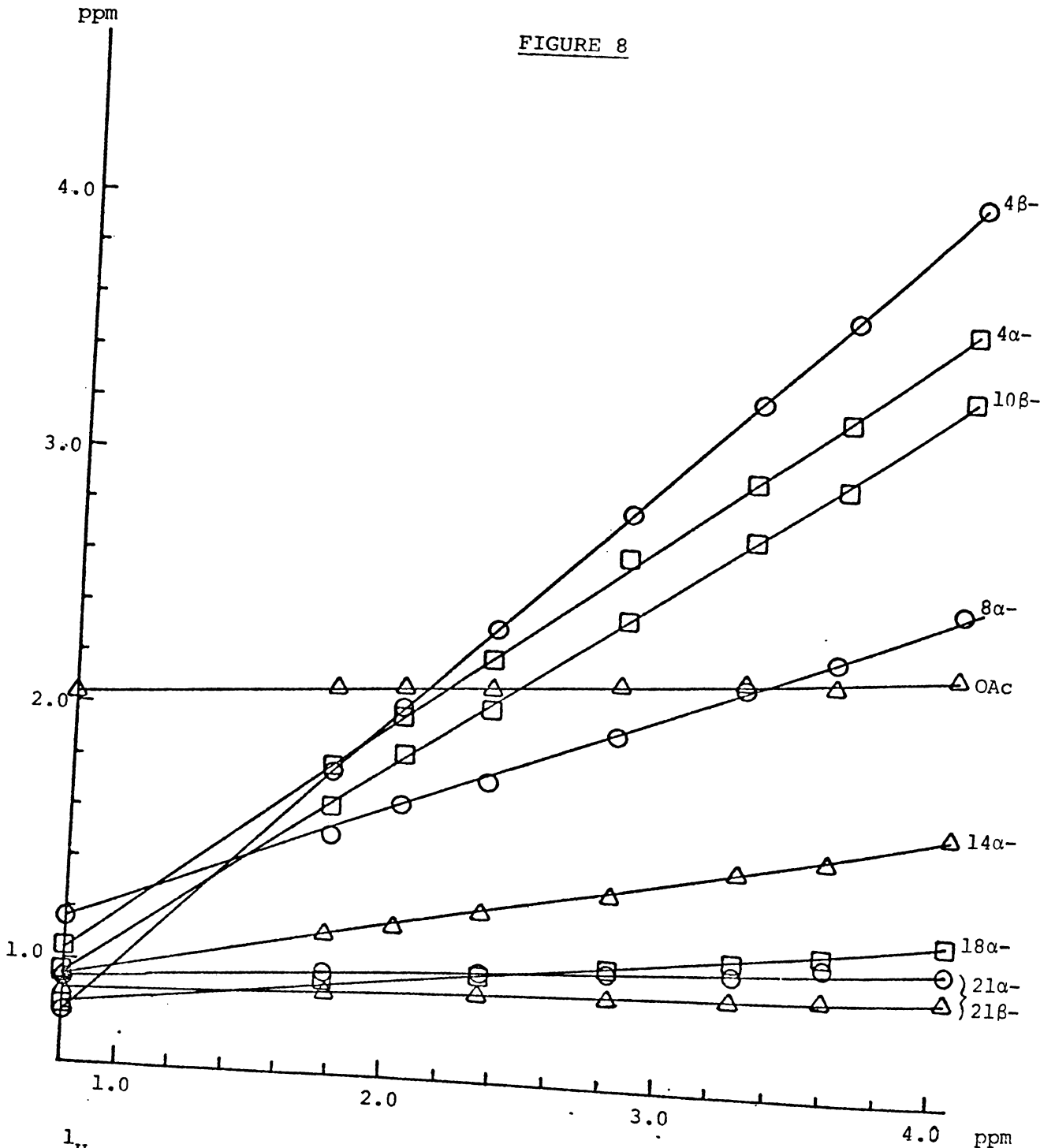
In accord with these observations Beierbeck and Saunders[88] have interpreted γ-effects as arising as a result of γ-hydrogen elimination rather than being due to the addition of a γ-substituent. This interpretation is supported by the observations that a) the γ-shift is independent of the number of hydrogen atoms carried by a substituent, b) account must be taken of the number of hydrogen atoms on a β-substituent (these will be γ-hydrogens) before a correlation between bond polarity and substituent effect is observed and c) removal of 1,3-*syn*-diaxial hydrogen alignment by bond rotation or hydrogen elimination has the same effect.

These findings formed the basis of another, exceptionally successful, empirical ^{13}C chemical shift prediction method,[89] which is applicable to 6-membered rings in chair conformation. According to this system, after degree of substitution and α-polar substituents have been taken into account, the largest single chemical shift-producing factor is γ-*gauche* interactions between protons (labelled HC). Thus, whereas Grover and Stothers[85] were unable to rationalise their observation that equatorial hydroxyl groups generally produce larger α-effects than axial hydroxyl groups, Beierbeck and Saunders[90] envisaged the difference as arising from the fact that although an axial hydroxyl group usually eliminates a deshielding HC relationship an equatorial hydroxyl group rarely does so. A parallel explanation was presented to account for the larger shielding effect of axial hydroxyl groups on γ-carbons possessing a proton which is 1,3-*syn*-diaxial to the hydroxyl group. Another feature of this system is that the β-effects of heterosubstituents are divided into two factors[90] (HX and CX) of which a β-carbon may possess any combination of the two depending on whether it has C-H and/or C-C bonds oriented *gauche* to the heterosubstituent .

Djerassi *et al.*[78] have also produced equations to predict the substituent effects of hydroxyl groups on α- and β-carbon atoms. Although the terms of reference and the values of the parameters differ yet both systems are basically analogous.

The advantages of the procedure of Beierbeck and Saunders[89] are a) model compounds are not required as a basis from which to begin (*cf.* Djerassi *et al.*[78]), b) it is generally applicable to 6-membered ring systems in chair conformations, c) it includes heterosubstituent effects (*cf.* Smith[91] and Dalling and Grant[83,84]),d) it is simple. However, the

FIGURE 8



^{1}H nmr LIS of the methyl group signals of 22α-acetoxystictane-2α,3β-diol (74)

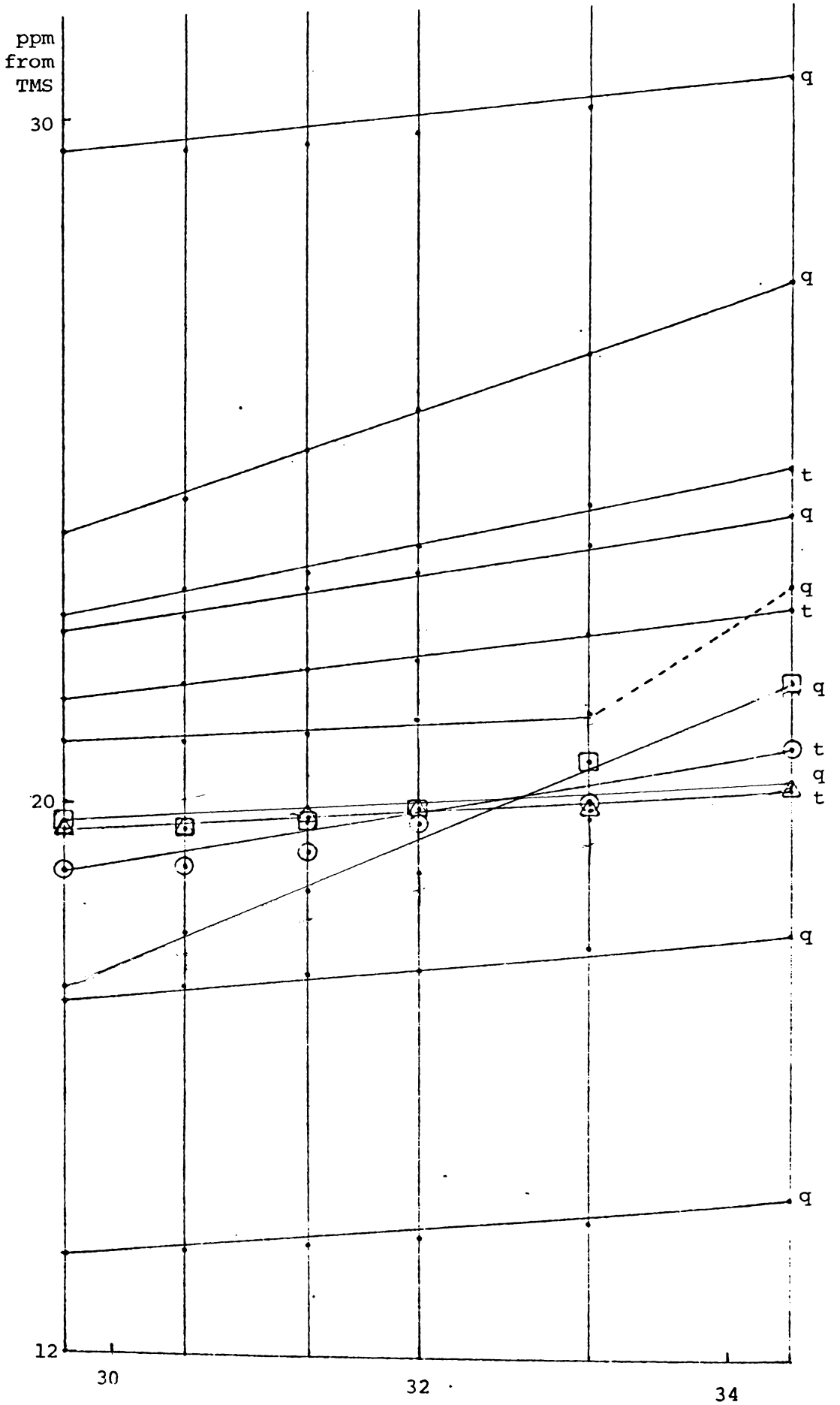
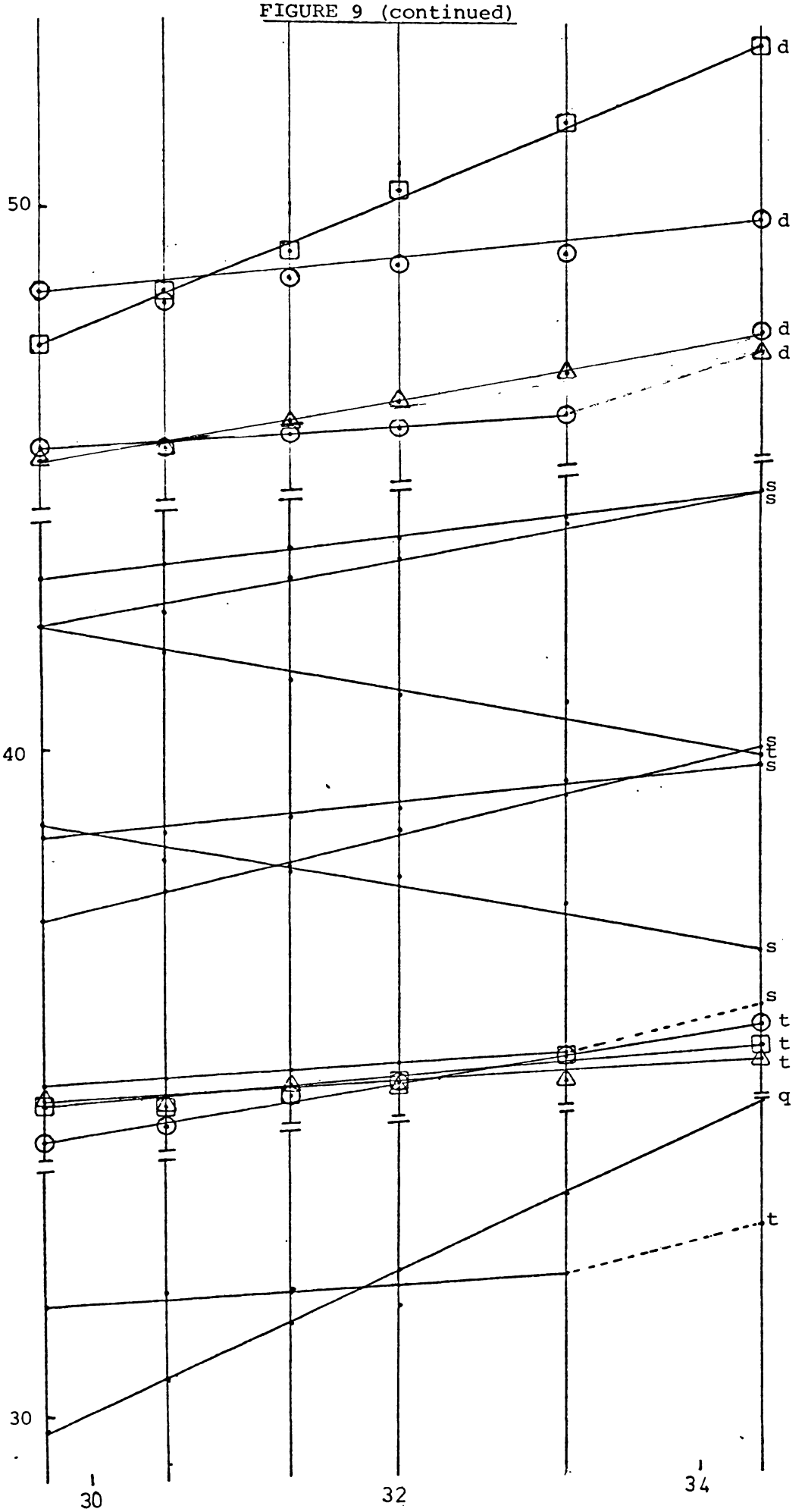


FIGURE 9

^{13}C nmr LIS of 22α-acetoxystictane-2α,3β-diol (74). The letters q, t, d and s, refer to the signal multiplicities in the SFORD spectrum as follows: q, quartet; t, triplet; d, doublet and s, singlet.

FIGURE 9 (continued)



last feature is also a disadvantage, since, having only the one γ-substituent parameter, this method fails to account fully for the γ-effect on methine carbon atoms, while it over-estimates the shielding effect on quaternary nuclei, which are often deshielded instead of shielded. In addition, although the δ-effect on other carbon atoms is often non-zero, yet the only case accounted for is where the hydroxyl group and carbon nucleus are 1,3-*syn*-diaxial (*cf.* the substituent effects calculated by Grover and Stothers[85]).

3.3 Lanthanide Induced Shift Experiments

(i) Introduction

A preliminary ^{1}H nmr study of the effect on 22α-acetoxystictane-2α,3β-diol (74) of adding increments of the lanthanide shift reagent (LSR) $Eu(fod)_3$, produced the linear plots, for each methyl group, shown in figure 8. This indicates that the two equatorial hydroxyl groups (2α- and 3β-) together behave as one co-ordination site and that there is little competition from the acetoxyl group.[92]

Following this demonstration of the suitablity of 22α-acetoxystictane-2α,3β-diol (74) as a substrate for lanthanide induced shift (LIS) studies ^{13}C experiments were performed on the same species. Figure 9 (in two parts) is a graph of the linear region of the LIS of each carbon nucleus (except the carbinyl and carbonyl carbons) plotted against the most shifted methyl group signal.

After the fifth addition of $Eu(fod)_3$ a tailing effect was observed on the carbons close to the co-ordination site, while the signals of some of the more remote carbon atoms experienced a sharp increase in deshielding which was especially marked in the case of the acetate methyl signal. That the latter effect was no artifact was demonstrated by a

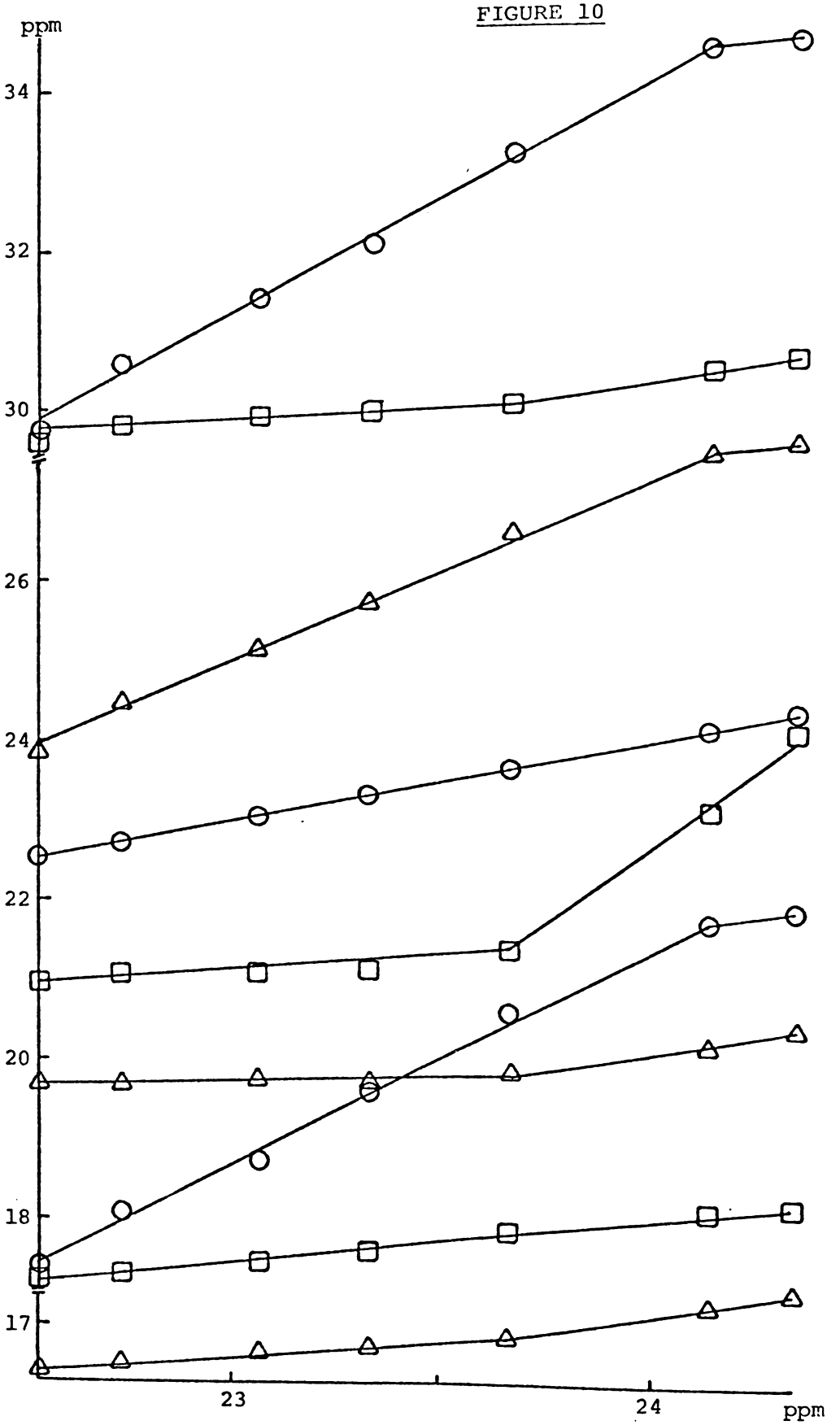
similar, though not so precipitous, effect on the carbonyl carbon signal. (table 5). This effect suggested that saturation or near-saturation of the ring A-co-ordination site had occurred such that the LSR subsequently complexed to the acetoxyl group.

In order to satisfactorily present the full LIS of each carbon nucleus a signal (the quartet at 22.9 ppm, from a central methyl carbon) that was considered to be steadily shifted throughout was selected as the standard by which to plot the LIS effects of the other signals. These graphs are presented in figures 10 - 13, in which the signals of the different categories (methyl, methylene, methine and quaternary) of carbon atom appear together (with one exception - the down-field methylene carbon signal in figure 13). These figures are placed further over in this section, where they are discussed. In many cases from the slopes of these plots, both before and after the change in co-ordination site, assignments could be made with a moderate degree of certainty.

The LIS effects on two carbon atoms were in the opposite sense to the rest - shielding instead of deshielding (figure 13). One of these signals (a triplet at 41.8 ppm) was ascribed to C-1, while the other (a singlet at 38.9 ppm) was ascribed to C-4. An explanation of this apparent anomally requires a brief analysis of lanthanide-induced shift theory.

Observed lanthanide induced shifts are summations of contact, pseudo-contact and complex-formation contributions. The complex-formation effect has been estimated by performing what are effectively "blank" experiments[93] using the equivalent lanthanum complex. Being diamagnetic lanthanum does not produce paramagnetic shifts. By this means, Chadwick and Williams[93] were able to determine that in alcohols (except adamantan-1-ol) only the α-carbon experienced a small (deshielding) complex-formation shift.

FIGURE 10



^{13}C nmr LIS of the methyl carbon signals of 22α-acetoxystictane-2α,3β-diol (74)

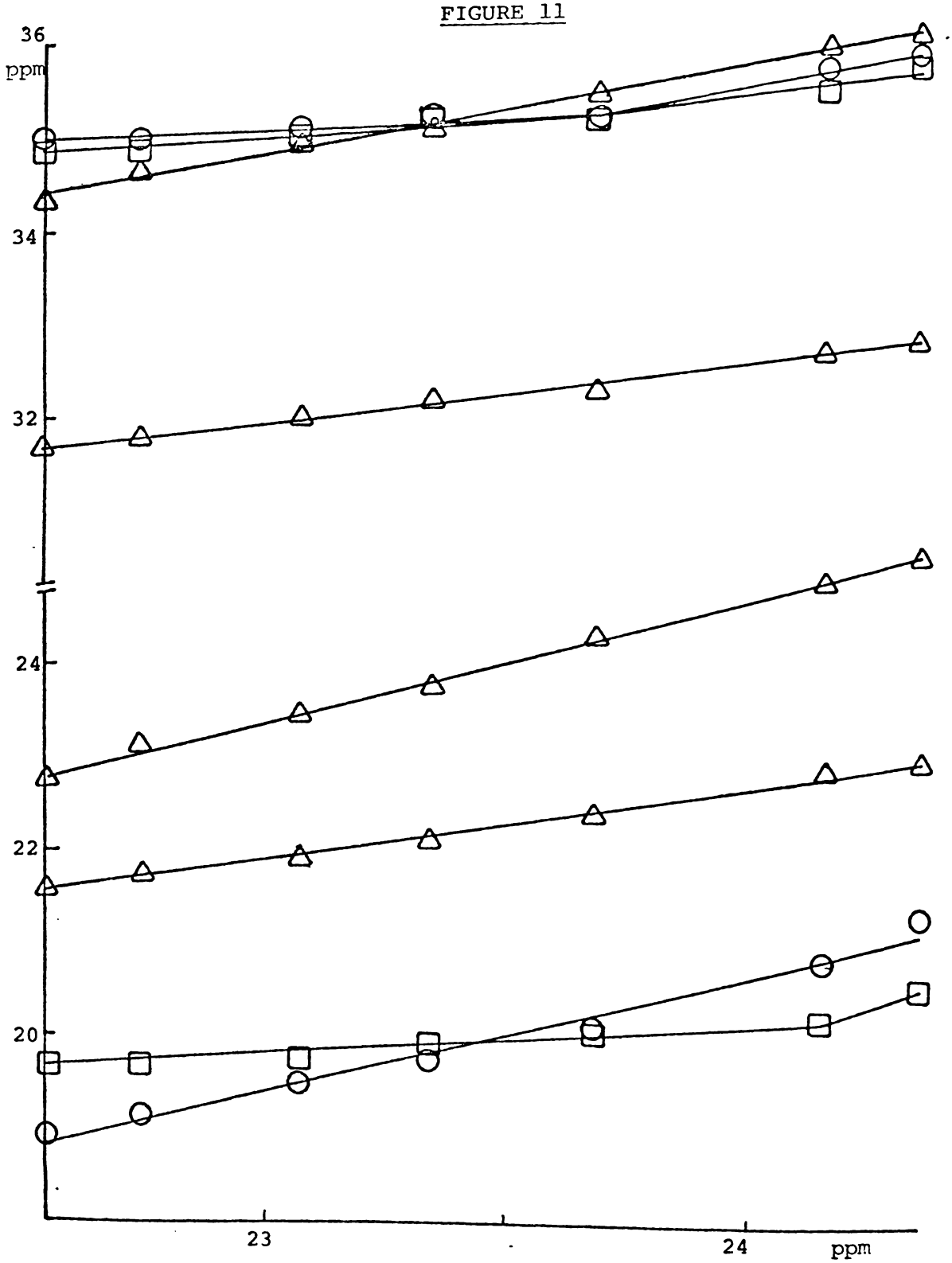
Pseudo-contact contributions, which operate through space, are proportional to $(3\cos^2\theta_i - 1)\, r_i^{-3}$, where r_i is the distance between the complexed lanthanide ion and the ith nucleus and θ_i is the angle between this radius vector (r_i) and the principal axis of the complex. Thus a shielding effect would be produced by the paramagnetic effect of $Eu(fod)_3$ if θ_i were greater[94] than 54.7°. However, it is difficult to conceive of a position for the lanthanide ion where the angle factor would not be greater, if not for C-24, then at least for C-23, than it is for C-4. Yet these two methyl carbons experienced a positive shift effect, second only to that observed for the carbinyl carbons, C-2 and C-3.

Fermi-contact shift effects, which have been invoked by several authors[94-98] to explain anomalous Eu(III)- and Pr(III)-induced shifts, operate through bonds and thus are significant only for nuclei up to four bonds from the co-ordination site. Generally when europium complexes are used with alcohols and amines the contact contribution has been found to produce an alternating effect,[97] alternately, reinforcing and opposing the pseudo-contact term until the former approaches zero for carbon atoms more remote from the complexation site. To account for the changing sign of the contact contribution hyperconjugative resonance forms have been proposed.[97] It would appear then that contact shift effects are the most likely origin of the shielding shifts experienced by C-1 and C-4. The generalisation [97] that more highly substituted carbons are more sensitive to contact effects was lent support by the observations that C-4 was shifted -35Hz compared with -28Hz for C-1.

(ii) The methyl group signals

The three methyl group signals (17.3, 23.9 and 29.7 ppm) which experienced a tailing-off effect (figure 10) were also those which were

FIGURE 11



^{13}C nmr LIS of the methylene carbon signals of 22α-acetoxystictane-2α,3β-diol (74)

most shifted initially, so these were assigned to the methyl groups about ring A. The similar degree of deshielding experienced by each of these carbon nuclei is in contrast with the clear distinction between the LIS effects experienced by the protons associated with these carbon atoms (figure 8) and suggests *[cf.* the LIS experienced by the parallel carbon nuclei in a lanostan-3β-ol[99] derivative : C-30 (≡ C-24) 20.4Hz, C-29 (≡ C-23) 18.5Hz, C-19 (≡ C-25) 11.1Hz*]* that the average location of the lanthanide ion was closer to C-2 than C-3 (contact shift effects would tend to augment the C-23 and C-24 shifts but not that of C-25).

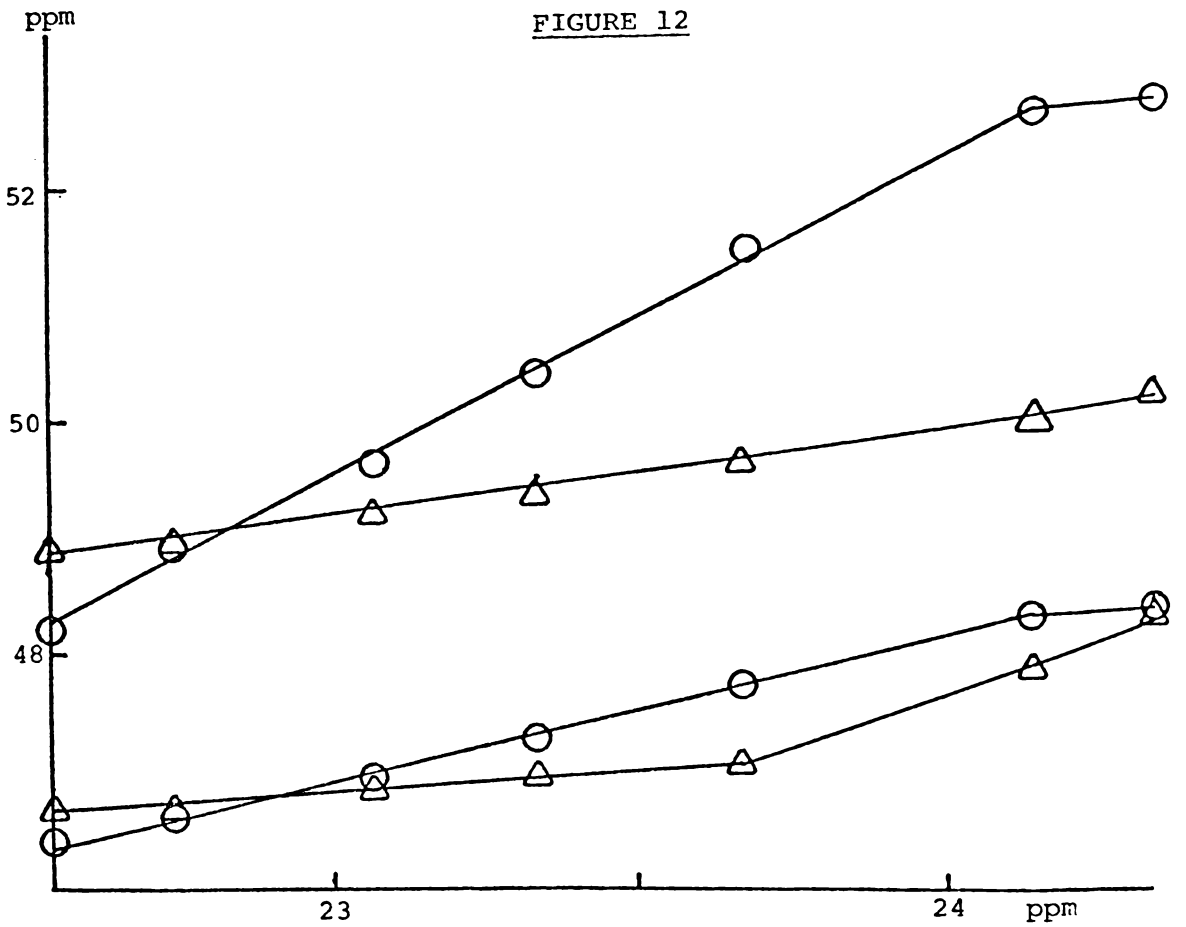
Two primary carbon signals, at 17.1 and 22.5 ppm, produced single straight-line plots (the latter was the signal by which the rest were standardised), which were assigned on the basis of their slope to C-27 and C-26 respectively. The remaining four methyl signals initially experienced almost negligible LIS but the deshielding effect increased more rapidly after the fourth addition of the LSR (figure 10). The most notable of these, at 20.9 ppm, has already been ascribed to the acetoxyl methyl group carbon, while the balance of the signals could not be distinguished by their LIS alone, so were approximately assigned to the angular methyl groups attached to ring E. These assignments can be summarised:

17.3)				13.4)	
)				)	
23.9)	C-23,-24,-25	22.5	C-26	19.7)	C-28,-29,-30
)		17.1	C-27	)	
29.7)				29.5)	

(iii) The Methylene carbon signals

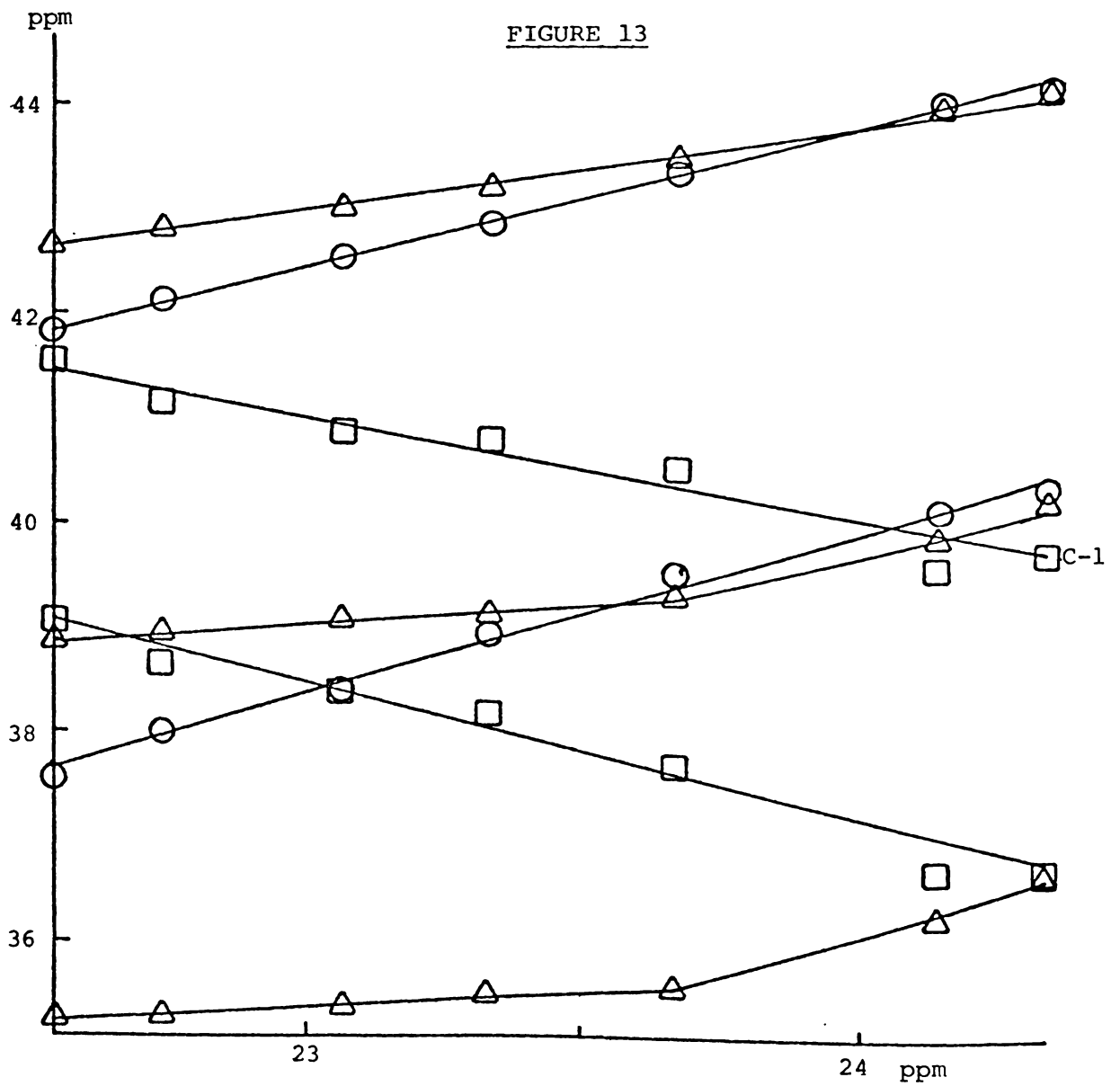
The negatively shifted methylene carbon signal (figure 13) has already been ascribed to C-1. Of the complement (figure 11) none experienced a tailing effect, but those signals which were subjected to a steady increase in deshielding could be grouped on the basis of the LIS

FIGURE 12



^{13}C nmr LIS of the methine carbon signals of 22α-acetoxystictane-2α,3β-diol (74)

FIGURE 13



^{13}C nmr LIS of the quaternary carbon signals and that of C-1 of 22α-acetoxystictane-2α,3β-diol (74)

produced as follows:

18.9)
)
22.7) C-6,-7,-11   21.5)
)   31.6) C-12,-15
34.3)

That the effect on each of C-6, -7 and -11 is so similar lends credence to the view that the lanthanide ion was, on the average, situated towards the C-1 end of ring A. The remaining secondary carbon signals experienced an increase in LIS after the fourth addition of the LSR, as is demonstrated by the change in the slope of their plots. Hence, the approximate assignment could be made:

19.6)
)
34.8) C-16,-19,-20.
)
34.9)

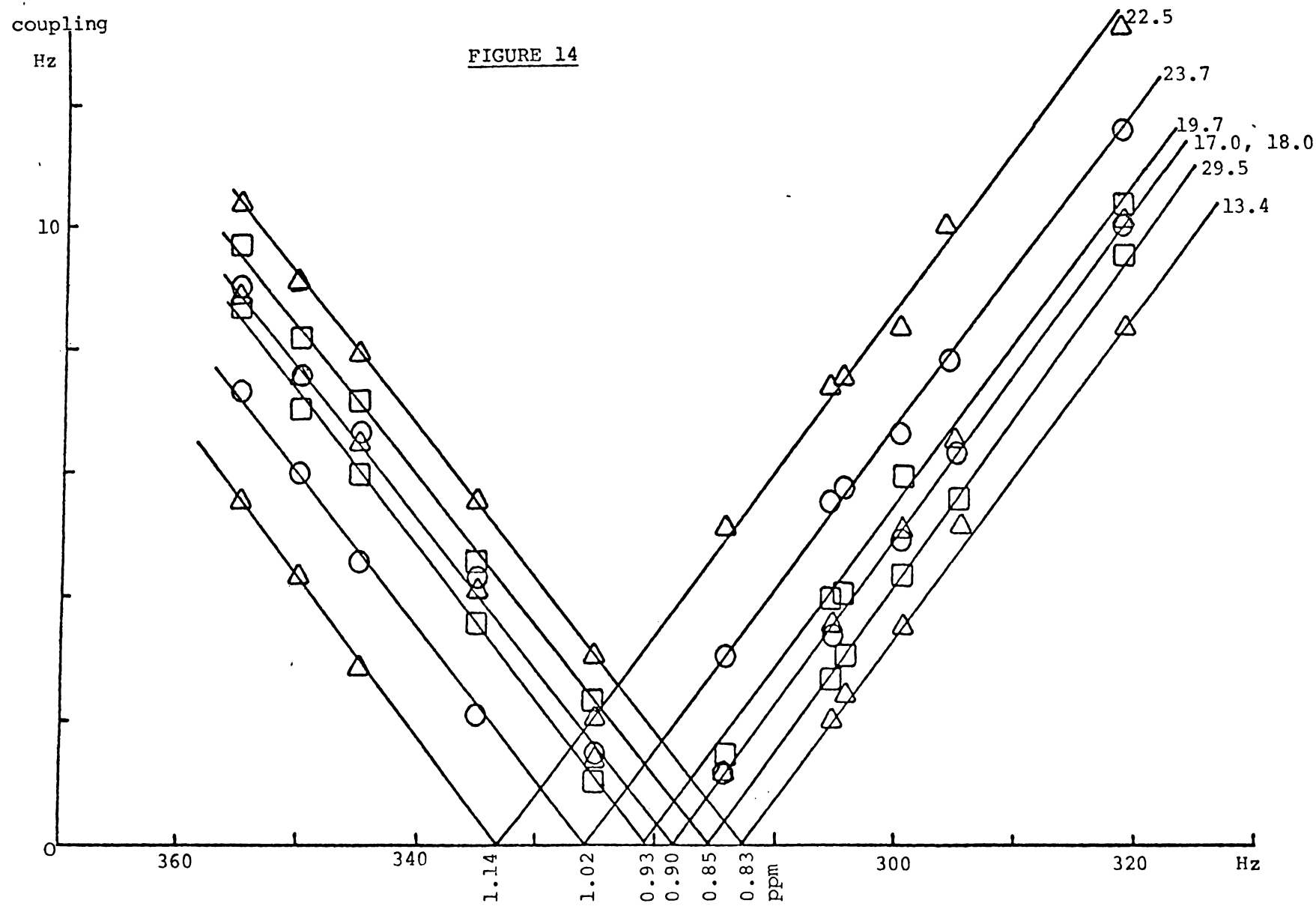
(iv) The methine carbon signals

The tertiary carbon signals can be unambiguously assigned purely on the basis of the results of the lanthanide shift experiments. Of the two signals (48.0 and 46.3 ppm) which experienced a tailing effect, one (48.0 ppm) was clearly more shifted initially (figure 12). One methine carbon signal (48.8 ppm) produced a single straight line, while the fourth (46.6 ppm) demonstrated an increase in the rate of change of deshielding after the fourth addition of LSR. Accordingly, assignments were made thus:

48.0	C-5	48.8	C-13
46.3	C-9	46.5	C-17

(v) The quaternary carbon signals

On the basis of its upfield LIS the signal at 38.9 ppm has already been assigned to C-4. The quaternary carbon signals, whose LIS plots produced a single straight line (figure 13) can be assigned by



Selective frequency decoupling experiments of the methyl groups of 2α,3β,22-triacetoxystictane (75)

comparing the slopes, which decrease in the order 37.5>41.8>42.5 ppm. The balance of the signals (35.2 and 38.7 ppm) cannot be assigned unambiguously because both the initial and final slopes of their LIS plots are very similar. The assignments can be set out:

38.9	C-4	37.5	C-10	35.1	)
					) C-18,-21
41.8	C-8	42.5	C-14	38.7	)

3.4 Selective Frequency Decoupling of the Methyl Groups

The aim of the selective frequency decoupling experiments[77,100] was to ascertain the relationship between the already assigned methyl group signals in the ^{1}H nmr spectrum and the methyl carbon signals of the ^{13}C nmr spectrum. This was achieved by moderate proton irradiation at selected frequencies over a range and noting the change in the residual $^{1}J_{^{13}C-^{1}H}$-values as observed in the primary carbon signals of the ^{13}C nmr spectrum. The frequency at which the coupling constant of a specific methyl carbon signal reaches zero, as determined from the graph of coupling constant versus irradiation frequency, is the frequency of the absorption of the protons attached to that carbon.

In figure 14 the results of sequential selective frequency decoupling on 2α,3β,22α-triacetoxystictane (75) are graphically presented. By this means, the assignments could be made as set out in table 6.

TABLE 6

Assignments from selective frequency decoupling experiments on 2α,3β,22α-triacetoxystictane (75)

^{1}H nmr signal	Methyl group	^{13}C nmr signal	carbon No.
0.80	18β-	13.4	28
0.85	21β-	29.5	29
0.90(x3)	4α-	17.1	23
	4β-	18.0	24
	14β-	29.5	27
0.93	21α-	19.7	30
1.02	10β-	23.7	25
1.14	8α-	22.5	26

The broadness of the signal at 29.5 ppm was consistent with coupling to a proton signal centered at *ca.* 0.90 ppm, although discrete coupling to the protons of another methyl group was not observed along with the 21β-methyl group coupling.

Comparing these results with the assignments made from the LIS experiments on 22α-acetoxystictane-2α,3β-diol (74) enables all of the primary carbon signals to be assigned except those originating from C-23 and -24, which in both cases must be bracketed due to accidental degeneracy.

3.5 Substituent Effects and Calculated Chemical Shifts

(i) The methyl carbon signals

From the outset it can be observed that the quartets in the SFORD spectra of the pentacyclic stictane derivatives fall into two distinct groups, so that six primary carbon signals occur between 13.0 and 25.0 ppm, while the remaining two resonate at *ca.* 29.0 ppm. Furthermore, in the seco-compounds one of these downfield signals has experienced a relatively large shielding effect and resonates in the higher field

region. In the stictane skeleton two of the eight methyl groups (4α- and 22β-) exist in an equatorial configuration, but this situation would be upset by cleavage of ring A, resulting in the erstwhile equatorial 4α-methyl group being now effectively attached to a side chain of ring B. A comparison of the ^{13}C nmr resonances of the methyl carbons of a number *gem*—dimethyl systems (table 7) reveals that equatorial methyl group

TABLE 7

^{13}C nmr resonances of *gem*—dimethyl carbons

Compound	axial	equatorial	ring	γ-substituent
pimara-8(14),15-diene[a]	22.5	34.5	A	-
3β-methoxylup-22(29)-ene[b]	16.1	28.0	A	OMe
hopane[c]	21.8	33.6	A	-
lanost-8-en-3β-ol[d]	14.8	27.4	A	OH
cycloartan-3β-ol[e]	14.0	25.4	A	OH
olean-12-en-3β-ol[f]	15.5	28.1	A	OH
olean-12-en-3β-ol[f]	23.6	33.2	E	-

[a]Ref. 101. [b]Ref. 102. [c]Ref. 103. [d]Ref. 104. [e]Ref. 105. [f]Ref. 106.

carbons do indeed typically resonate at significantly lower field regions than carbon nuclei of axial methyl groups. In addition the table demonstrates that a polar substituent in a γ-position has a shielding effect on both methyl groups.

TABLE 8

The methyl group signals (ppm from TMS)

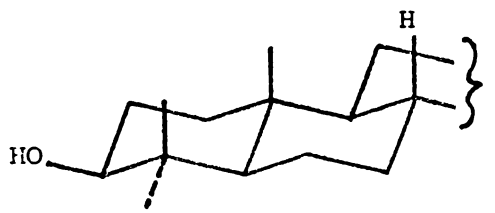
Compd.	Diol (72)	Triol (73)		Dihydroxy-acetate (74)	Tri-acetate (75)
Solvent	C_5D_5N	C_5D_5N	$CDCl_3$	$CDCl_3$	$CDCl_3$
23	29.7	30.1	29.8	29.7	29.5
24	17.1	18.1	17.3	17.3	18.0
25	22.9	24.1	24.0	23.9	23.7
26	22.9	22.6	22.6	22.5	22.5
27	17.5	17.4	17.2	17.1	17.1
28	13.9	13.6	13.6	13.4	13.4
29	30.6	30.5	29.8	29.5	29.5
30	19.3	19.2	18.6	19.7	19.7

This marked shielding of an axial methyl group relative to an equatorial methyl group has been attributed to a steric polarisation effect produced by γ-*gauche* protons.[107] However, doubt has fallen on this explanation as a result of the observation that 1,3-*syn*-diaxial methyl groups are mutually deshielding. Surprisingly, Allinger and Wertz[108] have suggested that, contrary to popular opinion, generally an equatorial position is more sterically crowded than an axial one and that the reason for the usually prefered conformation being that where the bulky groups are equatorial is that the ring protons prefer an axial position. In terms of Beierbeck and Saunders' method of predicting chemical shifts,[89] equatorial methyl groups generally experience a larger number of γ-*gauche*-proton interactions (HC), which have been given a deshielding value of 4.55 ppm (section 3.2).

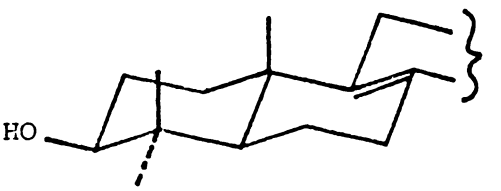
Accordingly, the two low field primary carbon signals *[*29.7 and 29.5 ppm in (74)*]* which have been clearly distinguished by both LIS and selective frequency decoupling experiments, are assigned to C-23 and -29, leaving the only unassigned methyl carbon signal at 18.0 ppm in the triacetate (75) to be ascribed to C-24.

The substituent effect on C-29 and -30 on acetylation of the 22α-alcohol (table 8) is consistent with that observed on the methyl group carbons of the 4,4-*gem*-dimethyl system on acetylation of both olean-12-en-3β-ol[106] and cycloartan-3β-ol.[105] C-28 also experienced a small δ-shift effect, but except for this substituent effect and a moderate solvent effect these ring E methyl groups remained unchanged in position. The C-26 and -27 signals are almost unshifted, as expected, except on cleavage of ring A. An analogy can be drawn from the oleanane series[106] as to the substituent effect of adding a 2α-hydroxyl group to a

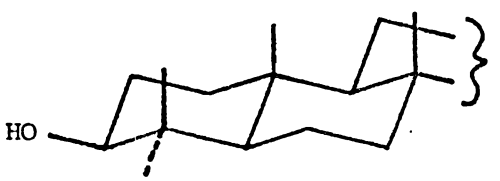
FIGURE 15



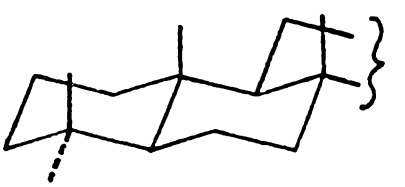
Lanostan-3β-ol



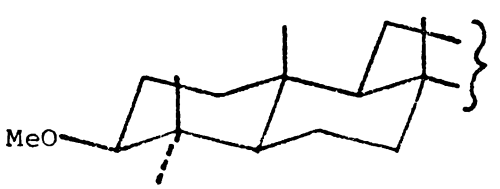
Lanost-8-en-3β-ol



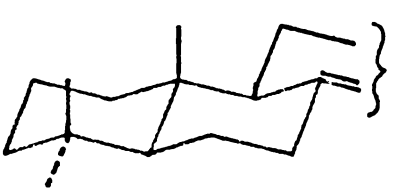
Olean-12-en-3β-ol



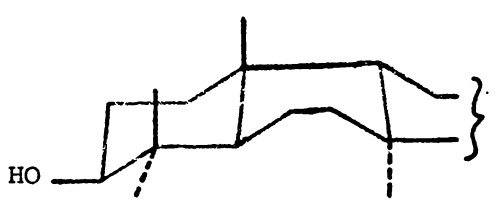
Hopane



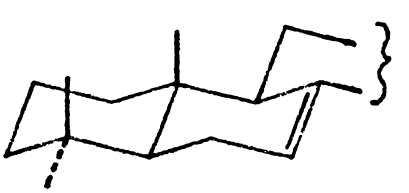
3β-Methoxylup-22(29)-ene



Pimara-8(14),15-diene



Stictane-3β;22α-diol (72)



IsoPimara-7,15-diene

3β-alcohol. There is no example in the literature of acetylation of a 2α,3β-diol system, but it is useful to compare the effect of acetylation

TABLE 9

Substituent effects on C-23, -24 and -25

		23	24	25
Δ 3β-OH→2α,3β-$(OH)_2$	(oleanane[a]	0.5	1.3	1.3
	(stictane	0.4	1.0	1.2
Δ 3β-OH→3β-OAc	oleanane[a]	0.0	1.3	0.2
Δ 2α,3β-$(OH)_2$→2α,3β-$(OAc)_2$	stictane	-0.2	0.7	-0.2

[a]Ref. 106.

of an isolated 3β-hydroxyl group (table 9). The first comparison confirms the assignments made, while the second indicates the presence of opposing γ- and δ-effects.

Comparing the absolute values of the chemical shifts of the ring A methyl carbons of a number of similar compounds (table 10) reveals that C-25 (≡ 10β-methyl carbon) in stictane-3β,22-diol (72) has an anomalous resonance frequency. (NB. The irregularity in the 4α- and 4β- carbon resonances of hopane,[150] pimara-8(14),15-diene and isopimara-7,15-diene[101] arises from the lack of γ-substituent as has already been mentioned). The 10β-methyl group of olean-12-en-3β-ol,[106] hopane[103] and 3β-methoxylup-22(29)-ene[102] possesses two 1,3-*syn*-diaxial methyl groups (figure 15), while the 10β-methyl group in stictane possesses only one of these interactions. However, such relationships are mutually deshielding as is demonstrated by the ^{13}C-shift of the 10β-methyl group

TABLE 10

^{13}C shifts of the 4α-, 4β- and 10β-methyl groups

	4α-	4β-	10β-
lanostan-3β-ol[a]	28.3	15.6	13.9
lanost-8-en-3β-ol[a]	28.1	15.4	18.3
olean-12-en-3β-ol[b]	28.1	15.5	15.5
hopane[c]	33.6	21.8	16.8
3β-methoxylup-22(29)-ene[d]	28.0	16.1	16.1
pimara-8(14),15-diene[e]	34.5	22.5	14.9
isopimara-7,15-diene[e]	33.9	22.6	15.2
stictane-3β,22α-diol (72)	29.7	17.1	22.9

[a]Ref. 105. [b]Ref. 106. [c]Ref. 103. [d]Ref. 102. [e]Ref. 101.

of lanostan-3β-ol. The downfield shift of the 10β-methyl group carbon in lanost-8-en-3β-ol can largely be attributed to the effect of the δ,γ-double bond, while that in pimara-8(14),15-diene and pimara-7,15-diene can be associated with the removal of a 1,3-*syn*-diaxial proton. There is some evidence[91] that protons 1,3-*syn*-diaxial to a methyl group have a shielding effect, but this effect is variable in magnitude. Thus in the stictane derivatives the removal of a 1,3-*syn*-diaxial proton-methyl group interaction, by ring B being in a boat conformation, would have a deshielding effect, but its magnitude would not fully account for the discrepancy observed between the C-25 resonance frequency in stictane derivatives and that of parallel carbons in related compounds. A recent X-ray crystallographic study[76] of stictane-2α,3β-diol (72) has indicated that ring B is severely twisted such that five carbon atoms are

approximately in the same plane. Hence, this deformation of ring B may be the origin of the unusually large chemical shift of C-25.

The results of calculating the chemical shifts of the methyl groups of the triol (73) after Beierbeck and Saunders[89] are compared with the experimental values (in $CDCl_3$) in table 11. To the Beierbeck and Saunders values for both C-26 and -27 was added a parameter (2.8 ppm)

TABLE 11

Calculated and experimental shifts of the methyl carbon of stictane-2α, 3β,22α-triol (73)

	23	24	25	26	27	28	29	30
Calc.	24.4	17.8	14.6	14.8	17.4	14.6	28.9	19.8
Obs.	29.8	17.3	24.0	22.6	17.2	13.6	29.8	18.6
Δ	5.4	-0.5	9.4	7.8	-0.2	-1.0	0.9	-1.2

to account for the mutual substituent effect of two antiperiplanar methyl groups. This value is not considered to be definitive however because it was derived from the much less rigid system 7β-hydroxy-2α,10-dimethyl-*trans*-decalin,[109] where one of the methyl groups is attached to a tertiary carbon atom. Comparing the calculated and observed chemical shifts it is evident that each pair agrees within 1.2 ppm except for C-23, -25 and -26. The discrepancy at C-23 is accounted for by neither the 2α,3β-diol system *[cf.* the diol (72), table 8*]* nor the boat ring (*cf.* the resonance position of the 4α-methyl group carbons of the related compounds, table 10). That both C-25 and -26 resonate considerably downfield of their calculated shifts (7-10 ppm) would appear to be a result of the twisted boat-ring B.

TABLE 13

The methylene group signals (ppm from TMS)

Compd.	Diol(72)	Triol(73)		Dihydroxy-acetate(74)	Tri-acetate(75)
Solvent	C_5D_5N	C_5D_5N	$CDCl_3$	$CDCl_3$	$CDCl_3$
1	33.7	42.7	41.6	41.8	39.0
2	30.1				
6	19.3	19.2	19.0	18.9	18.7
7	34.9	34.5	34.3	34.3	34.0
11	22.9	23.0	22.9	22.7	22.7
12	21.8	21.7	21.6	21.5	21.4
15	32.3	32.3	31.9	31.6	31.6
16	20.0	19.9	19.4	19.6	19.5
19	35.3	35.3	35.1	34.8	34.8
20	35.3	35.3	35.1	34.9	34.8

(ii) The methylene carbon signals

Of the secondary carbon signals only that pertaining to C-1 was unambiguously assigned on the basis of its LIS. However, calculating the chemical shifts, using the paramaters of Beierbeck and Saunders[89] and taking into account the results of the LIS experiments produced a guide *[*section 3.3 (iii)*]* for specific assignments (table 12). The only signal for which there was not a reasonable degree of agreement

TABLE 12

Calculated and experimental shifts of the methylene carbons of stictane-2α,3β,22α-triol (73)

	1	6	7	11	12	15	16	19	20
Calc.	40.7	17.6	28.6	22.2	22.2	33.1	20.9	33.1	36.4
Obs.	41.6	19.0	34.3	22.9	21.6	31.9	19.4	35.1	35.1
Δ	0.9	1.4	5.7	0.7	-0.6	-1.2	-1.5	2.0	1.3

between calculated and observed values was C-7, for which the boat ring evidently causes a downfield shift. This downfield boat-ring shift is in accord with the data of Dalling and Grant.[84]

A substituent effect on C-1 of approximately -8.0 ppm (steroidal example-ref. 110 *cf.* ref. 78 and oleanane example-ref.106) was expected between the triol (73) and the diol (72), enabling the secondary carbon signal at 33.7 ppm in the diol (72) (table 13) to be assigned to C-1, while the remaining methylene carbon signal (30.1 ppm) was assigned to C-2 *[*see also section 3.5 (v)*]*. These assignments are in accord with the respective calculated values (after Beierbeck and Saunders[89]) for C-1 and C-2 of the diol (72) which are 31.8 and 26.4 ppm. In addition the

effect (-2.8 ppm) experienced by C-1 on acetylation of the ring A alcohol functions is of the same order of magnitude as that induced in C-2 (-2.3 ppm) by acetylation of a steroidal 3β,4α-diol (ref.110 *cf.* ref.78).

C-6 and -7 were subjected to minor shielding effects (table 13) on both the introduction of the second ring A substituent and acetylation of the ring A hydroxyl groups, possibly reflecting a conformational adjustment induced by new steric requirements. By comparison C-6, and -7 in the oleanane series were unshifted by the addition of a 2α-hydroxyl group to a 3β-alcohol, whereas a similar substituent change in the steroid series produced a substituent effect of -1.1 ppm on C-6 (ref. 110 *cf.* ref. 78).

Acetylation of the C-22 hydroxyl group produced moderate γ- and δ-effects on C-20 and -19 respectively, while the γ-*gauche* effect on C-16 is smaller than the δ-effect on C-15. Perhaps the γ-*gauche* acetylation-effect reflects a summation of two opposing effects since it is observed to be zero on acetylation of the 6α-hydroxyl group of cholestan-6α-ol.[78]

(iii) The methine carbon signals

The signals which appear as ill-defined doublets in the SFORD

TABLE 14

The methine group signals (ppm from TMS)

Compd.	Diol(72)	Triol(73)		Dihydroxy-acetate(74)	Tri-acetate(75)
Solvent	C_5D_5N	C_5D_5N	$CDCl_3$	$CDCl_3$	$CDCl_3$
5	48.4	48.4	48.0	48.0	47.6
9	46.1	46.5	46.5	46.3	46.1
13	49.3	49.2	48.9	48.8	48.6
17	49.3	49.2	48.7	46.5	46.5

spectra have already been assigned by comparing their LIS effects *[*section 3.3 (iv)*]*, but these assignments can be independently confirmed by observing the substituent group effects. Thus, the tertiary carbon signal (table 14) which experienced a 2.2 ppm upfield shift on acetylation of the 22α-hydroxyl group (*cf.* 3.0 ppm for C-5 of 6α-acetoxycholestane[78]) had been assigned to the β-carbon, C-17. Substituent group changes in ring A produced minor effects on the signals allocated to C-5 and -9, but the C-13 signal is invariant (within experimental error) on substituent group changes.

TABLE 15

Calculated and experimental shifts of the methine carbons of stictane-2α, 3β,22α-triol (73)

	5	9	13	17
Calc.	45.8	43.4	51.6	48.2
Obs.	48.0	46.5	48.9	48.7
Δ	2.2	3.1	-2.7	0.5

Comparison of these assigned values with those calculated after Beierbeck and Saunders[89] (table 15) presents a reasonable degree of agreement, with the nuclei associated with ring B (C-5 and -9) again absorbing downfield of the predicted values. In contrast Dalling and Grant[84] observed that on an average the bridgehead carbon nuclei of boat-rings are shielded with respect to the analogous nuclei of chair-rings.

(iv) The quaternary carbon signals

Except for those corresponding to C-18 and -21 each of the six signals arising from the quaternary carbons of 22α-acetoxystictane-2α, 3β-diol (74) have been assigned from their LIS effects *[*section 3.3 (v)*]*.

TABLE 16

The quaternary carbon signals (ppm from TMS)

Compd	Diol (72)	Triol (73)		Dihydroxy-acetate (74)	Tri-acetate (75)
Solvent	C_5D_5N	C_5D_5N	$CDCl_3$	$CDCl_3$	$CDCl_3$
4	39.9	39.4	38.5	38.9	38.7
8	42.1	41.9	41.9	41.8	41.7
10	37.2	37.8	37.7	37.5	37.3
14	42.9	42.7	42.6	42.5	42.5
18	38.7	38.4	39.1	38.7	39.0
21	36.3	36.2	35.9	35.1	35.2

Of the two unassigned signals that absorbing at 35.1 ppm had experienced a typical β-carbon upfield shift (0.8 ppm) (table 16) on acetylation of the C-22 hydroxyl group (*cf.* a shift of *ca.* -1.0 ppm for C-4 on acetylation of both olean-12-en-3β-ol[106] and cycloartan-3β-ol [105]), and hence was assigned to C-21. On the other hand the signal resonating at 38.7 ppm had been subjected to a substituent group effect of -0.4 ppm on acetylation, which made the γ-carbon, C-18, a suitable assignment. The signals of C-8 and -14 were relatively invariant, but substituent group changes in ring A effected minor changes in the chemical shifts of C-4 and -10. Introduction of a second polar substituent to the 2α-position of methyl 3β-hydroxyolean-12-en-28-oate deshielded C-10 by 1.3 ppm and in the steroid parallel (ref.110 *cf.* ref. 78) by 2.0 ppm, in contrast C-10 is shifted by 0.6 ppm on going from the diol (72) to the triol (73). The marked reduction in substituent effect in the stictane series possibly reflects a restraint on C-10 imposed by the boat-ring.

Since the 8α- and 14β-methyl groups are antiperiplanar, the effect on C-10 in 7β-hydroxy-10-methyl-*trans*-decalin of adding a methyl group at the 1α-position (1.4 ppm) was added to the calculated chemical

TABLE 17

Calculated and experimental shifts of the quarternary carbons of stictane-2α,3β,22α-triol (73)

	4	8	10	14	18	21
Calc.	37.2	42.7	33.9	42.7	36.4	34.8
Obs.	38.5	41.9	37.7	42.6	39.1	35.9
Δ	1.3	-0.8	3.8	-0.1	2.7	1.1

shifts (after Beierbeck and Saunders)[89] of C-8 and -14 (table 17). Why the calculated and observed chemical shifts of C-18 differ so markedly is difficult to explain, but the other deviating signal arises from a bridge-head nucleus of the boat-ring, C-10, and so has experienced a concomitant downfield shift. C-8 is exceptional in this matter, being the only boat-ring carbon to resonate upfield of its calculated chemical shift.

(v) The carbinyl carbon signals

The carbinyl carbon signals were immediately identified by both their resonance positions (*ca.* 80 ppm) and their multiplicity in the SFORD spectra, in which they possessed better-defined doublets than the methine carbons, in the absence of second-order effects.[77]

The signal from C-22 was readily distinguished by the fact that it was the one carbinyl carbon signal that initially experienced little LIS (table 18). Its identity in the diol (72) and the triol (73) was indicated by the observation[78] that acetylation of equatorial hydroxyl

groups produces a downfield shift of approximately 2.0 ppm on a tertiary α-carbon.

TABLE 18

The carbinyl carbon signal (ppm from TMS)

Compd	Diol (72)	Triol (73)		Dihydroxy-acetate (74)	LIS (Hz)[b]	Tri-acetate (75)
Solvent	C_5D_5N	C_5D_5N	$CDCl_3$	$CDCl_3$		$CDCl_3$
2	30.1[a]	70.5	71.2	70.8	424	71.8
3	78.4	84.2	84.7	84.2	404	81.0
22	75.1	74.9	76.6	78.5	6	78.3

[a]Methylene group signal. [b]After four additions of $Eu(fod)_3$.

The signals of C-2 and -3 could not, of course, be distinguished by the LIS experiments unless it was assumed that, since evidence suggests that the lanthanide ion was closer to C-2 than C-3, then, C-2 would experience the greater LIS. However, the carbinyl carbon signal at 78.4 ppm in the spectrum of the diol (72) was assigned to C-3 by default and the signals of C-2 and C-3 of the triol (73) were assigned on the basis of three considerations. a) β-Hydroxyl groups are deshielding[78] *i.e.* C-3 would be expected downfield of its resonance position in the diol (72). b) The carbinyl carbon signals of methyl 3β-hydroxyolean-12-en-28-oate have been assigned:[106] 68.8 ppm to C-2 and 83.8 ppm to C-3. c) Calculations using the Beierbeck and Saunders[89] parameters indicated that the higher field signal arose from C-2 (table 19).

Since the ring A hydroxyl groups were 1,2-diequatorial an additional parameter (designated "OO" to be consistent with the labelling of Beierbeck and Saunders) was required. Djerassi *et al.*[110] observed that the chemical shifts of the carbinyl carbons of 1,2-diequatorial dihydroxysteroids differs by -4.0 ± 0.3 ppm from the values calculated from the summation of the individual substituent effects. In terms of Beierbeck and Saunders' parameters then: $-4.0 = OO - HO$

$$\therefore OO = 0.4,$$

since both carbon atoms will have lost an HO (= 4.4 ppm) interaction and gained an OO interaction by substitution of an α-equatorial hydroxyl group. Including this value for OO interactions the expected chemical shifts of the carbinyl carbons were calculated and are set out in table 19. Only an order of magnitude agreement is apparent between the

TABLE 19

Calculated and experimental shifts of C-2, -3 and -22 and their respective LIS effects

	Stictane-2α,3β,22α-triol			stictane-3β,22α-diol	
	2	3	22	2	3
Calc.	65.8	85.2	80.4	26.4	80.4
Obs.	71.2	84.7	76.6	30.1	78.4

calculated and observed chemical shifts, yet it is sufficient to confirm the assignments made.

Diacetylation shifted C-2 downfield but C-3 upfield (table 18). Usually the α-effect of acetylation is deshielding but acetylation of the 1,2-diequatorial hydroxyl groups in cholestane-3β,4α-diol shifted both

TABLE 20

^{13}C nmr resonances of stictane derivatives

Compd	Diol (72)	Triol (73)		Dihydroxy-acetate (74)	Tri-acetate (75)	Secodihydroxy-acetate (76)	Secotri-acetate (77)
Solvent	C_5D_5N	C_5D_5N	$CDCl_3$	$CDCl_3$	$CDCl_3$	$CDCl_3$	$CDCl_3$
1	33.7	42.7	41.6	41.8	39.0	39.0	34.8
2	30.1	70.5	71.2	70.8	71.8	60.8	62.9
3	78.4	84.2	84.7	84.2	81.0	72.3	73.0
4	39.9	39.4	38.5	38.9	38.7	39.8	38.4
5	48.4	48.4	48.0	48.0	47.6	45.5	46.4
6	19.3	19.2	19.0	18.9	18.7	23.0	22.8
7	34.9	34.5	34.3	34.3	34.0	32.3	32.3
8	42.1	41.9	41.9	41.8	41.7	41.6	41.5
9	46.1	46.5	46.5	46.3	46.1	45.4	45.3
10	37.2	37.8	37.7	37.5	37.3	40.3	40.2
11	22.9	23.0	22.9	22.7	22.7	22.8	22.7
12	21.8	21.7	21.6	21.5	21.4	21.8	21.9
13	49.3	49.2	48.9	48.8	48.6	48.8	48.7
14	42.9	42.7	42.6	42.5	42.5	42.1	42.3
15	32.3	32.3	31.9	31.6	31.6	31.4	31.4
16	20.0	19.9	19.4	19.6	19.5	19.6	19.5
17	49.3	49.2	48.7	46.5	46.5	46.4	46.4
18	38.7	38.4	39.1	38.7	39.0	38.7	38.6
19	35.3	35.3	35.1	34.8	34.8	34.5	34.5
20	35.3	35.3	35.1	34.9	34.8	34.8	34.8
21	36.3	36.2	35.9	35.1	35.2	35.1	35.1
22	75.1	74.9	76.6	78.5	78.3	78.5	78.1
23	29.7	30.1	29.8	29.7	29.5	26.3	25.3
24	17.1	18.1	17.3	17.3	18.0	24.4	25.1
25	22.9	24.1	24.0	23.9	23.7	21.6	21.5
26	22.9	22.6	22.6	22.5	22.5	20.3	20.5
27	17.5	17.4	17.2	17.1	17.1	16.5	16.5
28	13.9	13.6	13.6	13.4	13.4	13.5	13.4
29	30.6	30.5	29.8	29.5	29.5	29.3	29.3
30	19.3	19.2	18.6	19.7	19.7	19.6	19.5

carbons upfield. The shifts experienced in these cases would be the sum of α-and β-effects plus conformational adjustment shifts. The difference in effect between the stictane and steroidal examples could be due to the difference in position on ring A and/or the different steric requirements of a boat-ring B.

3.6 2,3-Seco-compounds

The allocation of the signals not associated with rings A or B was achieved with facility, by comparing the assignments of the pentacyclic derivatives (table 20).

The signals at 60.8 and 72.3 ppm in the spectrum of 22α-acetoxy-2,3-secostictane-2,3-diol (76) could be assigned to C-2 and -3 respectively by comparison with the carbinyl carbon signals of propan-1-ol and 2,2-dimethylpropan-1-ol[111] which appeared at 63.9 and 72.9 respectively. The assignments of the secotriacetate (77) carbinyl carbon signals follow directly (table 20). In addition, analogies may be drawn between C-23 and -24 on the one hand and the methyl carbons of both 2,2-dimethylpropan-1-ol[111] (resonating at 26.6 ppm) and *cis*- and *trans*-4-t-butylcyclohexanol[112] (resonating at 27.5 ppm) on the other. Hence the pair of methyl group signals at 26.3 and 24.4 ppm in the spectrum of the secodihydroxyacetate (76) and at 25.3 and 25.1 ppm in the spectrum of the secotriacetate (77) were assigned to C-23 and -24 respectively, where the lower field signal of the two is possibly derived from the methyl group oriented towards the 10β-methyl group, which would be pseudo-1,3-*syn*-diaxial. The converging chemical shifts in the secotriacetate (77) are probably a consequence of the steric constraints of the C-2 acetoxyl group, causing the two methyl groups to lie approximately equidistant from the 10β-methyl group. Although the

parameters of Beierbeck and Saunders[89] do not strictly apply to C-23 and C-24 (nor to C-1, -2, -3 and -4) yet a comparison with the calculated chemical shift (table 21) confirms the assignment made.

The two methyl group carbons, C-25 and -26 would be expected to be shifted upfield by the release of the boat-ring to a chair conformation [section 3.5 (i), especially table 11] with a possible modification of this effect on C-25 by its being equatorial. A tentative assignment based on calculations after Beierbeck and Saunders put C-26 of the secodihydroxyacetate (76) at 20.3 ppm and C-25 at 21.6 ppm (table 21), both of which correspond to a ring A-cleavage upfield shift of *ca.* 2.2 ppm (table 20). The C-25 and -26 assignments of the secotriacetate (77) follow (table 20).

TABLE 21

Calculated and experimental shifts of some carbons of 22α-acetoxy-2,3-secostictane-2,3-diol (76)

	4	5	6	7	8	9	10	23	24	25	26
Calc.	42.4	44.5	24.0	31.0	42.7	43.4	37.8	24.4	24.4	21.1	17.4
Obs.	39.8	45.5	23.0	32.3	41.6	45.4	40.3	(26.3	24.4)	21.6	20.3

The triplets (in the SFORD spectra) were assigned on the basis of their predicted chemical shifts (C-6 and -7), (table 21) and the substituent effect on acetylation (C-1, table 20). The methine carbon signals (from C-5 and C-9) were almost coincident in the spectrum of the secodihydroxyacetate (76), but were assigned on the basis of the expectation that the γ-effect (on C-5) of acetylation would be greater than the δ-effect (on C-9) (table 20). Purely on the basis of the predicted values the assignments of C-4 and -10 would be interchanged but the signal at 39.8 ppm was the only quaternary carbon signal, which suffered a significant

substituent effect on acetylation and so was assigned to the β-carbon, C-4.

3.7 Conclusion and Discussion

The stated object of this investigation was to obtain further evidence that ring B of the stictane skeleton is in a boat conformation. This evidence has been amply provided from several quarters.

The consistent discrepancy between the calculated and experimental chemical shift values of the carbons associated with ring B, in particular the anomalously low-field position of C-25 *[*section 3.5 (i)*]* in the absence of obvious deshielding substituent groups (*e.g.* double bonds), indicated that ring B was in an unusual, possibly boat or twist-boat conformation.

The presence of a boat-ring B was also indicated by a) the sensitivity of C-6 and -7 to substituent changes in ring A *[cf.* the oleanane series, section 3.5 (ii)*]*, b) the evident restraint on C-10, as demonstrated by the reduced effect *[cf.* the oleanane and steroid series, section 3.5 (iv)*]* of introduction of an additional ring A substituent and c) the α-effect of diacetylating the 2α,3β-dihydroxyl groups *[cf.* the steroid series, section *3.5 (v)]*.

The most notable difference between cleavage of ring A of cholestane-2α,3β-diol and 22α-acetoxystictane-2α,3β-diol (74) is the lack of sensitivity of C-6 and -7 in the cholestane skeleton *[*ref.113 *cf.*ref.110 Δ (C-6) -0.1 ppm, Δ (C-7) +0.1 ppm*]* compared with the stictane shifts of 5.1 and -2.0 ppm respectively (table 20). Comparison of the chemical shifts of the secocompounds with the pentacyclic stictane derivatives reveals that the bridgehead carbon nuclei[85,103] were most sensitive to long-range effects, so that even C-18, at the ring D/E junction, suffered a small

(0.3 ppm) shielding effect (table 20). This is in contrast to the short-range effect of cleavage of ring A of the cholestane derivative,[113] where although the bridgehead nuclei of ring B suffered considerable ($|6.8| - |0.9|$ ppm) shift effects yet those of the ring C/D junction experienced no significant change in resonance position. The marked shift in the resonance frequencies of C-6 and -7 to frequencies which concur with those calculated assuming a chair conformation for ring B (section 3.6) together with the long-range shift effect observed, suggests that, in the stictane series, a conformational change has occurred in ring B as a result of cleavage of ring A, indicating that the pentacyclic stictane derivatives possess a boat-ring B.

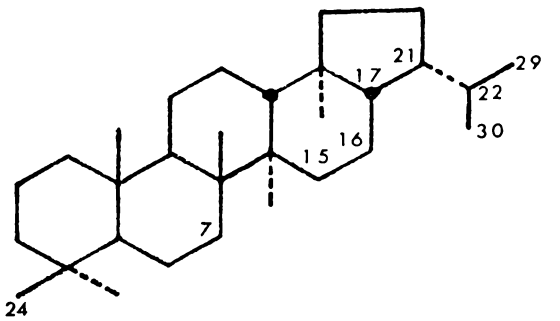


FIGURE 16

(5) 15α-Acetoxy-22-hydroxyhopan-24-oic acid

(9) 15α-Acetoxyhop-17(21)-en-24-oic acid

(10) 15α-Acetoxyhop-17(21)-en-24-ol

(12) 7β-Acetoxyhopan-22-ol

(13) 7-Oxohopan-22-ol

(14) 7-Oxo-22,29,30-trisnorhop-17(21)-ene

(18) 7,21-Dioxo-22,29,30-trisnor-17αH-hopane

(58) 15-Oxohopan-22-ol

(59) Hopane-15α,22-diol

(62) Hopane-7β,22-diol

(66) 15-Oxo-16β-phenylthiohopan-22-ol

(78) 7β-D_3-Acetoxyhopan-22-ol

(79) Hopane-7α,22-diol

(80) 22-Acetoxyhopan-7α-ol

(81) 15α-Acetoxyhopan-22-ol

(82) 15α-D_6-Acetoxyhopan-22-ol

(83) 15,21-Dioxo-22,29,30-trisnorhopane

(84) Hopane

(85) 21αH-Hopane

(86) 17αH-Hopane

(87) 17αH,21αH-Hopane

TABLE 22

IR absorptions (900 – 450 cm^{-1}) of hopane triterpenoids[a]

(62)	(12)	(78)	(79)	(80)	(13)	(14)	(18)
				895w			899m
886vs*	888vs	890vs	886sh		888m	884s	
			880s	870m			
855m	862w	866w	861w		860vs		858m
851m	854w	855w	852s	852vw		850s	852m
829s	831s	831vs		843w		838vs	
			824s*	836w			826m
814vw		813m	802vw	809w	804m	801vs	
798vw		802vw	799vw	790s	795m	787vs	795s
779m	781m	781m	777m	778m	783m		
750vw		760m	758w	754m	762s	758m	766w
728m	729m	729m	752m	738w	749vs	737w	739w
725m				727w	723vs		718w
713w			715w	717vw			691m
661w	647m		700w	700w	693vs	685s	686m
622s	624vs	620vs		654w			655m
615s	611s	611m		648w	632vs*	631vs*	636s
	601m	603w	605w	606vs*			608vs*
581w		580m			579vs	589vs	591vs
558w	562m	563s	553w		548vs	569m	
542w		536vs		544w	535vs	538s	541s
522w			523w	516m			524s
506w	509vs*	510vs*		511m	501vs		
							457m

*Strongest absorption in this region

TABLE 22 (continued)

(59)	(81)	(82)	(58)	(83)	(66)	(5)	(9)	(10)
896vw	898m	895s			897vs		893w	
	886w		883m	890vs	891m			
873s	873w	873sh	879m	872m	882w	878w		885w
		865vs				860s		
853vs*	856vs	855vs	859vs	855s	856s	855sh	853vs	853m
838s	836m	837m			840w	843vw		
817w	819w	823m	819vw	821m	826w	823vw	825s	
804w	802w	818m		815s	815w			816w
786w		802w	809w	803m	796m	795s	799vs	792w
771vw	773w		771w	773vw	779m			
760w	745m	744m	762m		754vs*	757vs*	763m	
730m	730m	729m	745m	749m	733vs	732sh	735m	736m
725m			727w	710m	721m		713m	715w
	719w	719w	691w	690w	708vs			
	685w	663w	667s		689vs	667m	648vs	650s
	639s	627vw	655w	655vs	665s	647m	634vs	
630w	617vs*	612vs	635vs*		634vs	625m	623vs*	624vs
611m	606vs	600w		611s	620m	610vs	615vs	608vs*
575w	572vs	578vs*	585vs		592vs	588w	588s	592m
561w	560vw			561s	556m	572w		
538w	538w	524w	545w	551vs*				523m
				540s		531m	525vs	516m
	502s	501m		517m	503vs	527m	517vs	501m
	485vs	467s	494m	472w	469m	486m		

*Strongest absorption in this region.

[a]The intensities of the absorptions have been standardised relative to the δ C-H band at *ca.* $\nu_{max.}$ 1460 cm^{-1} such that: vs>30%, 30%>s>20%, 20%>m>10% 10%>w>3% and vw<3%, while sh = shoulder peak.

CHAPTER FOUR

CORRELATIONS FROM THE INFRARED SPECTRA OF HOPANE AND STICTANE TRITERPENOIDS

4.1 Introduction

A part of this work, on hopane triterpenoids, has already been published[114,115] and is included as appendix 3. From the low frequency infrared region (900 - 450 cm^{-1}) studied, several correlations relating substitution position to infrared absorption patterns were made. The validity of these correlations was illustrated by their use in the structural elucidation of unknown compounds. This work has since been extended to include a number of additional hopane derivatives and the results of a similar investigation of stictane triterpenoids is herein described.

4.2 Hopane Derivatives

As a result of the discovery that the Jones' reagent oxidation of hopane diols, in addition to oxidising the secondary hydroxyl group, can oxidatively remove the isopropanol side chain (appendix 2) certain revisions of the published spectra[114,115] of 15-oxohopan-22-ol (58) and 7-oxohopan-22-ol (13) are required. These spectral revisions appear in table 22 together with the absorption maxima of a number of other hopane derivatives. (Figure 16 provides a key to the compound numbering system).

(i) 900 - 700 cm^{-1}

Examining the spectra of the 7-substituted derivatives, it was evident that the intense absorption ($\nu_{max.}$ 888 ± 2 cm^{-1}), characteristic[114,115] of 7β-substitution, was of reduced intensity and lower frequency in each of the 7α- and 7-oxo-derivatives [(79), (80), (13), and

(14)], except the 7,21-dione (18), from which it is apparently absent. The characteristic higher frequency and medium intensity of the absorption between $\nu_{max.}$ 770 and 781 cm^{-1} for 7β-substituted hopane derivatives was maintained by the 7α-derivatives (79) and (80), but the sp^2 hybridisation of C-7 in the 7-oxo-compounds apparently eliminated this absorption. 7-Oxo-22,29,30-trisnorhop-17(21)-ene (14) possessed an unusually large number of intense absorptions in the region 900 - 700 cm^{-1}, which could not be associated with the 7-ketone and were not consistently present in the spectra of the other two compounds [(9) and (10) table 22] with a double bond at the same position. Hence the absence of the side chain was implicated in the origin of these vibrations.

The peak ($\nu_{max.}$ 855 ± 4 cm^{-1}), characteristic of 15-substitution, was evident in the spectra of all three 15-ketone derivatives [(58),(83) and (66)]. Indeed the spectral patterns, between 900 and 830 cm^{-1}, in the spectra of the 15,21-dione (83) and the 15-oxohopan-22-ol (58) were very similar except for the increased intensity of the absorption of the dione (83) at $\nu_{max.}$ 890 cm^{-1}, compared with the medium intensity of the peak of the ketol (58) at $\nu_{max.}$883 cm^{-1}. At lower frequencies additional peaks ($\nu_{max.}$821 and 803 cm^{-1}) in the spectrum of the 15,21-dione (83) have analogues in that of the 7,21-dione (18) ($\nu_{max.}$826 and 795 cm^{-1}), while an intense absorption at $\nu_{max.}$815 cm^{-1} was not encountered elsewhere.

Intense bands in the region 760 - 680 cm^{-1} of the spectrum of 15-oxo-16β-phenylthiohopan-22-ol (66) were probably associated with the phenylthiol group, in accord with the observation[116] that, although acyclic C-S stretching vibrations generally absorb between 700 and 570 cm^{-1}, when the sulphur is attached to a ring carbon atom extensive coupling to the ring modes occurs [the bands below 680 cm^{-1} can be accounted for by invoking other vibrations - section 4.2 (ii)].

15α-Acetoxyhop-17(21)-en-24-oic acid (9) and 15α-acetoxyhop-17(21)-en-24-ol (10) have both maintained the absorption characteristic of a 15-substituent ($\nu_{max.}$ 855 ± 4 cm^{-1}). An intense band at $\nu_{max.}$ 799 cm^{-1} in the spectrum of the former would appear to be associated with the 24-carboxyl group since it is also present in the spectrum of 15α-acetoxy-22-hydroxyhopan-24-oic acid (5) (at νmax 795 cm^{-1}, table 22). The frequency of this absorption is probably too low for it to be associated with the OH deformation mode[117] of the carboxyl group (*ca.* $\nu_{max.}$ 930 cm^{-1}) but it could correspond to the out-of-plane CO skeletal deformation,[118] the absorption position of which varies widely.

(ii) Ketone group absorptions (700 - 500 cm^{-1})

The intense absorptions below 700 cm^{-1} nominally[119] associated with the in-plane vibrations of the ketone groups are set out in table 23. Each of the 7-oxo-derivatives *[(13), (14) and (18)]* possessed a series of four relatively invariant bands at $\nu_{max.}$ 689 ± 4, 633 ± 3, 585 ± 6 and 538 ± 3 cm^{-1}, with 7-oxohopan-22-ol (13) having additional low frequency

TABLE 23

IR absorptions associated with ketone groups[a]

7-CO-22-OH (13)	7-CO-trisnor-17(21)-ene(14)	7,21-$(CO)_2$ (18)	15,21-$(CO)_2$ (83)	15-CO-22-OH (58)	15-CO-16β-SPh-22-OH(66)
693vs	685s	686m		667s	665s
		655m	655vs		
632vs	631vs	636s		635vs	634vs
		608vs	611s		(620m)[b]
579vs	589vs	591vs		585vs	592vs
	569m		561s		556m
548vs			551vs		
535vs	538s	541s	540s		
501vs		524s			(503vs)[b]

[a] See note below Table 22 regarding intensities.

[b] Associated with the phenylthiol group.

absorptions at $\nu_{max.}$ 548 and 501 cm^{-1} and 7-oxo-22,29,30-trisnorhop-17,21-ene (14) absorbing at $\nu_{max.}$ 569 cm^{-1}. The bands in the spectrum of the 7,21-dione, which are not associated with the 7-ketone ($\nu_{max.}$ 655 and 608 cm^{-1}) are attributable to the 21-ketone since they also appear in the spectrum of the 15,21-dione (83) (at $\nu_{max.}$ 655 and 611 cm^{-1}).

Two peaks in the spectrum of 15-oxohopan-22-ol (58) ($\nu_{max.}$ 635 and 585 cm^{-1}) possess possible parallels in the spectrum of 7-oxohopan-22-ol (13) ($\nu_{max.}$ 632 and 579 cm^{-1}), possibly reflecting the local symmetry about ring C, making C-7 and -15 approximately equivalent (see appendix 3).

The comparability between the three spectra of the 7-oxo-derivatives was not unexpected since the substituent changes experienced in each case are remote from the site of the 7-ketone. However, due to the proximity to C-15 of an sp^2-hybridised carbon atom (C-21) in a 5-membered ring in the 15,21-dione (83) the absorption-additivity would be predicted to be reduced. Whence the possible source of the apparent shift to lower frequencies between 15-oxohopan-22-ol (58) and the 15,21-dione (83). Despite the presence of the 16β-phenylthiol group, adjacent to the 15-ketone group, the three in-plane vibrations of the carbonyl group in 15-oxohopan-22-ol (58) are virtually unshifted in the spectrum of 15-oxo-16β-phenylthiohopan-22-ol (66). The additional bands at $\nu_{max.}$620 and 503 cm^{-1} are assignable to the monosubstituted phenyl group.[120]

(iii) The acetoxyl group absorptions (650 - 450 cm^{-1})

The surprising sensitivity of these acetoxyl vibrations[121] to minor molecular changes was demonstrated by comparing the spectral patterns of 7β-acetoxyhopan-22-ol (12) and 15α-acetoxyhopan-22-ol (81) with those of the corresponding trideuteroacetates (78) and (82) (table 24).

TABLE 24

IR absorption bands associated with acetoxyl groups[a]

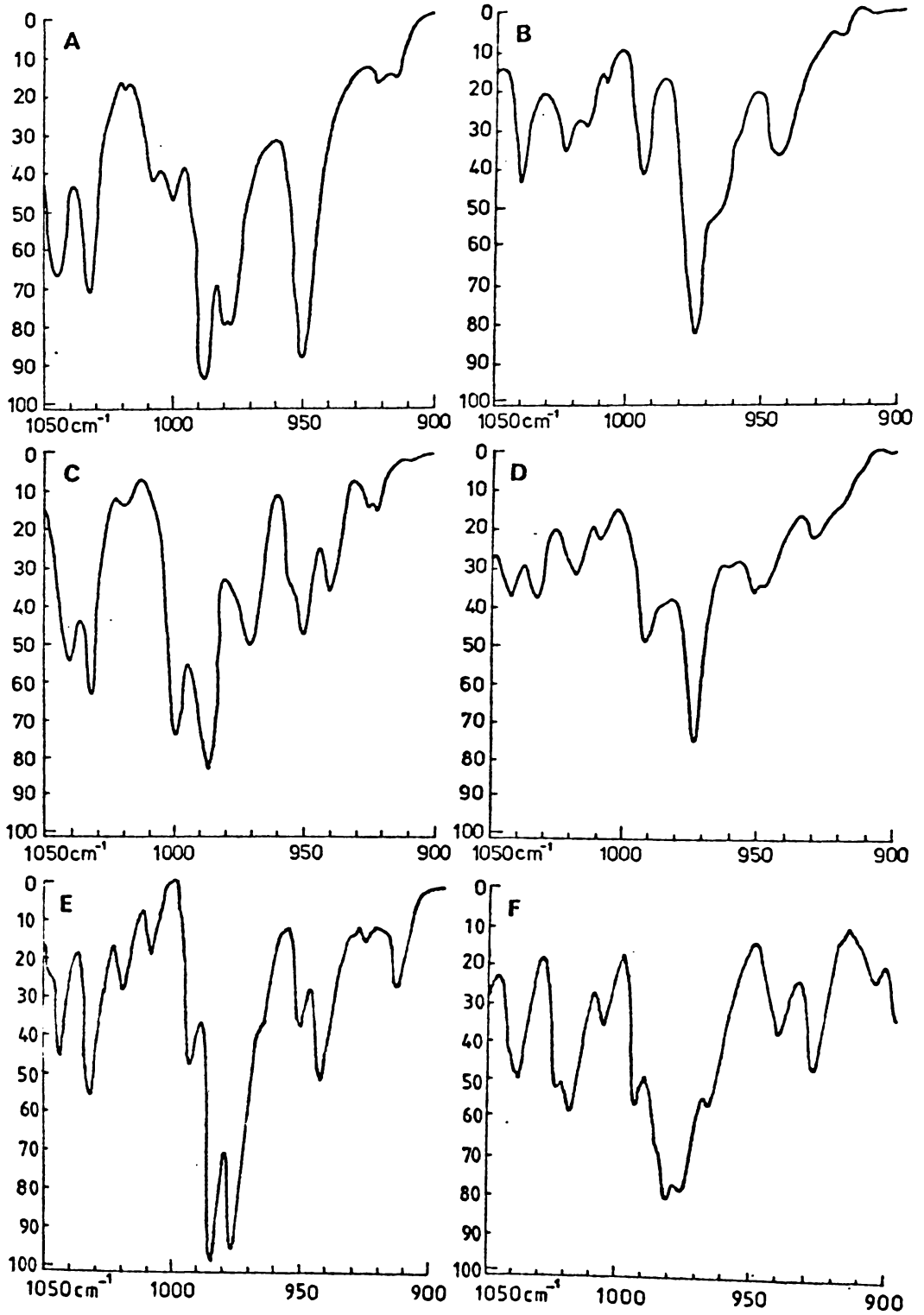
7α-OH-22-OAc (80)	7β-OAc-22-OH (12)	7β-D_3-OAc-22-OH (78)	15α-OAc-22-OH (81)	15α-D_3-OAc-22-OH (82)	4α-COOH-15α-OAc-22-OH (5)	4α-COOH-15α-OAc-17(21)-ene (9)	15α-OAc-17(21)-en-24-OH (10)
	647m				647m	648vs	650s
			639s			634vs	
	624vs[b]	620vs			625m	623vs	624vs
	611s[b]	611m	617vs	612vs	610vs	615vs	608vs
606s	601m		606vs				
		580m	572vs	578vs		588s	592m
	562m	563s					
		536vs			531m	525vs	523m
	509vs	510vs	502s	501m	527m	517vs	
			485vs	467s	486m		501m

[a]See note below Table 22 regarding intensities.

[b]Significant, but not so sharp, absorptions occur in this region of the spectrum of hopane -7β,22-diol (62).

Of the peaks apparently shifted, those nominally[121] configuration-dependent have in both cases been shifted to lower wavenumber values ($\nu_{max.}$624 to 620 cm^{-1}- from the 7β-acetoxyl; $\nu_{max.}$617 to 612 cm^{-1}- from the 15α-acetoxyl), while that at $\nu_{max.}$572 cm^{-1} in the spectrum of 15α-acetoxyhopan-22-ol (81) appeared to absorb at a higher frequency ($\nu_{max.}$ 578 cm^{-1}) on deuteration. The most shifted absorptions would appear to be those considered to be configuration independent at $\nu_{max.}$ 601 *[* from (12)*]* and 606 *[*from (81)*]* cm^{-1}, of which the former is shifted to $\nu_{max.}$

FIGURE 17



Infrared spectra (1050 - 900 cm^{-1}) of A. hopane (84), B. 17αH-hopane (85) C. 21αH-hopane (87), D. 17αH,21αH-hopane (87), E. 15,21-dioxo-22,29,30-trisnorhopane (83), F. 7,21-dioxo-22,29,30-trisnor-17αH-hopane (18). (Arbitrary absorption units have been used for the y-axis).

580 cm^{-1} and the latter is merged with the signal at $\nu_{max.}$ 578 cm^{-1}.

22-Acetoxyhopan-7α-ol (80), in accord with the location of the acetoxyl group on the isopropanyl group rather than on a ring carbon atom, possessed only a single absorption in this region intense enough to be associated with the acetoxyl group.

Comparing the acetoxyl bands of 15α-acetoxy-22-hydroxyhopan-24-oic acid (5) and 15α-acetoxyhop-17(21)-en-24-oic acid (9) with those of 15α-acetoxyhop-17(21)-en-24-ol (10) did not permit the assignment of any peaks in this region to the carboxylic acid group *[section 4.2 (i)]*. However, while the band near $\nu_{max.}$ 612 cm^{-1} is consistently present in the spectra of each of the 15α-acetates, that at $\nu_{max.}$ 502 cm^{-1} seemed to have moved to a higher frequency (*ca.* $\nu_{max.}$ 520 cm^{-1}) in these compounds *[(5), (9) and (10)]*. In addition all of the 15α-acetates with a 24-substituent, studied to date (see appendix 3) absorb strongly at $\nu_{max.}$ 646 ± 4 and 627 ± 4 cm^{-1}, suggesting that these may be breathing modes associated with ring A.

(iv) 1050 - 900 cm^{-1}

Each of the four isomeric hydrocarbons, hopane (84), 21αH-hopane (85), 17αH-hopane (86) and 17αH,21αH-hopane (87) possessed mainly weak absorptions below 900 cm^{-1} (appendix 3), but two peaks ($\nu_{max.}$ 854 ± 1 and (611 ± 7 cm^{-1}) were of significant intensity. Since the region 1050 - 900 cm^{-1} in the spectra of the hydrocarbons is uncomplicated by C-O stretching and O-H bending vibrations, it was found to have considerable structural significance as demonstrated by figure 17. In this region the most intense bands of hopane (84) and 21αH-hopane (85) were at $\nu_{max.}$ 988 and 985 cm^{-1} respectively and both compounds also absorbed strongly at $\nu_{max.}$ 1032 ± 1 cm^{-1}. However, the most intense absorptions of 17αH-hopane (86) and 17αH,21αH-hopane (87) occur at the lower frequencies of $\nu_{max.}$ 973 and 971 cm^{-1},

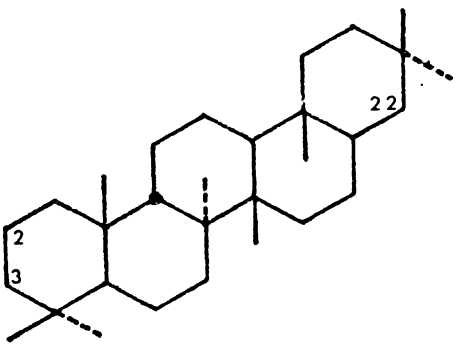


FIGURE 18

(71) Stictane

(72) Stictane-3β,22α-diol

(73) Stictane-2α,3β,22α-triol

(74) 22α-Acetoxystictane-2α,3β-diol

(75) 2α,3β,22α-Triacetoxystictane

(88) Stictan-22α-ol

(89) Stictan-22β-ol

(90) 22α-Acetoxystictane

(91) 22β-Acetoxystictane

(92) 22-Oxostictane

(93) Stictane-3β,22β-diol

(94) 3β,22α-Diacetoxystictane

(95) 3β,22β-Diacetoxystictane

(96) 3-Oxostictan-22α-ol

(97) 22α-Acetoxystictan-3-one

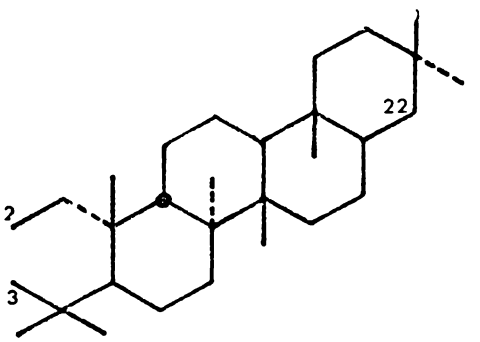
(98) 2α,22α-Diacetoxystictan-3β-ol

(99) 3β,22α-Diacetoxystictan-2α-ol

(100) 2α,3β-Diacetoxystictan-22α-ol

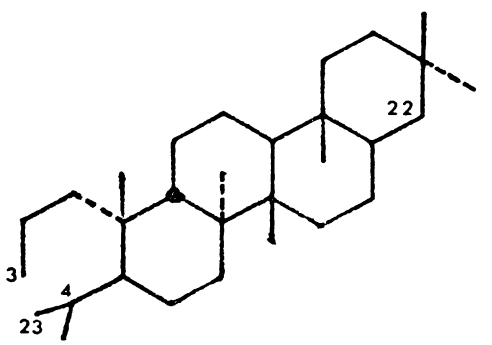
(101) 2α,3β-Diacetoxystictan-22β-ol

FIGURE 19



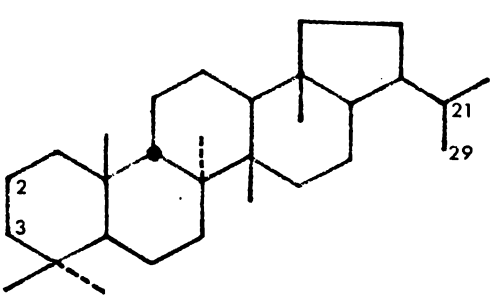
(76) 22α-Acetoxy-2,3-secostictane-2,3-diol

(102) 22α-Hydroxy-2,3-secostictan-2,3-diol



(103) Methyl 22α-acetoxy-3,4-secostict-4(23)-en-3-oate

(104) Methyl 22α-hydroxy-3,4-secostictan-3-oate



(105) 2α,3β-Diacetoxyflavic-21(29)-ene

respectively, while only weak bands were present in the vicinity of 1030 cm^{-1}.

An interesting application of this comparison was discovered after the realisation that since the two diones (18) and (83) possess no hydroxyl or acetoxyl functional groups this region of their spectra could also be compared with those of the hydrocarbons. Thus the similarity between the spectra of 17αH-hopane (86) and the 7,21-dione(18) (figure 17) suggested that isomerisation of the dione to the 17αH-epimer had occurred (see also section 1.5). In contrast the 15,21-dione (83) possessed five maxima ($\nu_{max.}$ 1044, 1031, 981, 973 and 942 cm^{-1}), which suggested a relationship with hopane (84) and hence appeared to have remained in the original 17βH- form (see also appendix 2).

4.3 Stictane Derivatives

Unlike that of hopane, the stictane skeleton has no local C_2-axis of symmetry (appendix 3) but possesses a boat-ring B. Thus one of the objects of the present study was, by comparing the infrared spectra of penta-cyclic stictane derivatives with those of ring A-cleaved derivatives, to locate absorptions associated with the boat ring. This possibility was suggested by the work of Anet *et al.*[122] who uniquely associated four relatively weak absorptions (at $\nu_{max.}$ 1150, 1000, 770 and 760 cm^{-1}) with vibrations of the twist-boat form of cyclohexane.

Stictane derivatives, in their lichen-source, are found variously substituted by hydroxyl or acetoxyl groups at the 2α-, 3β- and 22α-positions. Starting with these metabolites several compounds with additional combinations of substituents have been added to the series, and some of these compounds have been included in the present infrared investigations. (Figures 18 and 19 provide a guide to the numbering system).

TABLE 25

IR absorptions (1200 - 900 cm^{-1}) of stictane triterpenoids[a]

(71)	(88)	(89)	(90)	(91)	(92)	(72)	(93)	(94)	(95)	(96)
1192w	1192w		1192w	1190m	1193w	1195w	1192w	1187vw	1188m	1190m
1175w	1175w	1185m	1171w		1171w		1179m	1174w	1172sh	1176m
1162w						1167m	1160vw	1157w	1160m	1162vw
1140w	1136w	1140m	1141vw	1138m	1135m	1134m	1135m		1135m	1138m
		1129vw	1133vw	1122w			1126sh			
				1112w	1112w				1101m	1104s
1110w	1106w	1109m	1111w	1105w	1104m	1096m	1105m		1091m	
1080m	1181w		1080w	1085w	1082w	1085m	1090m		1082w	1084m
		1076m		1070vw		1075m	1075m	1075w	1072m	
1059m	1060m	1060s	1061m		1057m	1060w	1061m		1060s	1060m
		1040w	1055m	1055s	1052m	1054w	1043m		1041sh	1055m
1034w	1035m	1034m			1034s	1033sh	1036m			1033m
1029w		1027m	1023vs	1028sh	1020w	1025s	1023sh	1025vs	1027vs	1023m
	1017m	1021m		1018vs		1014vs	1016s	1001m	1018vs	1016m
1000m	993w	999w	998vw	998m	995m	999m	995m		999m	996m
981w	980m	984m	979m	980sh	977sh	993m	987sh	982m	985m	985m
973m	974m	975m		971s	969s	984m	977w	969m	971m	
	962m	958m	966s	963s	961m	966m	958w		959m	966m
		944m		940w		948w	942m		940m	
		934w	934w	933m	937w	939w		936m		
	929w	922w	924vw		925w	923w	921m		913w	929m
		908w	904w		903w	907w		905w	906sh	908vw

[a]The intensities of the absorptions have been standardised relative to the δ CH band at *ca.* $\nu_{max.}$ 1460 cm^{-1} such that; vs>100%, 100%>s>70%, 70%>m>30%, 30>w>10% and vw<10%, while sh = shoulder peak.

TABLE 25 (continued)

(97)	(73)	(74)	(98)	(99)	(75)	(100)	(101)	(76)	(102)	(103)	(104)
1190w	1194m	1190vw	1196w	1195w	1195m	1193w	1193w	1190w	1195w	1190w	
1177w	1175w	1175vw	1181w			1174vw		1179w	1176w		1176s
1162w	1157sh	1167w	1177w	1176w	1155m	1155w	1152w			1167m	1156w
1142w	1140m	1140m	1145vw	1157vw	1139w	1135w	1135m			1130vw	1134w
1132w	1130w		1135vw	1136w	1123w	1122vw				1120vw	1124w
1109w					1113w	1111w		1113w			1113w
	1105s	1106m	1104m	1100w	1103m	1099m	1106w				1093w
1080w	1084m		1083w		1099sh	1088m	1090w	1079w			1084m
	1072m		1069sh	1072m	1079m	1077m	1076m	1069sh	1070vw		1073w
	1062sh	1063m			1062sh	1062s	1059s		1058w	1051w	1063m
1056m	1051sh	1059m	1047vs	1054s	1045vs		1040sh	1044vs	1052w		1040sh
1039w	1044s	1045s	1042vs	1044vs	1034vs	1035vs	1032vs		1034w		1032m
1022s	1031s	1029vs	1020sh	1030vs	1026vs		1028vs	1020vs	1020m	1020m	1019m
	1020m		1000w		1007s	1002m	1007w				
	999w	995w	994w	997m	995sh	992m	999w	992sh	997w	994vw	996sh
979m	994sh		980m	983m	987m	984m	981m	980m	983m	981m	983m
	981s	980w			973m		971w	966m		964s	972w
969vs	966m	968m	961m	963m	967sh	961m	961m		964m		961m
	939m				961sh		952m				
933m		934w	932m	931m	933m	930m	934m	932m	930w	935m	929w
		921vw			916m	914m	913m				919w
908vw	908w	906vw	906m		905m			907m			906vw

(i) 1200 - 900 cm^{-1}

An intense band near 1020 cm^{-1} has been associated[123] with acetoxyl groups (C-O stretching) and has been found to appear at higher wave-numbers where the acetoxyl group is equatorial. In accord with this the equatorial mono- and di-substituted acetoxyl derivatives possessed an intense peak between 1030 and 1020 cm^{-1}, while the 22β-acetates (axially substituted) (91) and (95) absorbed between 1020 and 1010 cm^{-1} (table 25). In addition 22β-substitution was characterised by the presence of both of two bands of medium intensity at $\nu_{max.}$ 1137 ± 3 and 1074 ± 2 cm^{-1} (*cf.* ref. 124). Although both stictane-3β,22α-diol (72) and stictane-2α,3β,22α-triol (73) also absorbed to a medium intensity in both of the foregoing regions, these were readily distinguishable from the 22β-alcohols by the absence of an intense absorption below 900 cm^{-1} *[*section 4.3 (ii)*]*.

A series of strong peaks between 1070 and 1020 cm^{-1} was indicative of 2α,3β-disubstitution, hence the lack of diagnostic value, to the trisubstituted compounds, of the acetoxyl absorption at *ca.* $\nu_{max.}$ 1020 cm^{-1}.

(ii) 900 - 450 cm^{-1}

In the region below 900 cm^{-1} the spectrum of stictane (71) was virtually featureless (table 26), but substitution at the 22-position produced a medium-to-strong intensity absorption at $\nu_{max.}$ 830 ± 7 cm^{-1}. Each of the stictane derivatives possessed this absorption, but although it was not lost on cleavage of ring A to form either 2,3-seco- *[e.g.*(76) and (102)*]* or 3,4-seco- *[e.g.* (103) and (104)*]* derivatives, contraction of ring E to yield a flavicene derivative (105) removed this peak. In general substitution of a carbonyl group or an equatorial acetoxyl group

TABLE 26

IR absorptions (900 - 450 cm^{-1}) of stictane triterpenoids[a]

(71)	(88)	(89)	(90)	(91)	(92)	(72)	(93)	(94)	(95)	(96)	(97)
		888w	888w	893m	890vw		885m	896vw	900s		899w
875w	878vw	873vw			875vw	877vw	874w	879vw	895s		882w
	864w					861vw	858m	865w	865m	869vw	
854w	855w	856w	856w		855m	851vw					
					846s	841vw	844vw	843vw		849w	847w
	827s*	826s	834s	828s	836vs*	829s*	823s	825m	828vs	829s	833vs
807w	806w	805w	804w	806w	808w	805vw	806w		801w	805w	802w
		795vw	798vw	797vw	798vw						
770w	772w		774w	784w	785m				776w		
	752m		757w		768m	757m	770w		753m		750w
720w		743vs*	727w		751m		743vs*		690m	756s*	
690w	681w	688vw	682w	660m	700m	697vw	725w	690vw	680m		689m
		662m	654w	637s	662m	649w	684s	655m	658vs	669w	650m
631m*	634m		625m	626vw			622s	634w	635s		623vs
	616w	612m	612w	610vw		618vw			615m		611sh
		591m	603s*	599vs*	605m	601w		603m*	602vs*	609w	603vs*
	596w	589sh		588s	578s		591m	591w	590m	593w	
553m		552w	553w	563w			572w	568m	569s	579m	570s
534w	539m	545m	540w	553w		544vw	539m	539w	562s	542w	543s
516w	535m		525w	537w	516m				525w	527w	537m
503m			505m			505w		501m	506m	500vw	523m
			502m						471m	490w	503m
				466m	462m				464m		

*Strongest absorption in this region.

[a]See note below Table 22 regarding intensities.

TABLE 26 (continued)

(73)	(74)	(98)	(99)	(75)	(100)	(101)	(76)	(102)	(103)	(104)	(105)
886w			890w		900w	896vw		890m	893vs	886m	
				880m			880w		888vs	872m	882vs*
865m		869m	866w	876s	874m	875m	875w		882vs*	863w	
854m	856vs*	850w	857vw	853m		852s				845m	855w
835sh				848m	850w	840w	839sh	841w	841s	838m	844w
829s*	830m	831vs*	829s	829vs	826s	829s	829vs	829vs	832s	827s*	
819m		802vw	800vw	804vw		811w	807w		807m	810vw	
	798vs	787vw		791vw	790vw	792m	791m	797m		795w	
785w						779sh	776m		775w	776w	
755w	756w	754vs	754vs*	750w	752w	743vs*	749w	755vs*		760m	
	737m		741sh	735w		725w	736w	735w		754m	
690w	696m	702w	703w	675m			658w	691m		712vw	
	664w	666s	666w	662w	667s	667m	642m	666m	645m		674w
653w	644m	653s	654m	639vs	639s	638vs	633m	645vw	633m		643s
614vw	627w	617m	615sh	618vs*	613vs	615vs	619m	625w		624m	620w
604w	606vs	608m	600s	608vs			607vs*		607vs		607m
591w		602m	581m	599vs	595vs*	591vs	592m	600m		598m	599vs
561w	559w	554m	554w	577m	576m	553w			570s	566m	575m
543w	540m	540w	540m	536s	540m	540w	534m	540m	535m	541m	
533w	515vs	512m	514m	519s	517s	514m	511w				513w
500w	512vs		502s	501m	507m		501s	500s			
487vw	477w	485vw	489w	466s	501m	475m	485w			482m	475m
465m	464w	469m	469m	450w	465m	465s				454w	

at C-22 raises the position of this absorption, while axial substituents tend to reduce its frequency and equatorial hydroxyl groups produce an absorption in the middle of the range. These observations suggest that ring E is the origin of this absorption.

Of the compounds which are identically 2α,3β-disubstituted with either two acetoxyl or two hydroxyl groups three, (74), (75) and (101), possessed an additional strong absorption between 880 and 850 cm^{-1}, but (73) and (100) absorbed less intensely in this region. Conversely, inequivalent substitution at these positions apparently produced an intense band of distinct, broad shape at $\nu_{max.}$ 754 cm^{-1} as exemplified by (98) and (99). However, 3-oxostictan-22-ol (96) also had an intense absorption in the same region (table 26).

Generally the spectra of the mono-, di- and tri-hydroxy-compounds, below 800 cm^{-1}, were virtually featureless but those with an axial hydroxyl group (22β-) *[e.g.* (89), (93) and (101)*]* possessed a very intense peak at $\nu_{max.}$ 743 cm^{-1}, while stictane-3β,22β-diol (93) also absorbed strongly at $\nu_{max.}$ 684 and 622 cm^{-1}.

(iii) Acetoxyl and ketone group absorptions (700 - 450 cm^{-1})

The spectrum of 2α,3β,22α-triacetoxystictane (75) is characterised by a series of four remarkably intense absorptions between 630 and 590 cm^{-1}. Three of these peaks are also present in the spectra of both 2α,3β-diacetoxystictan-22α-ol (100) and 2α,3β-diacetoxystictan-22β-ol (101) (table 27). The absent peak, being thus associated with the 22α-acetoxyl group, is also present in the spectra of the other 22-acetoxylated species regardless of the configuration at C-22 (table 27), while the three peaks at *ca.* $\nu_{max.}$ 639, 615 and 595 cm^{-1} are assigned to the 2α,3β-diacetoxyl system. Each of the compounds *[*(94), (95), (98), and

TABLE 27

Acetoxyl absorptions of stictane derivatives

2α,3β,22α-(OAc)$_3$ (75)	2α,3β-(OAc)$_2$ 22α-OH (100)	2α,3β-(OAc)$_2$ 22β-OH (101)	22α-OAc (90)	22β-OAc (91)	2α,3β-(OH) 22α-OAc (74)
639vs	639s	638vs			
618vs	613vs	615vs			
608vs			603s	599vs	606vs
599vs	595vs	591vs			

(99)] in which there is one ring A acetoxyl group, either at the 2α- or 3β-position absorb at 655 ± 3 cm^{-1} and a single ring A functionality of a 3β-acetoxyl group [*e.g.* in (94) and (95)] appears to be characterised by an absorption at *ca.* $\nu_{max.}$ 568 cm^{-1}.

Neither a 22-acetoxyl substitituent on stictane nor a 22-acetoxyl substituent on hopane possess a β-methylene group yet both produce a unique acetoxyl absorption at $\nu_{max.}$ 603 ± 4 cm^{-1}. This is in the region in which Weinmann and Weinmann[121] found that all 3- and 17- acetoxy-steroids absorbed, independent of configuration, whereas the position of a second, higher frequency absorption indicated whether the substituent was axial or equatorial. These facts suggest that the higher frequency absorption is associated with the methylene group, while the lower wavenumber peak $\nu_{max.}$ *ca.* 605 cm^{-1} arises from an out-of-plane skeletal deformaton mode.

An absorption at $\nu_{max.}$ *ca.* 515 cm^{-1} would appear to indicate the presence of 3- and 22-ketones [table 26 - 3-ketones (96) and (97), 22-ketone (92)] but the number of compounds substituted in such a fashion is too few to draw any firm conclusions.

4.4 Conclusion

Both hopane (appendix 3) and stictane derivatives produced absorption patterns recognisable as being indicative of substitution at certain positions. Thus an absorption at $\nu_{max.}$ *ca.* 830 cm^{-1} demonstrates the presence of a 22-substituted stictane derivative, a cluster of strong absorptions between 1070 and 1020 cm^{-1} indicates 2α,3β-disubstitution, while axial 22-substituents were found to produce a series of absorptions at $\nu_{max.}$ 1137 ± 3 and 1074 ± 2 cm^{-1}. In addition a certain degree of additivity was demonstrated, in both series of compounds, among the acetoxyl and ketone group absorptions *[*700 - 450 cm^{-1} - sections 4.2 (ii), (iii) and 4.3 (iii)*]*.

The value of these observations was demonstrated recently, when a new lichen metabolite was isolated in these laboratories. The presence of infrared absorptions at $\nu_{max.}$ 832 and 607 cm^{-1} in the spectrum of the methylated acetate derivative (103) suggested[125] that the compound was a 22ξ-acetoxystictane derivative. Further investigation confirmed that the material was methyl 22α-acetoxy-3,4-secostict-4(23)-en-3-oate (103).

Considering the work of Anet *et al.*[122] it was hoped at the outset that it would be possible to observe the disappearance of certain diagnostic absorptions on cleavage of ring A and hence to devise a means of recognising boat-rings. However this has not been possible due to the probable coincidence of more intense substituent-associated vibrations. Furthermore, the near featurelessness of the stictane (71) spectrum together with the disappearance of the peak at $\nu_{max.}$ 830 ± cm^{-1} from the spectrum of the flavicene derivative (105) highlights the apparent requirement for substituents to enhance skeletal vibrations, probably by increasing the change in dipole moment experienced during a given vibration. (The ease of characterisation of axial groups, which are

approximately perpendicular to the molecular plane may be due to their effecting a larger change in the dipole moment than equatorial groups). The ring A substituents would be of limited use to demonstrate a change in the conformation of ring B, by enhancing skeletal vibrations, since the nature of their positions in the ring A-secocompounds is quite different from those in the pentacyclic derivatives, even ignoring the conformational change in ring B. Hence substituents in ring B or C should prove to be useful probes in this regard.

CHAPTER FIVE

DEPSIDONE CONSTITUENTS FROM THE QUINTARIA GROUP OF NEPROMA SPECIES

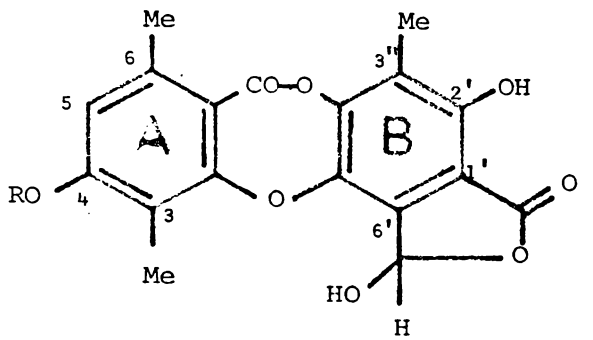
5.1 Introduction

Thin layer chromatography (toluene-dioxane-acetic acid, 90:25:4, TDA) revealed that one of the two chemical races of *Nephroma australe* possessed two metabolites which charred red with 10% sulphuric acid spray. These compounds were provisionally labelled Q-1 and Q-2, where the latter was the more polar of the two. The mass spectra of Q-1 and Q-2, showing sequential losses of water or carbon dioxide and up to four carbon monoxide units, suggested that they were depsidonal (see section 5.4).

Soon after the commencement of this work a publication[126] appeared describing the characterisation of two β-orcinol depsidones from a *Thelotrema* species. These were also described as charring red on t.l.c. plates sprayed with 10% sulphuric acid solution and were demonstrated to be identical to two compounds previously detected in *Pseudoparmelia neoquintaria* by Hale[127,128] and named PQ-1 and PQ-2 by Culberson.[129] The latter had demonstrated the presence of PQ-1 and PQ-2 among four metabolites (PQ-1, PQ-2, PQ-3 and PQ-4, of which all except PQ-3 developed a red colouration on the plates sprayed with 10% sulphuric acid solution) of *Xanthoparmelia quintaria*. Hence Keogh[126] was able to show that PQ-1 was hypostictic acid (106) and PQ-2 was hyposalazinic acid (107).

5.2 Isolation and Identification

Soxhlet extraction of *N. australe* with acetone (following hexane extraction to remove the less polar constituents) yielded a mixture



(106) R = Me

(107) R = H

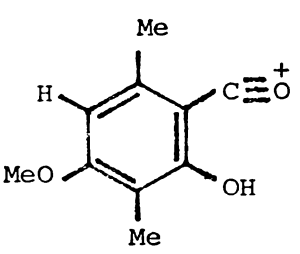
containing Q-1 and Q-2. This was then subjected to p.l.c. with TDA followed by rechromatography of the upper band (mainly Q-1) with hexane-ether-formic acid (12:13:2) and the lower band (mainly Q-2) with TDA.

The ^{1}H nmr spectrum of Q-1, thus obtained, indicated the presence of three aromatic methyl groups (signals *ca.* δ 2.6) and one methoxyl group (signal δ 3.83), while Q-2 had the methoxyl group missing and one methyl group significantly deshielded (0.14 ppm to absorb δ 2.81). In accord with this hypostictic acid (106) differs from hyposalazinic acid (107) in that the hydroxyl group at the 4-position (adjacent to the C-3 methyl group) is methylated.

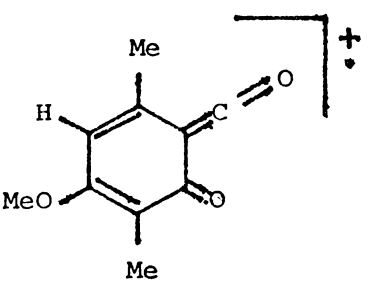
Acetoxylation of Q-1, in collaborative work[130] (with Wilkins and Moroney - see appendix 4), formed a diacetate on which several decoupling experiments analogous to those of Jackman *et al.*[131] were performed. Jackman *et al.* demonstrated that a long-range coupling of *ca.* *J* 0.5 Hz exists between a pair of mutually *para*-aryl methyl groups and that an aryl proton may couple to methyl groups *ortho* to it. Two methyl group signals (δ 2.24 and 2.44) of Q-1 diacetate were assigned to the acetoxyl groups on the basis of their sharpness (this assignment was later

confirmed by comparing the spectrum of Q-1 D_6-diacetate), while the methoxyl group signal was clearly distinguished by its resonance position. Thence irradiation of the aryl proton signal at δ 6.67 sharpened the signal at δ 2.53, whereas irradiation of the latter sharpened both the aryl proton signal and the methyl group signal at δ 2.17. The methyl group signals at δ 2.53 and 2.17 apparently therefore arise from mutually *para*-methyl groups and that resonating at δ 2.53 is *ortho* to the aryl proton. Thus the depsidonal ring A of Q-1 appeared to be identical to that of hypostictic acid (106).

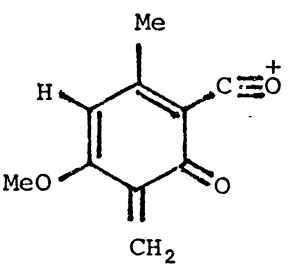
Furthermore the mass spectrum of Q-1 possessed three diagnostic peaks [*m/e* 179 (ion xxix), *m/e* 178 (ion xxx) and *m/e* 177 (ion xxxi)], while Q-2 had *m/e* 165 (ion xxxii), the non-methylated 4-hydroxyl equivalent of ion xxix. Ions xxxii and xxix also occur in the mass spectra of hypoprotocetraric acid (108) and its fully methylated analogue (109), respectively,[132] and have been demonstrated to arise from the depsidonal ring A.



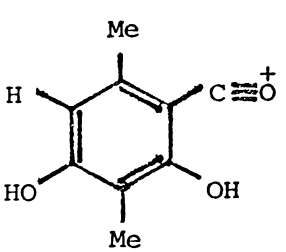
ion xxix *m/e* 179



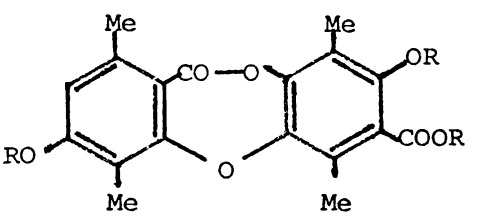
ion xxx *m/e* 178



ion xxxi *m/e* 177



ion xxxii *m/e* 165



(108) R = H

(109) R = Me

The carbonyl stretching band at $\nu_{max.}$ 1750 (Q-1) or 1720 (Q-2) cm^{-1} was assigned to the depsidonal ester linkage,[133] while the presence of only one surplus aryl methyl group signal, a carbonyl stretching band at $\nu_{max.}$ 1695 cm^{-1} and the mass spectral losses of both carbon dioxide and water from the molecular ion (section 5.4) suggested the presence of an α-hydroxylactol system as in the stictic acid series. Accordingly, the one-proton signal at δ 7.52 in the spectrum of Q-1 (δ 7.51 for Q-1 diacetate) was assigned to the 6'-C*H*(OH)-O proton of the lactol ring.

Hence, biosynthetic considerations, combined with the observation that Q-1 *di*acetylated indicated that Q-1 is identical to hypostictic acid (106) and Q-2 to its 4-desmethyl analogue, hyposalazinic acid (107). The ^1H nmr spectral assignments of Q-1 (106), Q-2 (107), Q-1 diacetate (110) and Q-1 D_6-diacetate (111) are presented in table 28. The signal of

TABLE 28

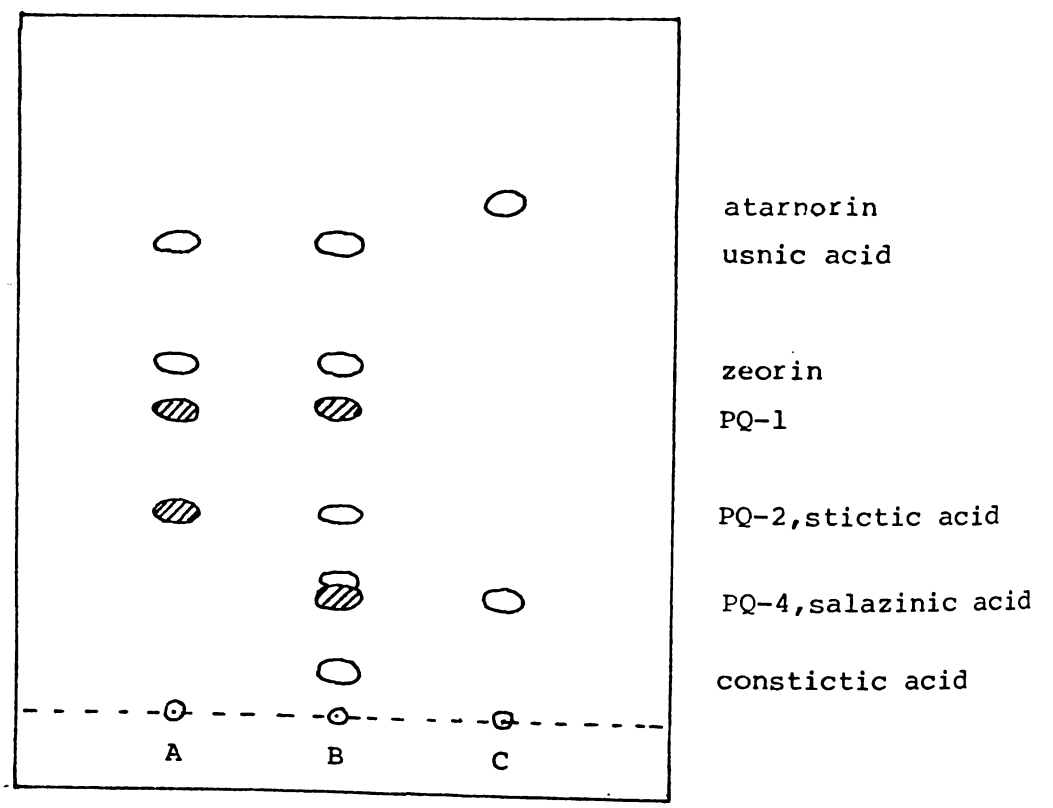
^1H nmr assignments (δ ppm)

Signal	Q-1 (106)[a]	Q-2 (107)[a]	Q-1 $(OAc)_2$ (110)[b]	Q-1 D_6-$(OAc)_2$ (111)[b]	PQ-4 $(OAc)_3$ (112)[b]
3-Me	2.67	2.81	2.17	2.18	2.18
6-Me	2.62	2.63	2.53	2.53	2.56
3'-Me	2.43	2.46	2.31	2.32	
3'-CH_2OAc					1.94
3'-CH_2OAc					5.30 (AB_q)[c]
2'-OAc			2.44		2.43
6'-CH(OAc)-O			2.24		2.25
6'-C*H*(OAc)-O			7.51	7.50	7.50
6'-C*H*(OH)-O	7.52	7.93			
4-OMe	3.83		3.91	3.91	3.92
5-H	6.82	7.03	6.67	6.67	6.67

[a] In C_5D_5N. [b] In $CDCl_3$. [c] AB_q, J = 11.5Hz (doublets centered at δ 5.45 and 5.15.)

FIGURE 20

T.l.c. plate (TDA) of A. *N. australe* B. *N. antarcticum* and C. standards of atarnorin and constictic acid. (Hatched spots charred red with sulphuric acid spray).



hyposalazinic acid (107) at δ 7.03 was assigned to the aryl proton by consideration of the substituent effect[134] of methylating an hydroxyl group (+ 0.17 ppm).

5.3 PQ-4

A t.l.c. comparison of *N. antarticum* with *N. australe* revealed the presence of PQ-1 (or Q-1) and the uncharacterised PQ-4 in the former lichen species (figure 20 - the identity of the lower sulphuric acid-charred red spot from *N. antarticum*, as being the same as that named PQ-4 by Culberson,[129] was indicated by its t.l.c. R_F being similar to that of salazinic acid).

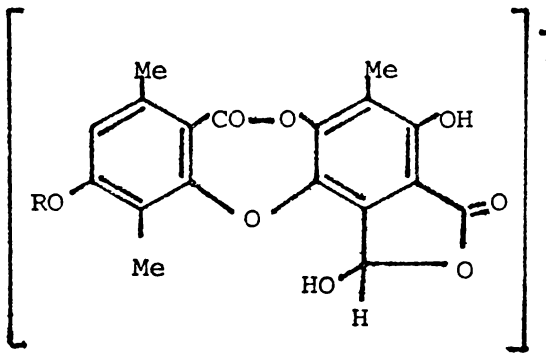
PQ-4 due to its polarity could not be satisfactorily isolated[130] directly from the acetone extracts of *N. antarticum*. Instead the extracts were acetylated and PQ-4 triacetate was isolated and demonstrated (see appendix 4) to be 3-methylconstictic acid triacetate (112) (see table 28 for ^{1}H nmr assignments).

5.4 Mass Spectra

On the basis of the monumental paper of Huneck and Djerassi *et al.*[132] the suggested structures for the mass spectral ions of hypostictic acid (106), hyposalazinic acid (107), hypostictic acid diacetate (110), hypostictic acid D_6-diacetate (111) and 3-methylconstictic acid triacetate (112) have been assigned (schemes 13 - 15).

Deuteration studies [hypostictic acid D_6-diacetate (111) - discussed later] confirm that ions xxxiii and xxxiv are formed as a result of loss of water from the molecular ions of hypostictic acid (106) and hyposalazinic acid (107) as indicated (scheme 12). The loss of carbon dioxide from compounds containing a lactol function although not

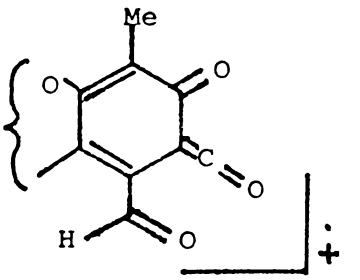
SCHEME 12



(106) R = Me, *m/e* 372 ($M^{+\cdot}$)

(107) R = H, *m/e* 358 ($M^{+\cdot}$)

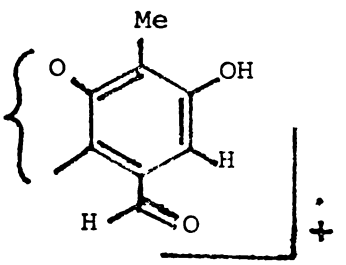
$- H_2O$



(106) ion xxxiii *m/e* 354

(107) ion xxxiv *m/e* 340

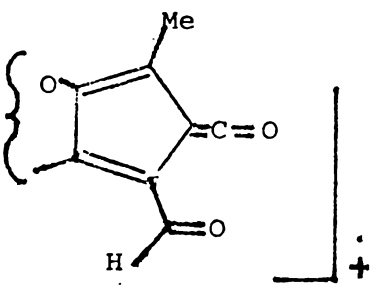
$- CO_2$



(106) ion xxxv *m/e* 328

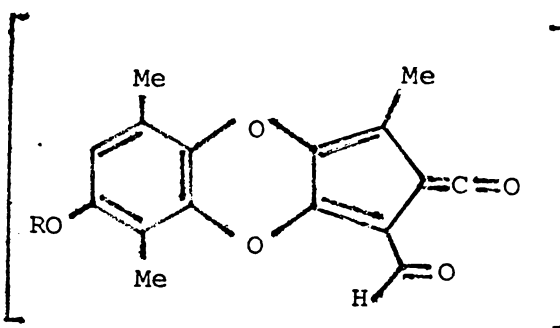
(107) ion xxxvi *m/e* 314

$- CO$



(106) *m/e* 326

(107) *m/e* 312



(106) *m/e* 270

(107) *m/e* 256

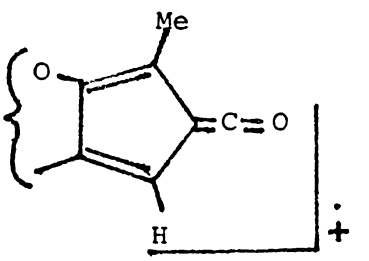
$- CO$

(106) *m/e* 242

(107) *m/e* 228

$- CO$

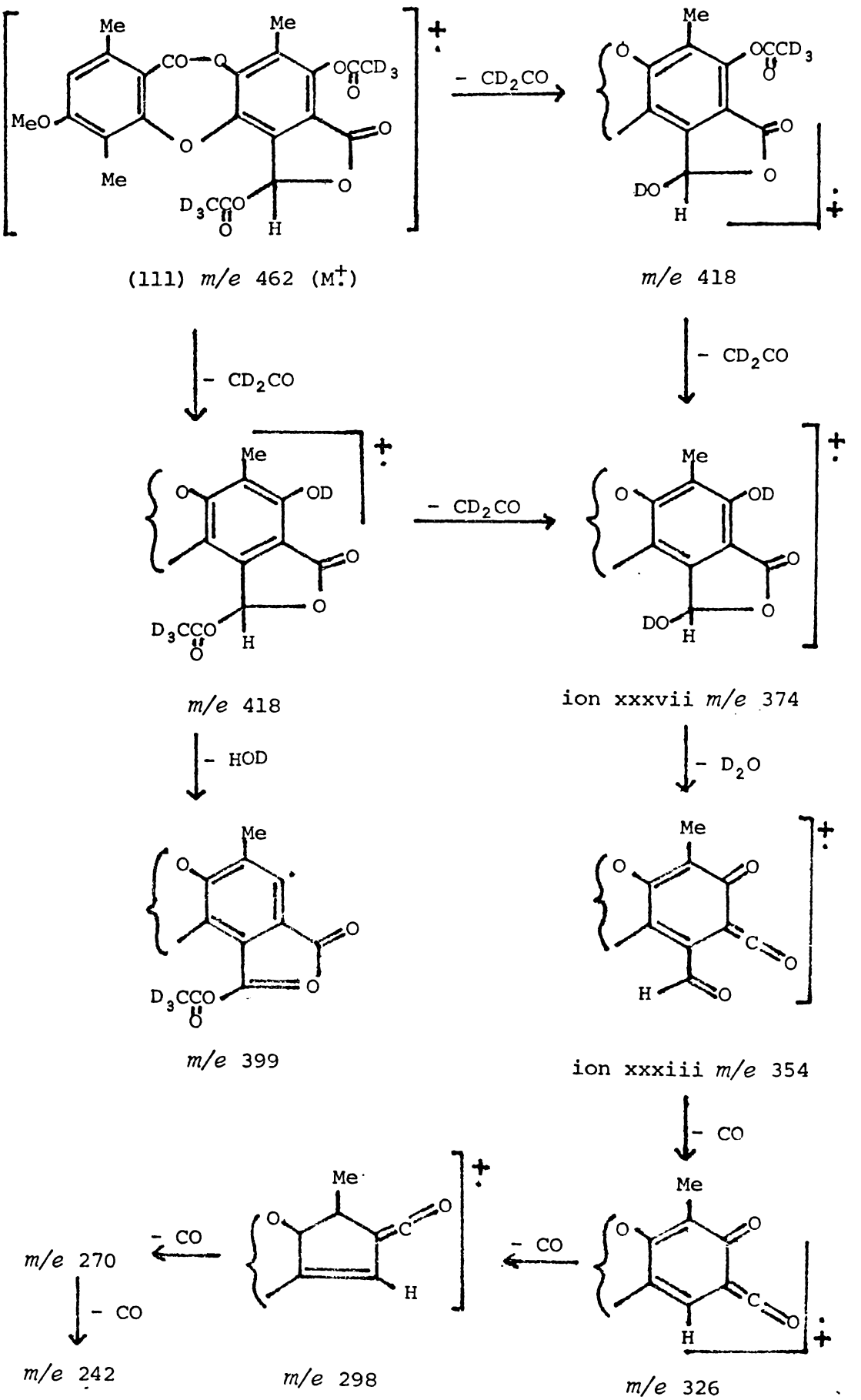
$- CO$



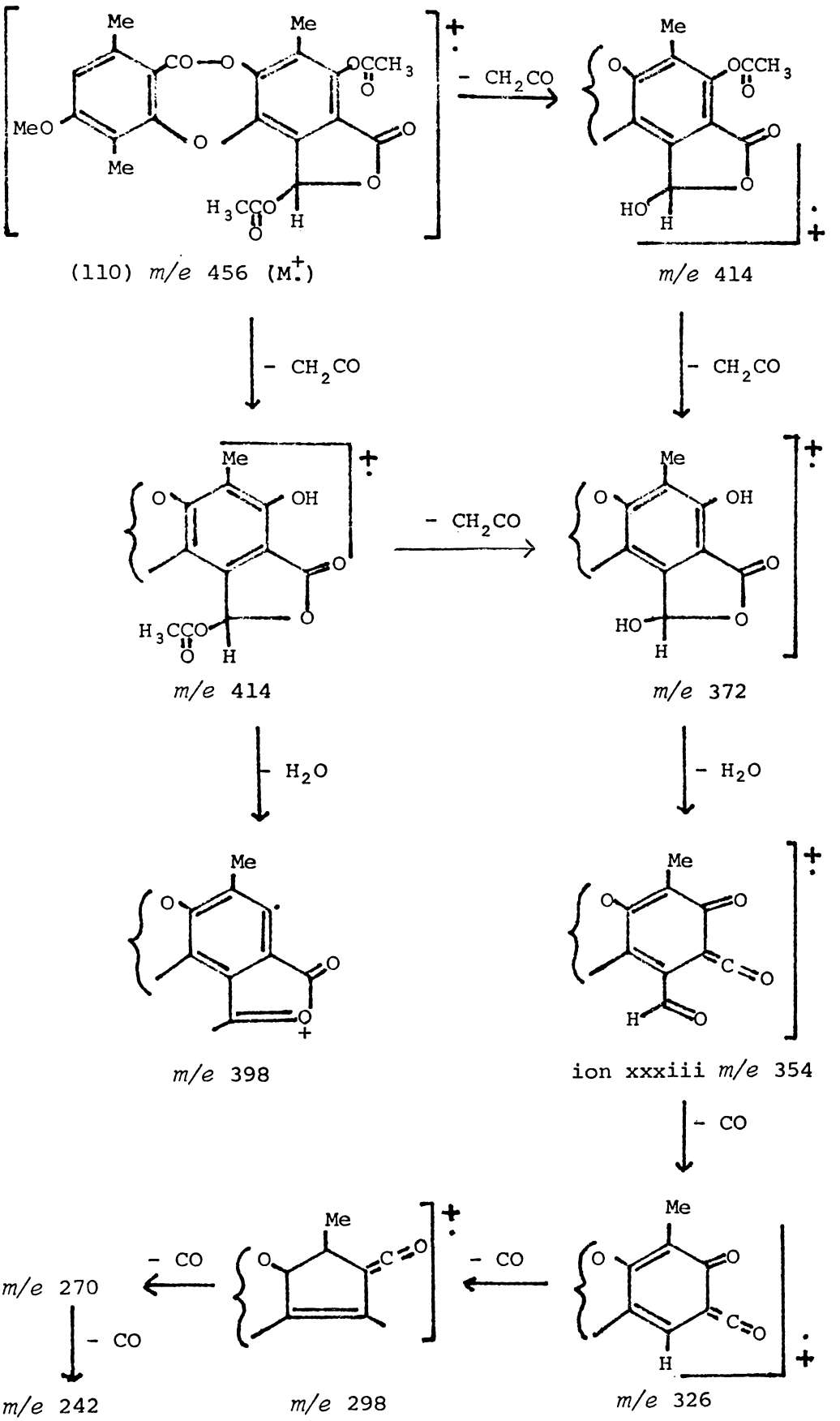
(106) *m/e* 298

(107) *m/e* 284

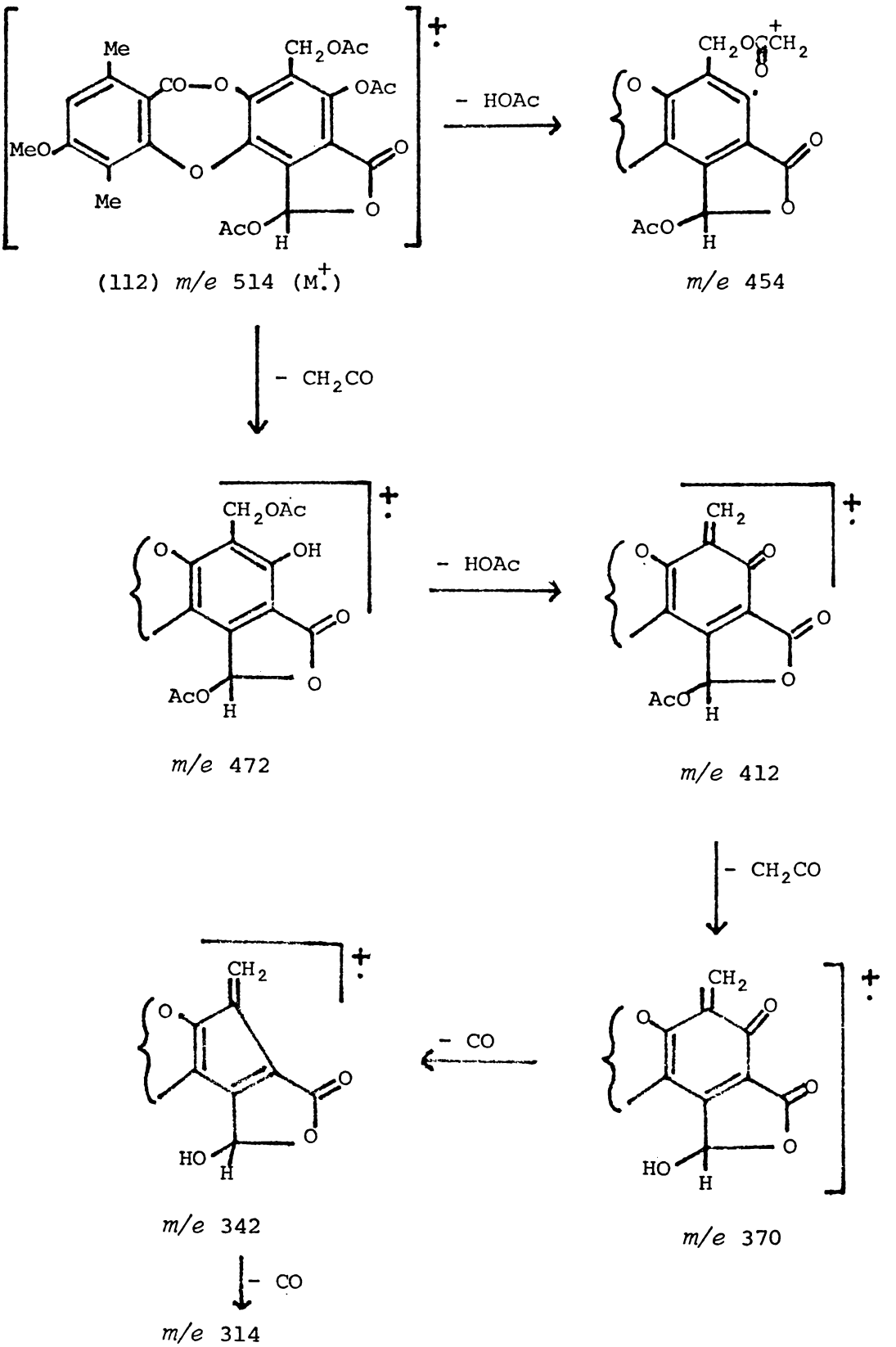
SCHEME 13


- CD2CO
(111) m/e 462 (M+·)
m/e 418
- CD2CO
- CD2CO
m/e 418
ion xxxvii m/e 374
- HOD
- D2O
m/e 399
ion xxxiii m/e 354
- CO
- CO
- CO
m/e 270
- CO
m/e 242
m/e 298
m/e 326

SCHEME 14


(110) m/e 456 (M.+)
- CH2CO
m/e 414
- CH2CO
- CH2CO
m/e 414
m/e 372
- H2O
- H2O
m/e 398
ion xxxiii m/e 354
- CO
- CO
- CO
m/e 270
- CO
m/e 242
m/e 298
m/e 326

SCHEME 15



(112) *m/e* 514 ($M^{+\cdot}$)

m/e 454

m/e 472

m/e 412

m/e 370

m/e 342

m/e 314

unprecedented[132] has not been assigned a resulting ion structure, so ions xxxv *[from (106)]* and xxxvi *[from (107)]* are suggested as a possible assignment. The peaks corresponding to losses of carbon monoxide subsequent to the loss of carbon dioxide are much less intense than those following the loss of water. The sequence presented in scheme 12 is not intended to infer that this is the only order in which the losses occur.

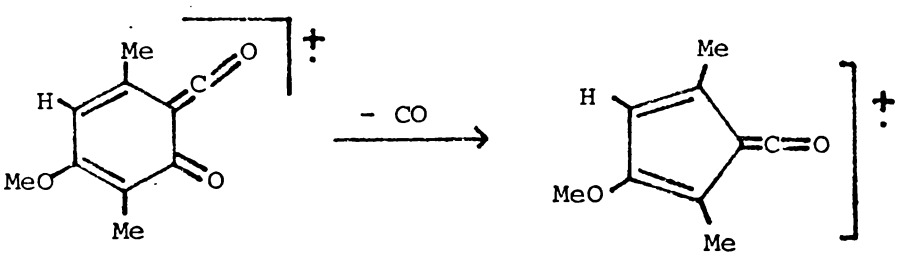
The origin of the well-established[132] loss of ketene ($H_2C=C=O$, 42 m.u.) from depsidonal acetates is confirmed by comparing the mass spectrum of hypostictic acid D_6-diacetate (111), in which losses of 44 m.u. occur instead (scheme 13). The loss of D_2O from ion xxxvii (subsequent to two losses of 44 m.u. from the molecular ion) results in ion xxxiii, which is also formed by loss of water from the molecular ion of hypostictic acid (106) (scheme 12) and hence demonstrates the source of the water lost. Subsequent losses of carbon monoxide from this ion in both mass spectra *[*of (106) and (111) - schemes 12 and 13*)]* produce the same series of peaks (*m/e* 326, 298, 270 and 242). Ion xxx in the mass spectrum of hypostictic acid D_6-diacetate (111) appears to have lost carbon monoxide resulting in ion xxxviii, although none of the other compounds, even hypostictic acid diacetate (110) have demonstrated a parallel cleavage. In addition, the source of the proton on the C-2 oxygen of ion xxix is demonstrated by its replacement by a deuterium atom in the mass spectrum of (111) to form ion xxxix.

A loss of 60 m.u. (≡ HOAc) following a loss of ketene in the mass spectrum of 3-methylconstictic acid triacetate (112) is envisaged to have occurred between the C-3' acetoxymethyl group and the C-2' hydroxyl group (scheme 15). A peak at *m/e* 221 would appear to be ion xl, the acyl-migrated analogue of ion xxix.

5.5 Conclusion

It has been established that the metabolites of *N. australe*, Q-1 and Q-2, are hypostictic acid (106) and hyposalazinic acid (107) respectively, and hence are identical to the metabolites of *X. quintaria* designated PQ-1 and PQ-2 by Culberson.[129] In addition the identity of PQ-4 as 3-methylconstictic acid has been demonstrated.[130]

Mass spectral studies have produced further support for the cleavages suggested by Huneck and Djerassi *et al.*[132]



ion xxx *m/e* 178

(111) ion xxxviii *m/e* 150

(111) ion xxxix *m/e* 180

(112) ion xl *m/e* 221

CHAPTER SIX

EXPERIMENTAL

6.1 General Experimental Conditions

Alumina refers to BDH aluminium oxide, activity II, standardised according to Brockmann. T.l.c. was conducted on Merck silica gel G (type 60) and p.l.c. on Merck silica gel (type 60 $PF_{254+366}$). References to E and H apply to ether and hexane (or petroleum ether) respectively. P.l.c. fractions are always discussed in order of increasing polarity.

Melting points were determined on a Gallenkamp hot stage and are uncorrected. ^1H nmr spectra were obtained in $CDCl_3$ (unless otherwise specified) on a Jeol C-60 HL spectrometer. Ir spectra were determined as potassium bromide discs on a Perkin-Elmer 180 spectrometer. Mass Spectra were measured with a Varian MAT CH5 instrument.

Benzene and toluene were purified according to Vogel[135] and stored with sodium wire. Ether was used freshly distilled from sodium-benzophenone while dioxan was purified by passing through a column of freshly activated alumina (500° for 8 h).

"Work-up" refers to a process of dilution of the reaction mixture with *ca.* three times the volume of water and extraction with ether or chloroform several times. Evaporation of the solvent, under reduced pressure, to a small volume is followed by chromatography on a short column of alumina.

6.2 Preparation of 15α-Acetoxyhop-17(21)-en-24-ol (10)

Extraction of Pseudocyphellaria amphisticta — The air-dried lichen material (89 g) (collected from the shores of Lake Waikare-iti, Urewera National Park, New Zealand,) was ground to a fine powder and extracted in a Soxhlet apparatus with chloroform for 8 h. On concentration of the extract, crystals precipitated. Filtration and repeated washing of the crystals yielded crude filtrate (2.84 g) and crystalline 15α-acetoxy-22-hydroxyhopan-24-oic acid (5) (5.12 g) (identical, ^{1}H nmr, to that isolated previously[8,9] from the same lichen).

15α-Acetoxyhop-17(21)-en-24-oic acid (9) — To a cold solution of 15α-acetoxy-22-hydroxyhopan-24-oic acid (5) (1.8 g) in AnalaR acetic acid (100 ml) was added slowly with swirling a cold solution of AnalaR sulphuric acid (8 ml) in AnalaR acetic acid (12 ml). The resultant solution was left at room temperature for 24 h before being worked up in the usual way except that chromatography on alumnia was replaced by chromatography on a short column of silica gel to yield crude *15α-acetoxyhop-17(21)-en-24-oic acid (9)* (1.77 g), m.p. 218.5-223° (from acetone 2x); $\nu_{max.}$ 1725 (OAc), 1690 (COOH), 1245 (OAc) cm^{-1}; δ 2.01 (3H, OAc), 4.95 (1H, m, $W_{\frac{1}{2}}$ 20Hz, C*H*OAc), 9.90 (1H, m, $W_{\frac{1}{2}}$ 30Hz, COOH); *m/e* 483 (M^+ - $\cdot CH_3$) 455, 438, 423, 395, 187 (100%). (Found: C, 76.7; H, 10.5%. $C_{32}H_{50}O_4$ requires C, 77.1; H, 10.0%).

15α-Acetoxyhop-17(21)-en-24-ol (10) — Crude 15α-acetoxyhop-17(21)-en-24-oic acid (9) (2.0 g) was dissolved in redistilled dry benzene (15 ml) in a flask fitted with a calcium chloride drying tube and cooled to 0°. After addition of oxalyl chloride (5.5 ml), the effervescing solution was maintained at 0° for 45 min, followed by 45 min at room temperature. After removal of the excess oxalyl chloride under reduced pressure, the crude acid chloride was dissolved in dioxan (15 ml) and sodium borohydride (1.4 g) was added. After 10 min the resulting suspension was refluxed for 30 min and worked up in the usual way, including washing with 2M hydrochloric acid. The product (1.9 g) was adsorbed from chloroform on to Merck silica gel G (type 60) (3 g) and loaded on to a column of the same (54 g). Elution in 100 or 200 ml aliquots gave the following fractions a) H-E (17:3, 400 ml), b) H-E (4:1, 400 ml), c) H-E (4:1, 100 ml), d) H-E (3:2, 300 ml), e) H-E (3:7, 200 ml), f) ether (400 ml). Fraction b) (112 mg), contained 15α-acetoxyhop-17(21)-en-24-oic acid (9). Fraction c) (104 mg) contained 15α-acetoxyhop-17(21)-en-24-oic acid (9) and 15α-acetoxyhop-17(21)-en-24-ol (10). Fraction d) (1.01 g) crystallised from hexane to give *15α-acetoxyhop-17(21)-en-24-ol (10)*, m.p. 120-1.5°; $\nu_{max.}$ 1740, 1235 (OAc); δ 2.02 (3H, OAc), 3.45, 3.74 (1H, each, pair of d, J_{AB} 11Hz CH_2OH), 4.98 (1H, m, $W_{\frac{1}{2}}$ 20Hz, CHOAc); *m/e* 469 ($M^+ - {}^{\bullet}CH_3$), 453, 424, 409, 381, 187 (100%). Found *m/e* 424.3689. Require for $^{12}C_{30}\ ^{1}H_{48}\ ^{16}O$ (M^+-HOAc) *m/e* 424.3705).

6.3 Hypoiodite Reactions

Using lead tetraacetate on 15α-acetoxyhop-17(21)-en-24-ol (10) —A mixture of benzene (150 ml), lead tetraacetate (4.0 g), calcium carbonate (1.5 g) and iodine (2.0 g) in an atmosphere of nitrogen was brought to reflux and a solution of 15α-acetoxyhop-17(21)-en-24-ol (10) (470 mg) in benzene (20 ml) was added. Irradiation (160 W Hg lamp) under reflux for 7 h, yielded, after work-up including reduction of excess iodine with sodium thiosulphate solution, a crude product mixture (560 mg). P.l.c. (H-E, 9:1) gave four fractions, A, B, C (*ca.* 10 mg each) and D (230 mg) each of which were intractable mixtures(t.l.c. and g.l.c.).

Using mercuric acetate on 7-oxohopan-22-ol (13) — a) A mixture of 7-oxohopan-22-ol (13) (220 mg, 0.5 mmol), mercuric acetate (320 mg, 1 mmol), iodine (510 mg, 2 mmol) and calcium carbonate (500 mg) in absolute benzene (60 ml) was refluxed for 2.5 h, filtered and worked up in the usual way. The crude product was chromatographed by p.l.c. (H-E, 17:3) to yield, as the major product, *7-oxo-22,29,30-trisnorhop-17(21)-ene (14)* (50 mg), m.p. 227-9° (sublimed sample); $\nu_{max.}$ 1695 (C=O) cm^{-1}; δ 5.18 (1H, S, $W_{\frac{1}{2}}$ 5Hz, C=CH); *m/e* 382 (M^{+}, 100%), 367, 289, 275, 233, 220, 207, 205, 147. (Found *m/e* 382.3234. Require for $^{12}C_{27}{}^{1}H_{42}{}^{16}O$ *m/e* 382.3238).

b) A mixture of 7-oxohop-22-ol (13) (550 mg, 1.25 mmol), mercuric acetate (1.6 g, 5 mmol), iodine (1.2g, 5 mmol) and calcium carbonate (1.2 g) in benzene (100 ml) was refluxed for 2.5 h, filtered and worked up in the usual way. The product mixture was separated by p.l.c (H-E, 9:1) to yield three fractions, A (34 mg), an intermediate fraction (220 mg) and a polar fraction (380 mg). Rechromatography of the intermediate fraction (H-E, 19:1, 2x) gave fractions B (28 mg) (identical to

A), C, D, E and F (total 32 mg) (discarded). Rechromatography of the polar fractions gave fractions G (21 mg), H (54 mg), I (39 mg) and J (35 mg).

Fraction A was identical to the major product of reaction a) 7-oxo-22,29,30-trisnorhop-17(21)-ene (14).

Fraction G appeared to contain 16ξ-acetoxy-22,29,30-trisnorhop-17(21)-ėn-7-one (21), *m/e* 440 (M^{+}), 425, 380, 365, 233, 220, 207, 205, 145 (100%).

Fraction H (crystallised from acetone) appeared to contain 21ξ-acetoxy-22,29,30-trisnorhopan-7-one (19), δ 0.84 (6H), 0.93 (3H), 1.00 (3H), 1.10 (3H), 1.18 (3H) (CH_3), 2.10 (3H, OAc); *m/e* 442 (M^{+}), 382, 233, 220, 207, 205. Hydrolysis of crude H with 10% potassium hydroxide in refluxing ethanol yielded two fractions after p.l.c. (H-E, 3:2). The less polar fraction was discarded, but the remaining fraction appeared to contain 21ξ-hydroxy-22,29,30-trisnorhopan-7-one (20), δ 0.87 (9H), 1.02 (6H), 1.23 (3H); *m/e* 400 (M^{+}), 382, 367, 233, 220 (100%), 207, 205.

Fraction I was *7,21-dioxo-22,29,30-trisnor-17αH-hopane (18)*, m.p. 251-2° (sublimed sample); $\nu_{max.}$ 1724, 1690 (C=O) cm^{-1}; *m/e* 398 (M^{+}), 233, 220, 207, 205 (100%). (Found C, 81.4; H, 11.0%. $C_{27}H_{42}O_2$ requires C, 81.4; H, 10.6%).

Fraction J appeared to be a mixture of two acetoxyketones, δ 2.07 and 2.10 (3H each, OAc); *m/e* 454 (M^{+}?), 394 and 438 (M^{+}?), 378.

Using mercuric acetate on 15α-acetoxyhop-17(21)-en-24-ol (10) — A mixture of 15α-acetoxyhop-17(21)-en-ol (10) (430 mg), mercuric acetate (900 mg), iodine (1.1 g) and calcium carbonate (800 mg) in benzene was refluxed in a nitrogen-atmosphere for 2 h. Filtration followed by work-up, including washing with sodium thiosulphate solution, gave a complex

mixture of products. Separation by p.l.c. (H-E, 9:1) yielded three fractions A (93 mg), B (28 mg) and C (26 mg) (an intractable mixture). Re-chromatography of fractions A (H-E, 9:1) and B (H-E, 4:1) yielded only three fractions, A(1), A(2) and A(3) (2 mg each), which appeared (by t.l.c.) to consist of one component. Fraction A(1) had *m/e* 400, 388, 386, 373, 369, 263, 262, 261, 249, 248, 247, 245, 235, 234, 233, 232, 231, 217, 216, 215, 203, 202, 201, 187, 175, 174, 173, 165, 161 (100%), 159, 151. Fraction A(2) had *m/e* 434, 279, 231, 203, 201, 187, 186, 185, 179, 173 (100%). Fraction A(3) had *m/e* 434, 405, 391, 231, 201, 200, 187, 186, 185, 173 (100%).

6.4 Preparation of 7-Oxohopan-22-ol (13) and 15-Oxohopan-22-ol (58)

Hydrolysis of 7β-acetoxyhopan-22-ol (12) — A solution of 7β-acetoxyhopan-22-ol (12) (5 g) in ethanol (250 ml) was refluxed with potassium hydroxide (20 g) for 5 h. Filtration of the precipitated crystals (4.35 g) and work-up of the filtrate yielded hopane-7β,22-diol (62) (5.19 g), identical (t.l.c., nmr) to an authentic sample.

Jones reagent oxidation of hopane-7β,22-diol (62) — Excess Jones reagent was added dropwise over a period of 1 h to a stirred solution of hopane-7β,22-diol (62) (2.5 g) in acetone (150 ml) at 35°. Work-up, after stirring for a further 1 h, yielded, after purification by p.l.c. (H-E, 1:1) and recrystallisation (from acetone), 7,21-dioxo-22,29,30-trisnor-17αH-hopane (18) (430 mg), identical (^{1}H nmr, ir, ms) to that contained in fraction I of the hypoiodite oxidation of 7-oxohopan-22-ol (13) (section 6.3).

Jones reagent oxidation of hopane-15α,22-diol (59) —

Excess Jones reagent was added dropwise to a stirred solution of hopane-15α,22-diol (59), (580 mg) in acetone-ethylacetate (3:1, 80 ml) at room temperature. After a total reaction time of 20 min work-up in the usual way, followed by purification by p.l.c. (H-E, 1:1) and recrystallisation (from acetone) gave *15,21-dioxo-22,29,30-trisnorhopane* (83) (200 mg), m.p. 258° (sublimed sample); $\nu_{max.}$ 1737, 1698 (C=O) cm^{-1}; *m/e* 398 (M^{+}.) 383, 207, 205, 192, 191, (100%), 175, 135, 123. (Found C, 81.6; H, 10.7% $C_{27}H_{42}O_2$ requires C, 81.4; H, 10.6%).

Alkaline oxidation of hopane-7β,22-diol (62) —

A solution of hopane-7β,22-diol (62) (2.84 g) in pyridine (180 ml) was added to a suspension prepared by adding chromium trioxide (2.0 g) piece-wise to pyridine (20 ml). After being stirred for 44 h at room temperature the reaction mixture was worked up in the usual way including washing with 4 M hydrochloric acid to yield 7-oxohopan-22-ol (13) (1.7 g) identical (^{1}H nmr, ms, ir) to an authentic sample.

Alkaline oxidation of hopane-15α,22-diol (59) — The oxidation of hopane-15α,22-diol (59) (2.5 g) was carried out as for hopane-7β,22-diol (62) to yield 15-oxohopan-22-ol (58) (1.9 g), identical (^{1}H nmr, ms, ir) to an authentic sample.

6.5 Sulphenylation Reactions

General — All reactions were performed using glassware which had been flamed under a stream of nitrogen immediately prior to use. Throughout the reaction period the reaction mixture was mechanically stirred and a positive pressure of dry nitrogen was maintained. The n-butyllithium used was a solution of 15% w/w in hexane (Aldrich). Diphenyldisulphide was prepared after Yiannios and Karabinos.[136] THF was used freshly distilled from sodium-benzophenone, while, unless otherwise specified, HMPA and dicyclohexylamine were used distilled from potassium hydroxide. A temperature of -82° was achieved using an ethylacetate slush bath.

Standard procedure — To a solution of dicyclohexylamine and THF (5 ml) at -82° was added n-butyllithium in hexane. After 25 - 30 min a solution of the ketone in THF-HMPA (2:3) (5 ml) was added dropwise. After 30 min at -82° the reaction mixture was allowed to warm to 0° over a period of 30 min before diphenyldisulphide in THF-HMPA (1:1 (5 ml) was added dropwise. Work-up included washing with 2 M hydrochloric acid solution, filtering (to remove the dicyclohexylamine hydrochloride) and extracting the filtrate with ethylacetate.

a) Pilot scale attempted sulphenylation of 15-oxohopan-22-ol (58) — (Unpurified dicyclohexylamine and HMPA were used). n-Butyllithium solution (1.5 ml) was added to dicyclohexylamine (3 ml, 1.5 mmol) in THF (5 ml) at 0°. After 20 min the solution was cooled to -82° and 15-oxohopan-22-ol (58) (150 mg, 0.4 mmol) in THF (5 ml) added dropwise. After 30 min at -82^{0}, diphenyldisulphide (400 mg, 2 mmol) in HMPA (5 ml) was added dropwise and the solution was allowed to warm to room temperature. An aliquot (2 ml) was removed after 2 h, while the remaining reaction mixture was held at 90° for 1 h. Both portions, after work-up, produced evidence of only unchanged starting material (t.l.c., ms).

b) Sulphenylation of cyclohexanone — (unpurified dicyclohexylamine and HMPA were used). The reaction was carried out according to the standard procedure except that the ketone (cyclohexanone) was added in THF (5 ml) and diphenyldisulphide in HMPA (5 ml).

Quantities used:

dicyclohexylamine, 6 ml in THF, 8 ml,

n-butyllithium in hexane, 5 ml,

cyclohexanone, 0.2 ml,

diphenyldisulphide, 470 mg.

After addition of the diphenyl disulphide the reaction mixture was stirred for 1 h at room temperature before work-up. P.l.c. (H-E, 4:1, 2x) of the product mixture gave two fractions A and B. Fraction A contained 2-phenylthiocyclohexanone, *m/e* 206 (M^{+}), 97, 96 and 2-phenylthiocyclohexanol, *m/e* 208 (M^{+}), 99, 98. Fraction B contained 2,6-diphenylthiocyclohexanol, *m/e* 316 (M^{+}, 100%).

c) Sulphenylation of 15-oxohopan-22-ol (58) — 15-Oxohopan-22-ol (58) (220 mg, 0.5 mmol) was reacted as outlined in the standard procedure, employing a ketone-base-disulphide ratio of 1:2:2. After diphenyldisulphide in HMPA (2 ml) had been added to the solution of the lithium enolate the reaction mixture was left at room temperature for 30 min and then held at 80° for 1 h. Work-up followed by separation by p.l.c. (H-E, 1:1) yielded three fractions, A (90 mg), B (80 mg) and C (10 mg).

Fraction A contained 15-oxo-16β-phenylthiohop-21-ene (63), δ 4.23 (1H, m, $W_{\frac{1}{2}}$ 16Hz, C*H*SPh), 7.45 (5H, m, $W_{\frac{1}{2}}$ 22Hz, SPh); *m/e* 532 (M^{+}), 422 (100%), 407, 243, 229, 218, 216, 203, 201, 191.

Fraction B contained unchanged starting material (ms).

Fraction C consisted mainly of 15-oxohop-16-en-22-ol (64) *m/e* 440, 422 (M^{+}) 407, 229, 218, 216, 203 (100%), 201, 191.

d) Attempted sulphenylation of 7-oxohopan-22-ol (13) — 7-Oxohopan-22-ol (13) (220 mg, 0.5 mmol) was reacted as in c) except that a ketone-base-disulphide ratio of 1:2:3 was employed. Work-up and p.l.c. (H-E, 3:2) gave four fractions, A (90 mg), B (80 mg), C (80 mg) and D (30 mg) each of which proved to be intractable mixtures, containing variable proportions of compounds having *m/e* 548 (M^{+}), 546 (M^{+}), 532 (M^{+}), 530 (M^{+}), 489, 438, 423, 395 (100%), 381. Further p.l.c. and recrystallisation failed to effect any separation.

e) Attempted sulphenylation of 7-oxohop-17(21)-ene (65) — 7-Oxohop-17(21)-ene (65) (220 mg, 0.5 mmol) was reacted as in c) except that a ketone-base-disulphide ratio of 1:2:4, was employed and, after the addition of diphenyldisulphide, the reaction mixture was maintained at 0^{o} for 1 h and at room temperature for 2 h. Work-up followed by p.l.c. (H-E, 17:3) produced one major fraction (150 mg) which was rechromatographed (H-E, 17:3) to obtain two major fractions, A (52 mg) and B (76 mg). Fraction A contained recovered 7-oxohop-17(21)-ene (65) (ms). Fraction B had *m/e* 490, 454, 438, 395, 151 (100%).

f) Sulphenylation of 15-oxohopan-22-ol (58) — 15-Oxohopan-22-ol (58) (660 mg, 1.5 mmol) was reacted according to the standard procedure, employing a ketone-base-disulphide ratio of 1:2:3. After the addition of diphenyldisulphide, the reaction mixture was kept at room temperature for 30 min and then held at 45^{o} for 3.5 h. Work-up followed by column chromatography on alumina produced the following fractions: a) hexane (300 ml) (discarded), b) H-E (9:1, 103 ml), c) H-E (9:1, 200 ml), d) H-E (9:1, 100 ml), e) H-E (9:1, 100 ml), f) H-E (2:3, 400 ml) (discarded). Fraction b) (283 mg) was further separated by p.l.c. (E-H, 1:4, 2x) into two fractions, b)(1) (41 mg) and b) (2) (127 mg). Fraction b)(1) contained 15-oxo-16α-phenylthiohopan-22-ol (67), *m/e* 550 (M^{+}), 489 261, 245 (100%). Fraction b)(2) contained 15-oxo-16β-phenylthiohopan-22-ol (66).

Fraction c) (455 mg) contained 15-oxo-16β-phenylthiohopan-22-ol (66).

Fraction d) was further separated by p.l.c. (E-H, 1:4, 2x) into two fractions, d)(1) (86 mg) and d)(2) (57 mg). Fraction d)(1) contained

15-oxo-16β-phenylthiohopan-22-ol (66) and fraction d)(2) recovered 15-oxohopan-22-ol (58) (t.l.c., ms).

Fraction e) (56 mg) contained recovered 15-oxohopan-22-ol (58) (t.l.c., ms).

Since the samples of 15-oxo-16β-phenylthiohopan-22-ol (66) were yellow they were purified by p.l.c. (toluene-ether, 19:1). *15-Oxo-16β-phenylthiohopan-22-ol (66)* had m.p. 201-3° (from acetone); $\nu_{max.}$ 1682 (C=O), 620 and 503 (Ph) cm^{-1}; δ 4.15 (1H, d, *J* 14Hz, C*H*SPh), 7.35 and 7.58 (5H, m, SPh); *m/e* 550 (M^{+}), 532, 423, 422, 261, 243 (100%), 227, 203, 191. (Found C, 78.5; H, 10.0. $C_{36}H_{54}O_2S$ requires C, 78.5; H, 9.8).

6.6 Reduction of 15-oxo-16β-phenylthiohopan-22-ol (66)

Using sodium borohydride — 15-Oxo-16β-thiophenylhopan-22-ol (66) (30 mg) was heated under reflux in ethanol (30 ml) with sodium borohydride (30 mg) for 2.5 h and left for three nights at room temperature. Work-up yielded a mixture of hop-16-ene-15ξ,22-diol (68) (minor component) *m/e* 442 (M^{+}), 424, 191, 163 and 15-oxohop-16-en-22-ol (69) (major component) *m/e* 440 (M^{+}), 422, 382, 367, 191, 189, 176, 161.

Using lithium aluminium hydride — A solution of 15-oxo-16β-thiophenylhopan-22-ol (66) (16 mg) and lithium aluminium hydride (40 mg) in ether (5 ml) was kept at room temperature for 29 h. Work-up followed by p.l.c. (H-E, 3:7) yielded hopane-15α,22-diol (59) (4.5 mg) (identical, t.l.c. and ^{1}H nmr to an authentic sample) as the major product.

Lithium triethylborohydride — Unsuccessful attempts were made to prepare the precursor, triethylboron after Brown.[137] Reaction, in each case, of the resulting solution with lithium hydride produced no evidence (ir) of lithium triethylborohydride, nor would the preparation reduce 15-oxo-16β-phenylthiohopan-22-ol (66).

Using sodium-isopropanol — HMPA (0.5 ml) and sodium (0.9 g) were added to a solution of 15-oxo-16β- phenylthiohopan-22-ol (66) (60 mg) in toluene (30 ml). When the resulting mixture was brought to reflux it became a green solution and isopropanol (11 ml) was added. After 20 min the green colour had faded and the amount of sodium was much diminished, so further sodium (0.75 g) was added. After a total reaction time of 2.5 h work-up, followed by recrystallisation (from acetone), gave hopane-15α,22-diol (59) (45 mg) identical (t.l.c and ^{1}H nmr) to an authentic sample.

Using aluminium hydride — Aluminium chloride (800 mg, 6 mmol) was added to a solution of lithium aluminium hydride (50 mg, 0.5 mmol) in ether (25 ml). After standing for 30 min, the reaction mixture was heated under reflux for 15 min prior to addition of 15-oxo-16β-phenylthiohopan-22-ol (66) (30 mg) in ether (5 ml). After refluxing for 75 min the aluminium hydride was destroyed by adding water dropwise. Work-up followed by separation by p.l.c. (E-H, 3:7) produced two major fractions, A (2.1 mg) and B (1.2 mg). Fractions A and B appeared to contain 16β-phenylthiohopane-15ξ,22-diol (70), *m/e* 552 ($M^{+\cdot}$), 443, 369, 355, 295, 221, 191.

6.7 Attempted Preparation of a hydrazone of 7-oxohopan-22-ol (13)

p-Toluenesulphonylhydrazide — *p*-Toluenesulphonylhydrazide was prepared after Albert and Royer,[138] using hydrazine hydrate (5 ml) and *p*-toluenesulphonylchloride (10 g).

Attempted preparation of the p-toluenesulphonylhydrazone of 7-oxohopan-22-ol (13) — A solution of 7-oxohopan-22-ol (13) (80 mg) and *p*-toluenesulphonylhydrazide (60 mg) in toluene (60 ml) was heated under reflux with the aid of a Deane and Stark trap for 44 h. Work-up yielded unchanged 7-oxohopan-22-ol (13) (t.l.c., ^{1}H nmr).

6.8 ^{13}C nmr Spectra

The spectra were determined in $CDCl_3$ or C_5D_5N solution on a Jeol FX-60 spectrometer at the University of Auckland or the University of Otago.

6.9 Infrared Spectra

The spectra were determined as potassium bromide discs (*ca.* 2 mg/80 mg KBr) on a Perkin-Elmer 180 spectrometer with an abscissa expansion of 5, while the ordinate expansion was varied to give reasonable (>60%) intensity to peaks of maximum absorption.

6.10 Depsidones from *N. australe* and *N. antarticum*

Extraction of N. australe — The finely ground lichen material (4.8 g) (collected in December 1977 and May 1978 from the vicinity of Lakes Waikaremoana and Waikareiti, Urewera National Park, New Zealand) was extracted in a Soxhlet apparatus with petroleum ether for 17 h and then with acetone for 2.5 h. The petroleum ether extracts were demonstrated to consist largely of usnic acid and zeorin (hopane-6α,22-diol). Separation of the acetone extracts (490 mg) by p.l.c. with toluene-dioxane-acetic acid (TDA) (90:25:4) gave two fractions which were subjected to further p.l.c., with petroleum ether-ether-formic acid (12:13:2) (Q-1 fraction, faster moving band) or with TDA (Q-2 fraction, slower moving band) to give hypostictic acid (106) (Q-1) (44 mg) and hyposalazinic acid (107) (Q-2) (16 mg) respectively.

Hypostictic acid (1a) had m.p. 260-262° with decomposition from 220° (lit.[126] 264° with decomposition); $\nu_{max.}$ 1750, 1695, 1605, and 1560 cm^{-1}; *m/e* 372 ($M^{+\cdot}$) 354, (100%) 328, 327, 326, 300, 299, 298, 283, 272, 271, 270, 255, 244, 243, 242, 216, 179, 178, 177.

Hyposalazinic acid (106) had m.p. 274° with decomposition from 219° (lit.[126] 280° with decomposition); $\nu_{max.}$ 1720, 1695, 1610 and 1580 cm^{-1}; *m/e* 358 ($M^{+\cdot}$), 340 (100%) 314, 313, 312, 286, 285, 284, 269, 258, 257, 256, 230, 229, 228 and 165.

Hypostictic acid diacetate (110) and hyposalazinic acid triacetate — The acetone extracts of *N. australe* (203 mg from 1.8 g of lichen) were dissolved in pyridine-acetic anhydride (1:1) (3 ml) and stirred for 16 h at room temperature. Work-up and purification by p.l.c. with benzene-ether (93:7) gave hypostictic acid diacetate (110) (21 mg) m.p. 240°

(lit.[126] 244°) *m/e* 456, and hyposalazinic acid triacetate (8 mg) m.p. 201° (lit.[126] 203-205°).

Hypostictic acid D_6-diacetate (111). — Repetition of the acetylation experiment described above with a portion of the acetone extracts from *N. australe* (170 mg from 1.46 g of lichen) and D_6-acetic anhydride (0.5 ml) in pyridine (2 ml) gave, as the major product, hypostictic acid D_6-diacetate (111) (17 mg), *m/e* 462 (M^{+}), 418 (100%) 399, 374, 354, 345, 335, 326, 322, 301, 298, 285, 271, 270, 245, 180, 179, 178, 150.

Extraction of N. antarcticum — The finely ground lichen material (0.9 g) *[*from the Herbarium of the British Museum (Natural History), London, (J.D.Hooker, Cape Horn)*]* was extracted in a Soxhlet apparatus with petroleum ether for 24 h and then with acetone for 6 h. The petroleum ether extracts were demonstrated to consist largely of usnic acid and zeorin. T.l.c. analysis (TDA as solvent) established the presence in the acetone extracts of three major components, two of which gave distinctive red colourations when charred with sulphuric acid. Pilot scale experiments established that the more polar of these substances could not be satisfactorily recovered from p.l.c. plates developed by the procedures employed for the separation of the *N. australe* extracts.

Reaction of the acetone extracts (120 mg) with pyridine-acetic anhydride (1:1) (5 ml) for 24 h at room temperature gave a gummy residue which was shown by t.l.c. analysis *[*petroleum ether-ether (3:2) as solvent*]*

to consist of three major components, two of which gave red colourations when charred with sulphuric acid. Separation of the latter components by p.l.c. with petroleum ether-ether (3:2) gave hypostictic acid diacetate (110) (22 mg) (higher R_F-value). 3-Methylconstictic acid triacetate (112) had m.p. 190-192°; $\nu_{max.}$ 1780, 1743, 1710, 1634, 1610, 1564, 1456, 1343, 1185, 1152, 1137, 1056, 986 and 913 cm^{-1}; *m/e* 514 (M^{+}), 472, 454, 412, 370, (100%), 342, 314, 221, 179, 178. (Found: *m/e* 514.1129. $^{12}C_{25}{}^{1}H_{22}{}^{16}O_{12}$ requires 514.1111).

APPENDIX ONE

REACTION OF MERCURIC ACETATE WITH UNSATURATED COMPOUNDS

1'.1 Introduction

At the outset this literature survey was intended to investigate the value of using mercuric acetate in the hypoiodite reaction of 15α-acetoxyhop-17(21)-en-24-ol (10). However, it developed into a review of the reactions of mercuric acetate with unsaturated compounds, particularly those in which intramolecular substitution results yielding products analogous to those obtained by the hypoiodite reaction of alcohols.

Although it is recognised that other mercury (II) salts often yield analogous products, this review is restricted to the reactions of mercuric acetate and, while it is not exhaustive, it is intended to present an overview.

Products of mercuric acetate reactions may be the result of either addition across the double bond (oxymercuration) or a redox reaction in which the olefin is oxidised and the mercuric acetate is reduced. Oxidation occurs when the substrate itself functions as the solvent or the reaction is performed in acetic acid.[139] High temperatures and extended reaction periods[139] may also be required depending on the nature of the substrate. The precise course of reaction depends on a) steric hindrance, b) suitably situated nucleophilic neighbouring groups and c) solvent nucleophilicity, but the overall routes of the two alternative processes are presented in Figure 21.

FIGURE 22

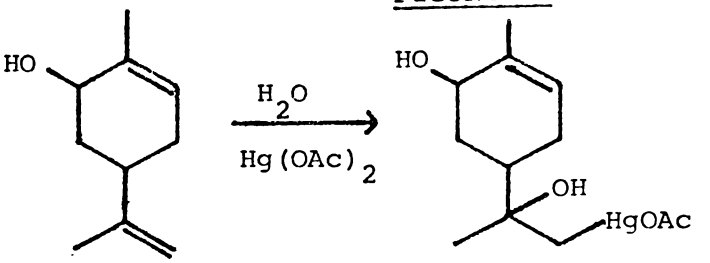


FIGURE 23

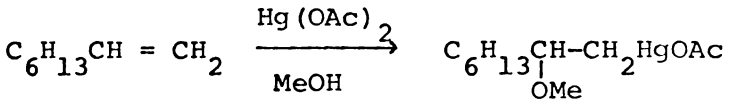


FIGURE 24

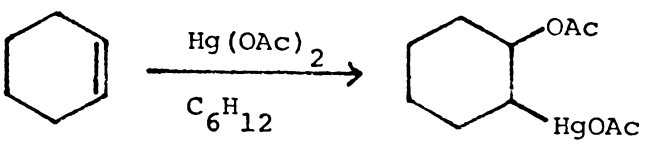


FIGURE 25

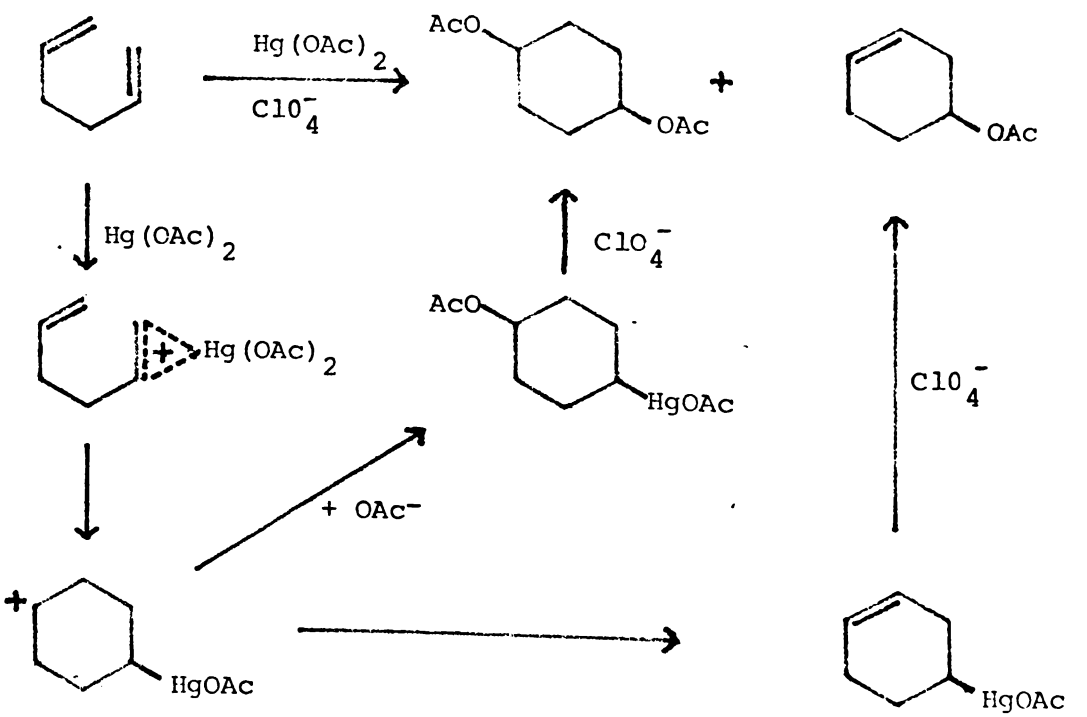
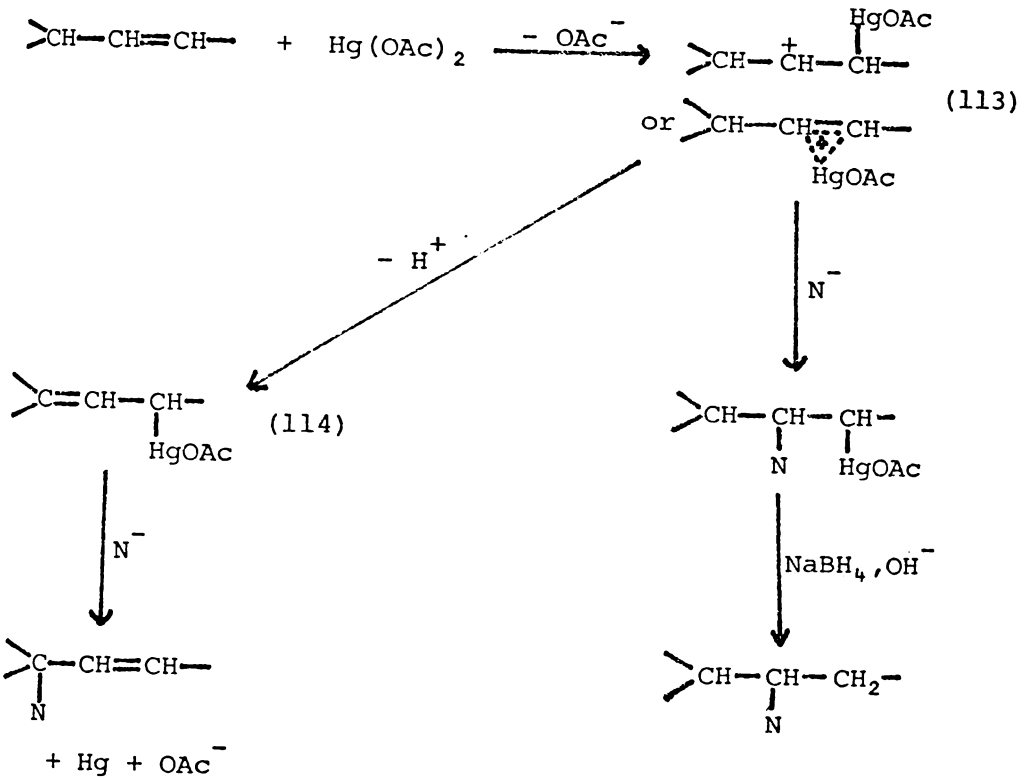


FIGURE 21



1'.2 Oxymercuration

Examples of addition to the double bond are supplied in Figures 22 - 24, where in Figures 22[140] and 23[141] the solvent functions as the nucleophile (solvomercuration), whereas mercuric acetate itself adds across the double bond in the reaction of cyclohexene[142] in petroleum ether (Figure 24). The solvomercuration of alkenes, yielding products from which the metal can be readily removed by alkaline sodium borohydride reduction, has been employed[143] as a convenient procedure for Markovnikov addition. (Conversely, anti-Markovnikov addition is achieved using a hydroboration-mercuration-iodination sequence).[144]

FIGURE 26

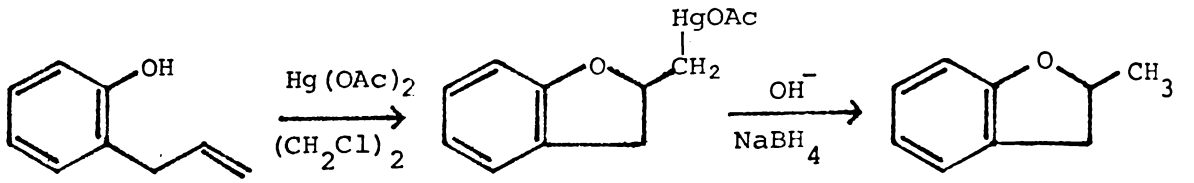


FIGURE 27

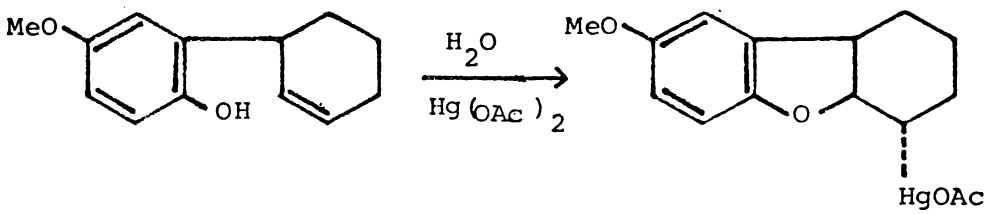


FIGURE 28

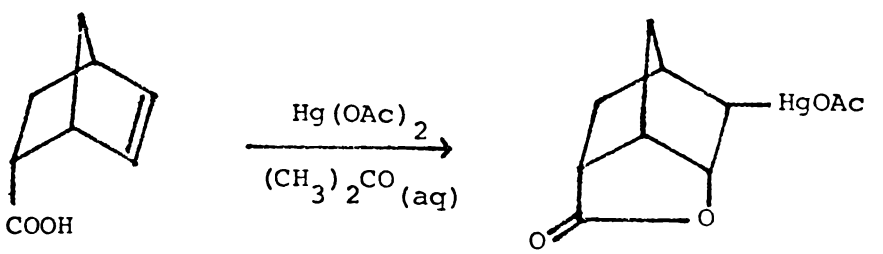


FIGURE 29

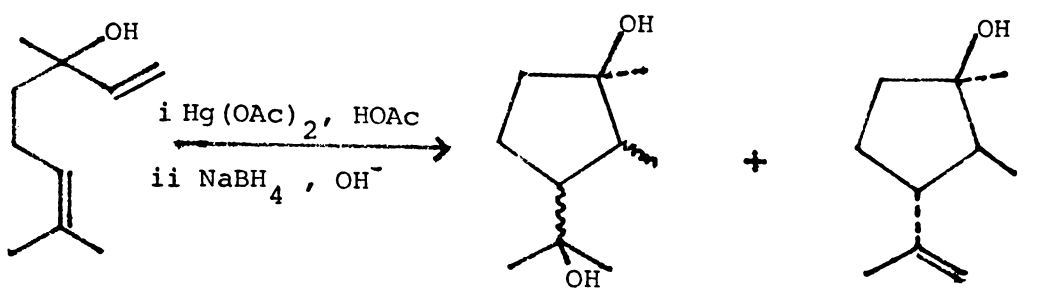


FIGURE 30

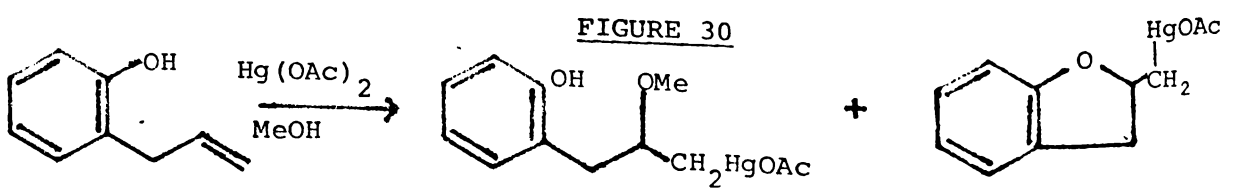
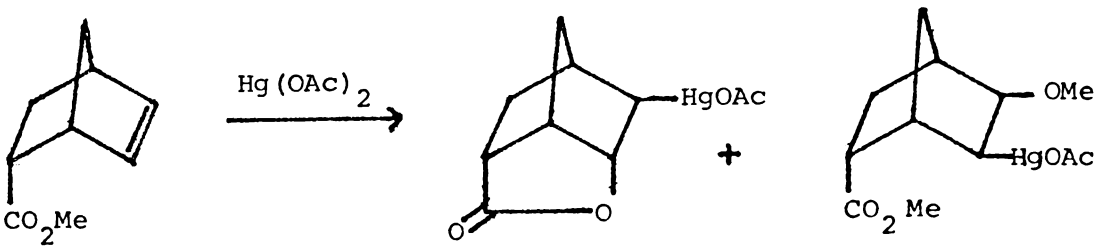
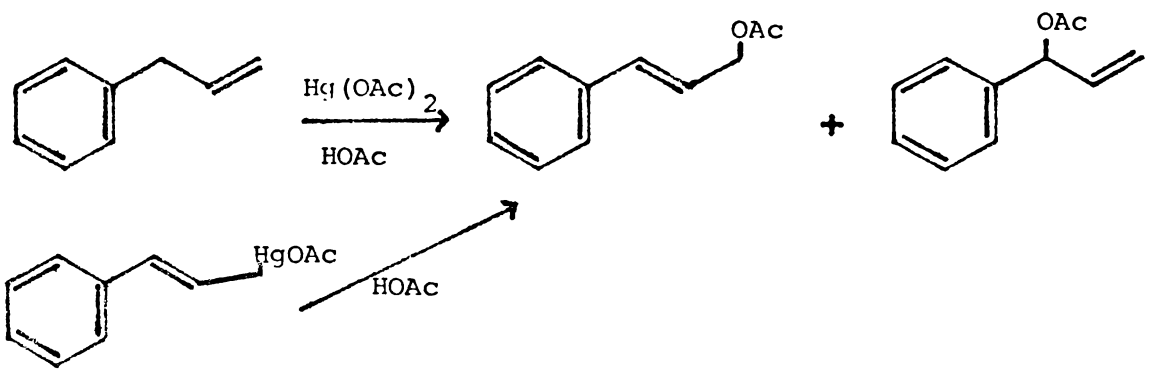


FIGURE 31



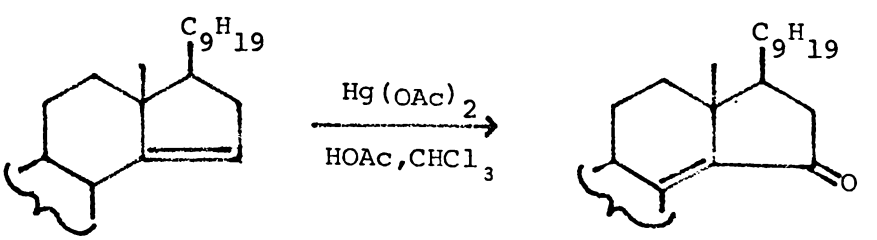
aqueous $(Me)_2CO$	90%	0%
anhydrous MeOH	0%	100%
HOAc	0%	80%

FIGURE 32



(115)

FIGURE 33



When certain neighbouring groups are placed in suitable positions mercuration of the double-bond may also be accompanied by intramolecular nucleophilic addition (Figure 21).

In the reaction of hexa-1,5-diene[145] (Figure 25) the unmercurated double-bond functions as the nucleophile and adds to the terminal carbon atom. The oxidation step, catalysed by the perchlorate ion is said to proceed subsequent to and hence is distinct from the addition/cyclisation steps. Neighbouring hydroxyl groups[146,147] (Figures 26 and 27) and carboxylic acid groups[148] (Figure 28) may also add to the mercurinium ion intermediate (113), resulting in cyclisation to an ether or a lactone respectively. Reaction of linool[149] with mercuric acetate (Figure 29) having two options, intramolecular nucleophilic attack by either an unsaturated carbon atom or an hydroxyl group, yielded products from the former alternative only.

Competition between the solvent and the neighbouring group for the mercurinium ion (113) has been observed in the reaction of 2-(prop-2-enyl)phenol in methanol[146] (Figure 30 *cf.* Figure 26). However, by contrast the oxymercuration of endo-5-carbomethoxy-2-norbornene[148] (Figure 31) is accompanied solely by intramolecular addition , when water is present, even though the carbomethoxyl group must first be hydrolysed and in spite of the strong nucleophilicity of the solvent.

1'.3 Allylic Oxidation

Mercuric acetate reactions which proceed *via* an allylic oxymercurial intermediate (114) (Figure 21) (implicated by the work of Rappoport *et al.*)[150] was initially called Treibs' reaction and has been reviewed from a mechanistic point of view by Arzoumanian and Metzger.[151]

FIGURE 34

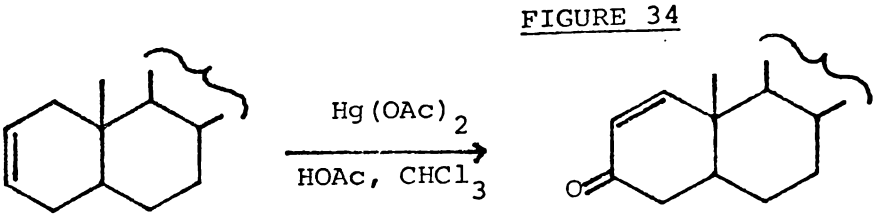


FIGURE 35

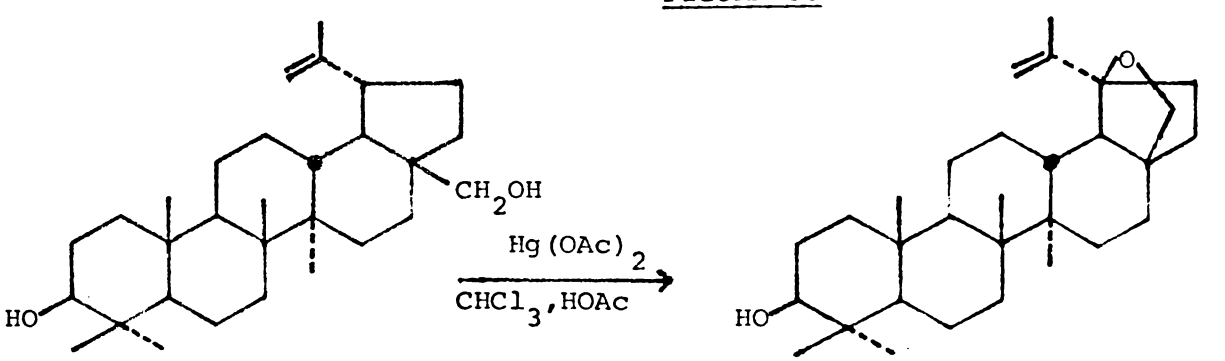


FIGURE 36

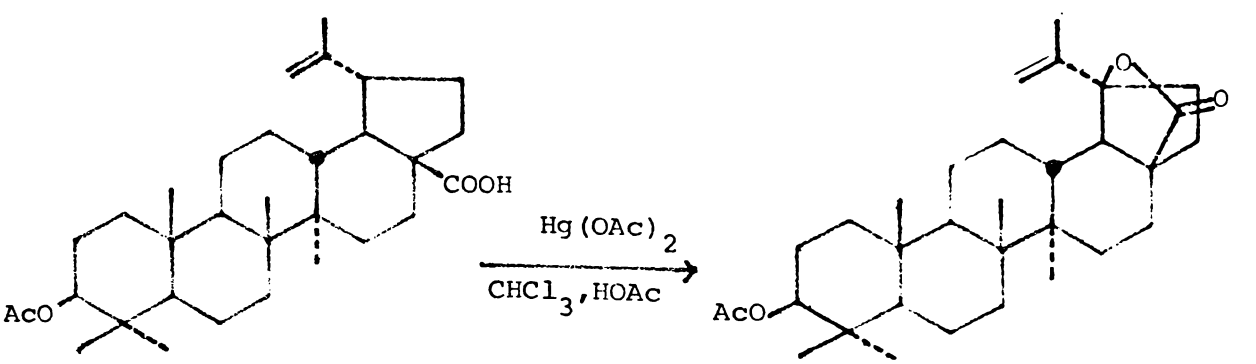


FIGURE 37

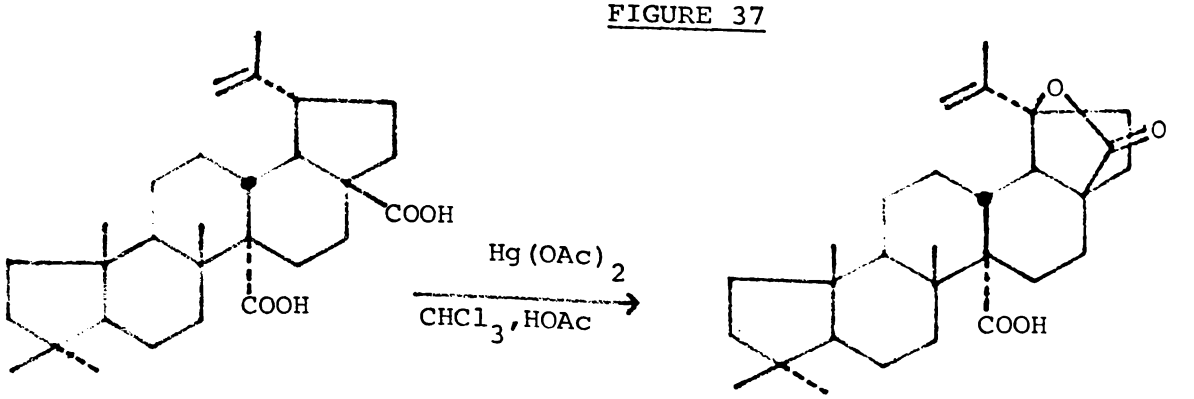


FIGURE 38

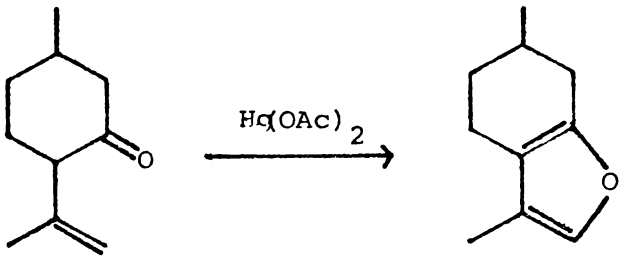


FIGURE 39

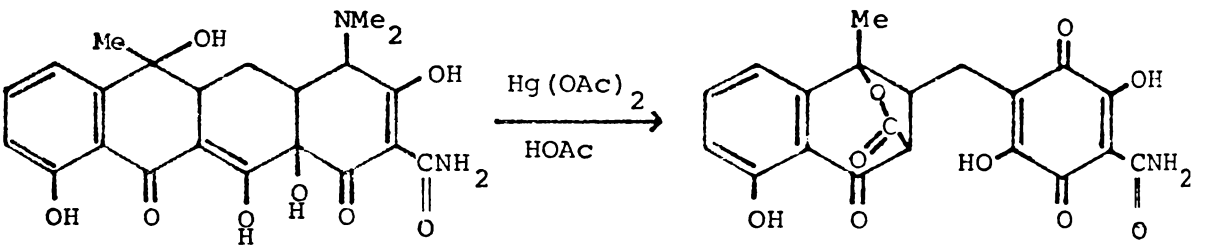


FIGURE 40

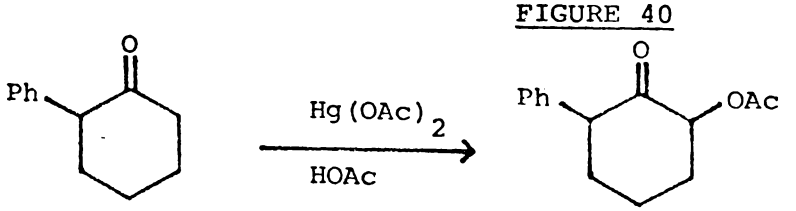
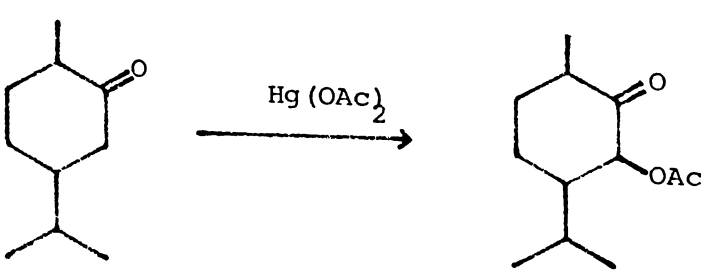


FIGURE 41



This reaction may be distinguished from the foregoing oxymercuration by the absence of alkyl mercurial products and the precipitation of mercurous acetate (if mercuric acetate is in excess) or metallic mercury (if the substrate is in excess). Furthermore, the reaction is accelerated by the addition of excess mercuric acetate, but the products are no different.[153]

That an allylic intermediate is involved in the oxidation of allylbenzene[150] (Figure 32) has been demonstrated by the fact that cinnamylmercuric acetate (115) forms identical products on acetolysis in acetic acid.

The mercuric acetate oxidations of ergost-14-ene[153] (Figure 33) and cholest-2-ene[153] (Figure 34) are unusual in that over-oxidation would seem to have occurred, forming products which are analogous to those formed by the action of acidic aqueous solutions of mercuric sulphate or nitrate on terminal olefins.[151]

Intramolecular nucleophilic addition allylic to the double-bond occurred in the mercuric acetate oxidation of both betulin[154] (Figure 35) and acetyl betulic acid[155] (Figure 36). The necessity of the 20(29)-ene was in part, demonstrated by the reaction of dihydroceanothenic acid[156] (Figure 37), in which cyclisation to a lactone involved the exclusive participation of the C-17 carboxyl group. The oxidation of isopulegone[140] (Figure 38) probably occurred by attack of mercuric acetate on the terminal unsaturated carbon atom, followed by the formation of an allylic oxymercurial intermediate (114) and a concerted deprotonation/cyclisation step initiated by the carbonyl-oxygen atom and accompanied by precipitation of metallic mercury. The reaction of tetracycline[157] (Figure 39) is more complex, but that it occurs *via* the allylic oxidation mechanism of

FIGURE 42

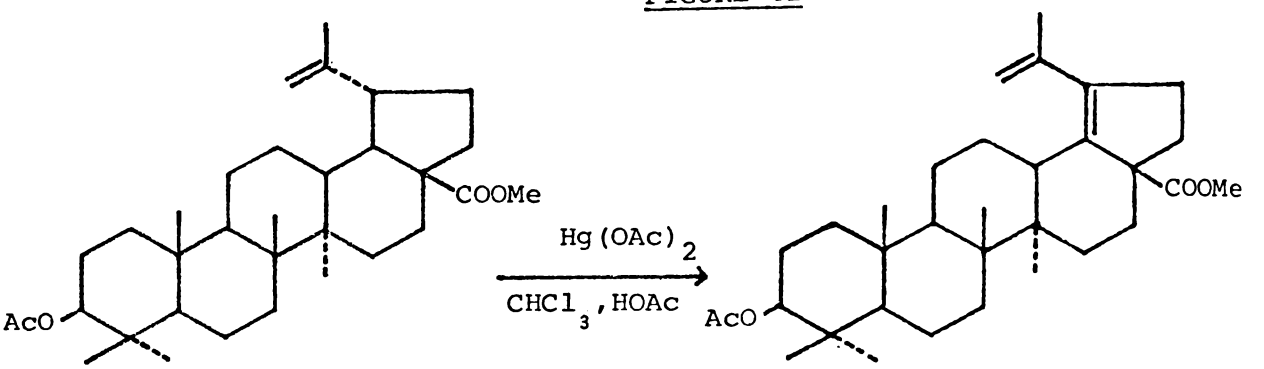


FIGURE 43

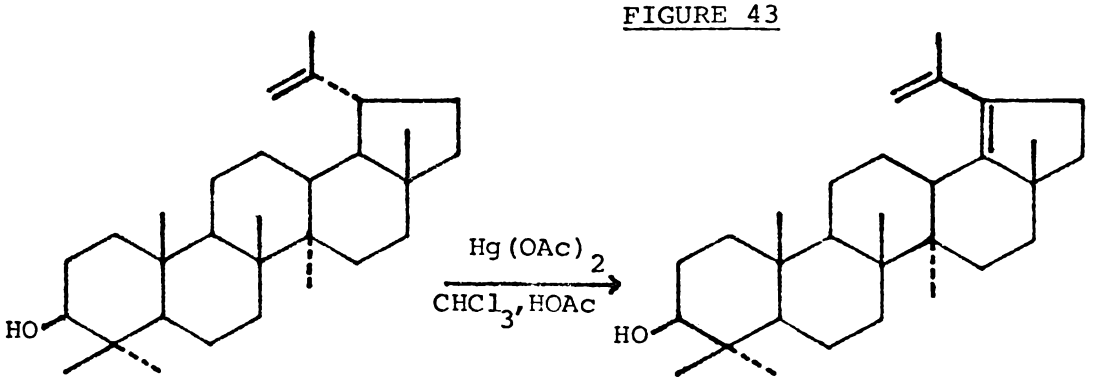


FIGURE 44

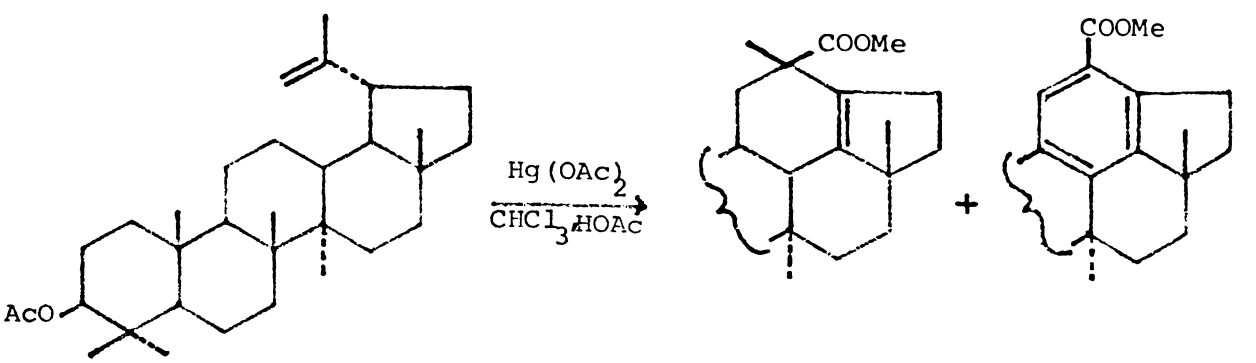


FIGURE 45

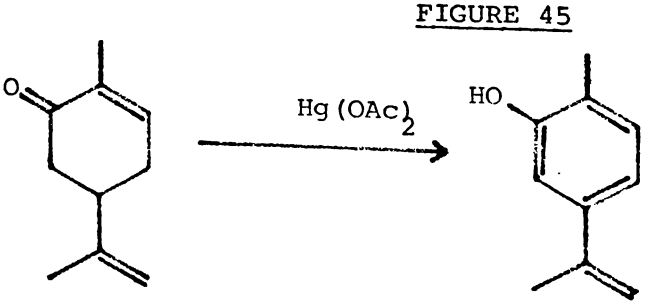


Figure 21, is suggested by the precipitation of mercurous acetate during the course of the reaction.

Mercuric acetate also adds an acetoxyl group α- to carbonyl groups (Figures 40 and 41).[158,159] This reaction may be considered to be initiated by attack of the mercuric acetate on the carbonyl-oxygen and thence proceed *via* a mechanism analogous to that for allylic oxidation of olefins (Figure 21). This proposal is supported by the observation that mercuric acetate inhibits the reduction of 3β,5α-diacetoxy-7α-bromocholestan-6-one[160] with sodium borohydride in methanol-dioxane and leads instead to debromination.

Reaction of unsaturated species with mercuric acetate, in the absence of suitable nucleophiles, has resulted in dehydrogenation and in selected cases, led to aromatisation. Thus the treatment of methyl acetylbetulinate[161] (Figure 42) and lupeol[162] (Figure 43) has been reported to yield the 18,20(29)-diene as the major product. However, additional more polar compounds have resulted from the mercuric acetate oxidation of a similar species, lupenyl acetate[163] (Figure 44). One of these products had derived an extra carbon atom from the chloroform solvent, while another contained an aromatic nucleus. Figures 45[140] and 46[164] present further examples of aromatisation. The direction of the latter reaction has been demonstrated to be dependent on the presence of the nitrile, the isopropenyl group and the position of the skeletal double-bond.

FIGURE 46

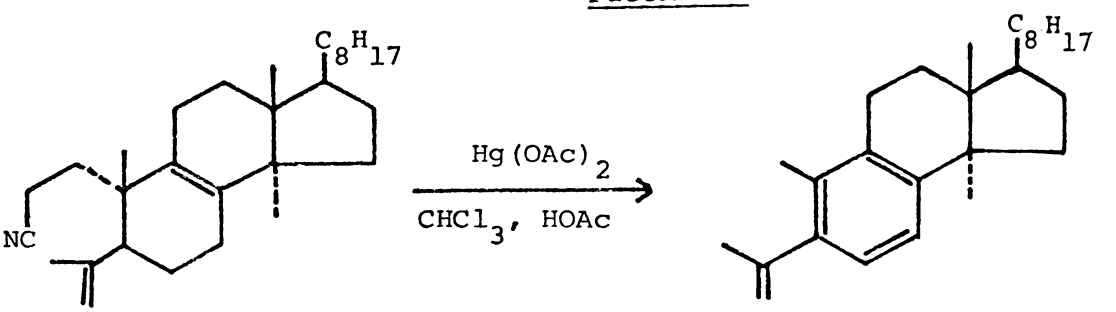
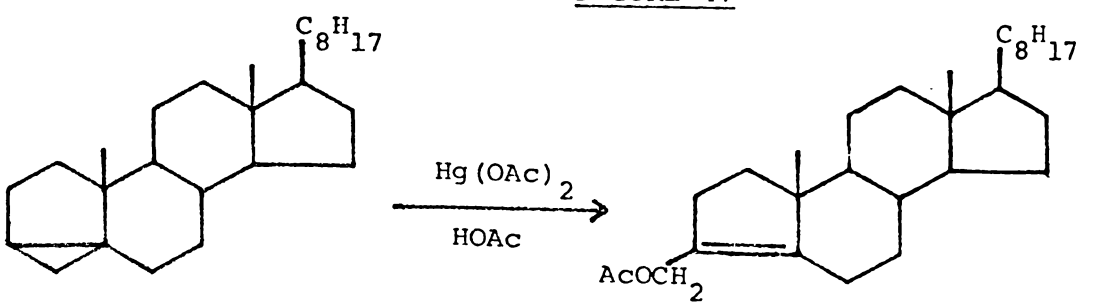


FIGURE 47



Cyclopropane rings, which can be regarded as unsaturated centres also react with mercuric acetate, for example, the oxidation of 3α,5-cyclocholestane[165] is presented in Figure 47. Macchia *et al.*[166] have studied in some detail the effect of solvent and type of Hg (II) salt on the stereochemical result of reaction with arylcyclopropanes.

1'.4 Conclusion

Apart from the review of Arzoumanian and Metzger,[151] it seems that most workers have acknowledged only one or other of the types of mercuric acetate reaction with unsaturated compounds and indeed, oxymercuration has been loosely[141,146] called oxidation. However, this survey has briefly sought to categorise and distinguish between oxymercuration and allylic oxidation by mercuric acetate.

APPENDIX TWO

PRODUCTS OF THE JONES REAGENT OXIDATIONS

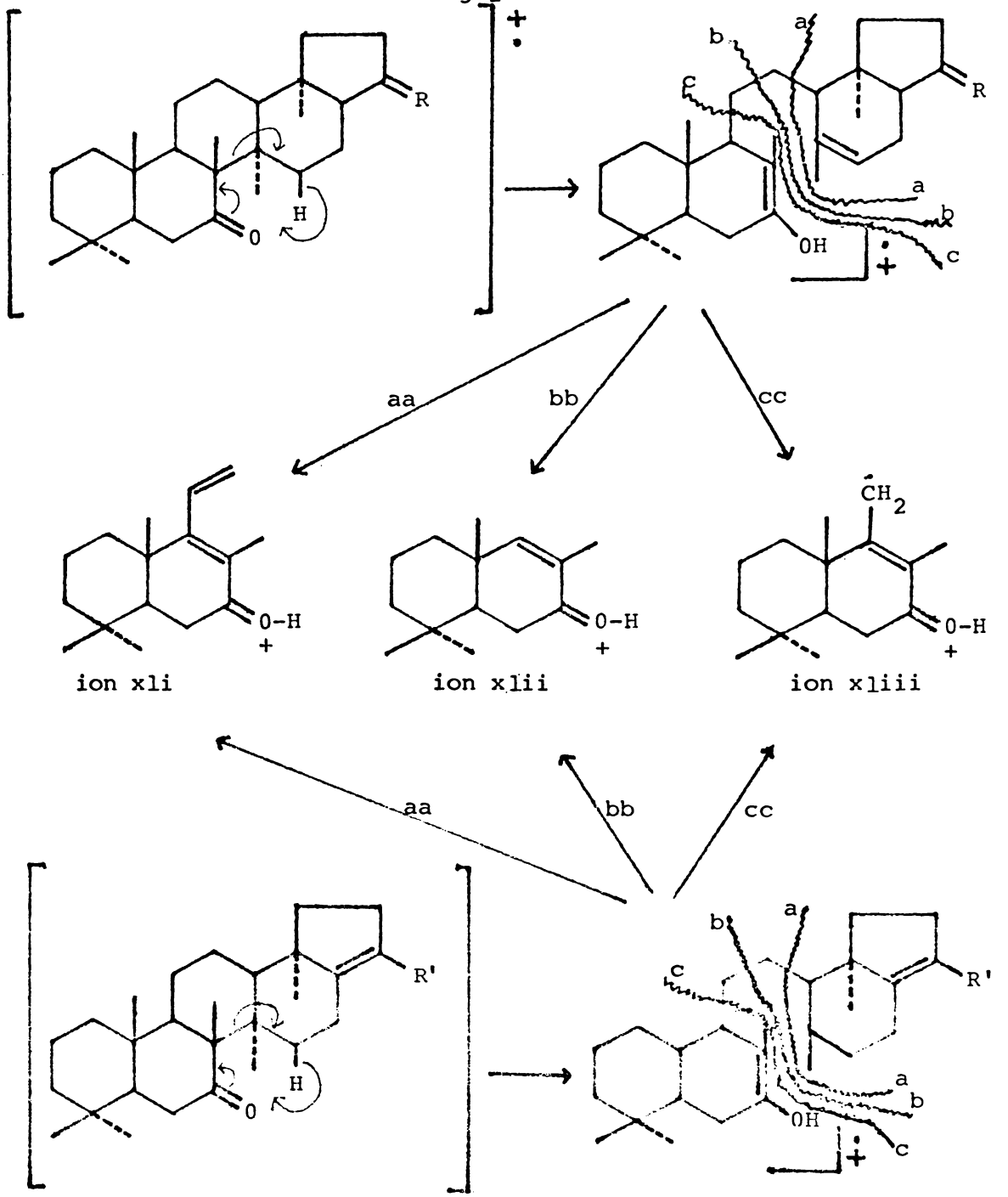
2'.1 The Jones Reagent Oxidation

Jones reagent, which has often[9,12,167] been used to oxidise (within 30 minutes) primary and secondary triterpenoidal alcohols to aldehydes and ketones, respectively, is generally considered to leave tertiary alcohols untouched.[9] However, in the present investigation, hopane-7β,22-diol (65), after 45 minutes in an excess of Jones reagent was incompletely reacted and there was little evidence of 7-oxohopan-22-ol (13) (by t.l.c.). Continuing the reaction for a further six hours resulted in a diminution of starting material yet t.l.c. gave little evidence of any products. A subsequent reaction was halted after 15 minutes reaction time in an effort to trap, if possible, the ketol before it underwent over-oxidation (as it was supposed was occurring). Column chromatography produced fractions containing 7-oxohopan-22-ol (13) (15%) an unidentified material and unchanged starting material.

Hopane-15α,22-diol (59) appeared to react more quickly, all evidence (t.l.c.) of the starting material having disappeared after a reaction period, with Jones reagent, of 20 minutes at room temperature. However, in spite of the shorter reaction time, there was still very little yield of the desired ketol and even performing the reaction at 0^{o} had no noticable effect on the yield.

FIGURE 48

(18) R = :O, (13) R = H, α-C(CH$_3$)$_2$OH, (14) R' = H, (65) R' = CH(CH$_3$)$_2$

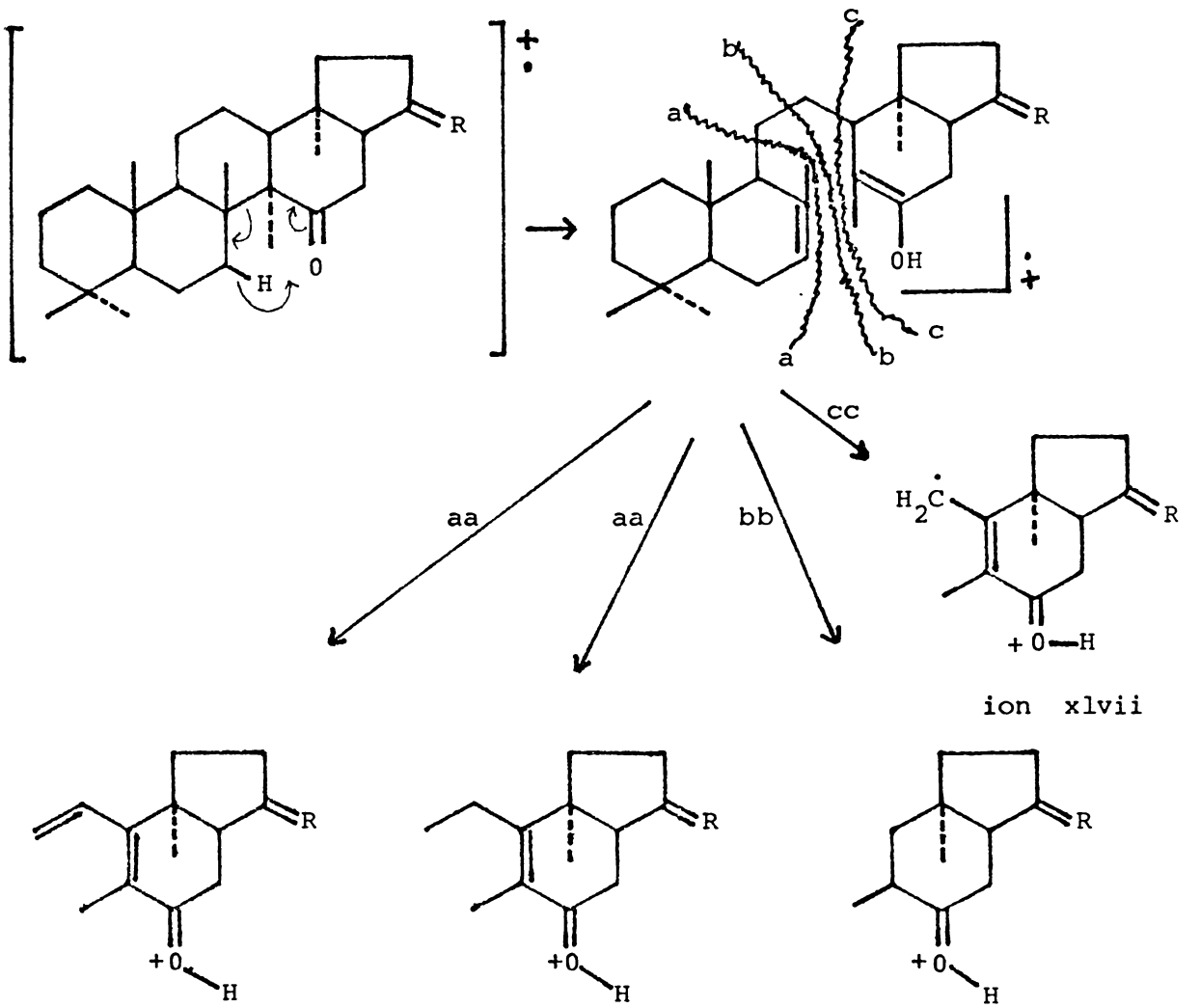


Compound	ion *m/e*	xli %	ion *m/e*	xlii %	ion *m/e*	xliii %
7,21-dione (18)	233	26	207	36	220	73
7-oxo-22-ol (13)	233	29	207	50[a]	220	53
7-oxo-17(21)-ene (65)	233	5	-	-	220	16
7-oxo-trisnor-17(21)-ene (14)	233	30	-	-	220	57

[a]This peak also arises from the ring D/E fragment.

FIGURE 49

(83) R = :O, (58) R = H, α-C(CH$_3$)$_2$OH



ion xlvii

ion xliv ion xlv ion xlvi

Compound	ion xliv *m/e*	%	ion xlv *m/e*	%	ion xlvi *m/e*	%	ion xlvii *m/e*	%
15,21-dione (83)	205	64	207	16	179	36	192	26[a]
15-oxo-22-ol (58)	249	42	-	-	223	6	236	13[b]

[a]This peak also arises from the ring A/B fragment (*m/e* 191) + 1 m.u.

[b]There is also a peak (*m/e* 218, 5%) corresponding to ion xlvii - H_2O.

2'.2 Identification of Products

The identity of the major product of the Jones reagent oxidation of hopane-7β,22-diol (62) was determined by comparison with 7,21-dioxo-22,29,30-trisnor-17αH-hopane (18) derived from 7-oxohopan-22-ol (13) by the hypoiodite reaction (section 1.5). The two products were found to be identical in ^{1}H nmr spectrum, mass spectrum and melting point, indicating that, in addition to the oxidation of the 7β-alcohol function to a ketone, the isopropanol group had been oxidatively removed to be replaced by a doubly-bonded oxygen (section 2'.3).

Since the Jones oxidation product of hopane-15α,22-diol (59) had the same molecular ion (*m/e* 398) as the foregoing dione (18), it was thought to be the analogous 15,21-dioxo-22,29,30-trisnor-17αH-hopane (116), but infrared and ^{1}H nmr spectral evidence suggested [section 2'.2 (ii) and (iii)] that it was 15,21-dioxo-22,29,30-trisnorhopane (83) and that no epimerisation at C-17 had occurred.

(i) Mass spectra

The base peaks of (18) and (83) at *m/e* 205 and 191, respectively, arise from the well-documented[12,15] ring A/B fragments, while a number of the other peaks can be explained in terms of the McLafferty rearrangement (Figures 48 and 49). The tables below the schemes compare equivalent peaks of other 7- or 15-ketones. Although ion xliii is the 7-ketone analogue of ion xlvii yet the former is much more significant in intensity. Conversely, the 15-ketone peak, corresponding to ion xliv is much more intense than its 7-ketone equivalent at *m/e* 233 (ion xli). The hydrogen radical abstraction by the carbonyl group (Figures 48 and 49) is envisaged to occur from the endocyclic 1,4-carbon atom rather than from the

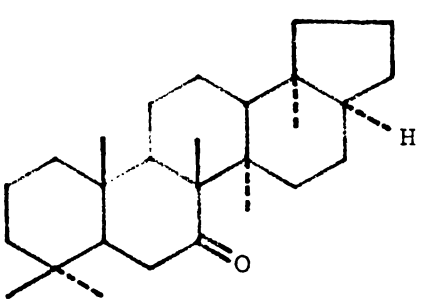
TABLE 29

^{1}H nmr methyl group signals (δ ppm)[a]

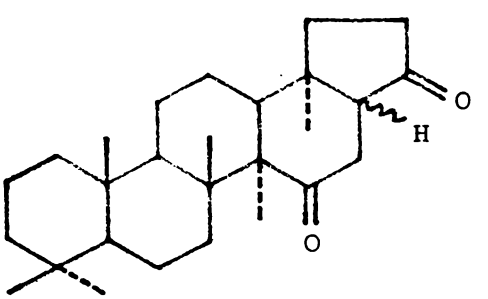
	4α-	4β-	10β-	8β-	14α-	18α-
21-oxo-22,29,30-trisnor-17αH-hopan-7β-ol[b]	0.81	0.81	0.88	0.89	1.15	1.18
- Δ for 7β-OH[c]			-0.09	-0.04	-0.11	-0.02
+ Δ for 15-oxo[c]				0.09	0.24	0.11
∴ Calc. for 15,21-dioxo-22,29,30-trisnor-17αH-hopane (116)	0.81	0.81	0.79	0.94	1.28	1.27
Obs. for 15,21-dione	0.88	0.82	0.82	0.99	1.12	1.16
21-oxo-22,29,30-trisnorhopan-7β-ol[b]	0.84	0.84	0.89	1.03	1.07	0.74
- Δ for 7β-OH[c]			-0.09	-0.04	-0.11	-0.02
+ Δ for 15-oxo[c]				0.09	0.24	0.11
∴ Calc. for 15,21-dioxo-22,29,30-trisnor-hopane (83)	0.84	0.84	0.80	1.08	1.20	0.83
Obs. for 15,21-dione	0.88	0.82	0.82	1.12	1.16	0.99

[a] Incorporates revision after Ageta *et al.* (Ref.19). [b] Ref.15.

[b] Ref. 15. [c] Ref. 14.



(18)



(83) 17βH

(116) 17αH

1,3-axial methyl group as suggested by Corbett and Young.[15] The position of abstraction depends on whether it is proposed to occur prior or subsequent to cleavage of the 8(14)-bond.

(ii) ^{1}H nmr spectra.

The ^{1}H nmr spectrum of 7,21-dioxo-22,29,30-trisnor-17αH-hopane (18) has already been discussed in section 1.5.

In table 29 the methyl group resonances calculated for 15,21-dioxo-22,29,30-trisnor-17αH-hopane (116) and 15,21-dioxo-22,29,30-trisnorhopane (83) are compared with those obtained for the 15,21-dione. In neither case can a comparison establish the structure of the 15,21-dione, although the agreement is better for the 17βH-epimer (83). Possibly the proximity of the carbonyl groups (γ-diketone) has a conformational effect.

(iii) Infrared spectra.

The carbonyl stretching frequency of the 21-ketone of the 7,21-dione (18) (*cf.* 21-oxo-22,29,30-trisnor-17αH-hopan-7β-ol, table 30) confirms that it has isomerised to the 17αH-epimer, while the 21-ketone absorption of the 15,21-dione (*cf.* 21-oxo-22,29,30-hopan-7β-ol, table 30) suggests that it has remained in the 17βH-configuration. The values for the androstane derivatives are included in the table to demonstrate that the shift in the carbonyl stretching frequency, observed in the spectrum

TABLE 30

Carbonyl stretching frequencies

Size of ring	5-ring	6-ring
7-Oxohopan-22-ol (13)		1686
15-Oxohopan-22-ol (58)		1686
7,21-Dioxo-22,29,30-trisnor-17αH-hopane (18)	1724	1690
15,21-Dione	1737	1698
21-Oxo-22,29,30-trisnor-17αH-hopan-7β-ol[a]	1722	
21-Oxo-22,29,30-hopan-7β-ol[a]	1730	
11-Oxoandrostane[b]		1707
17-Oxoandrostane[b]	1742	
11,17-Dioxoandrostane[b]	1747	1713

[a]Ref. 15. [b]Ref. 168.

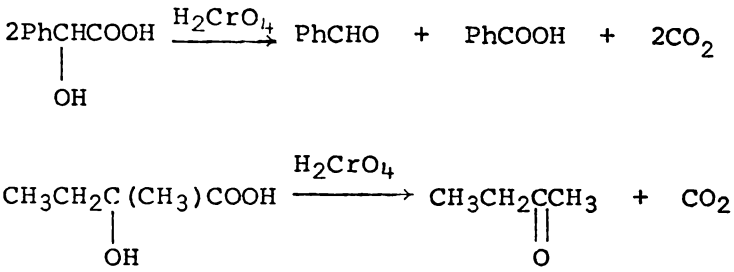
of the 15,21-dione for each ketone group is of the order expected (*ca.* 7 cm^{-1}) for a γ-diketone, assuming no configurational change.

A similar conclusion can be drawn from the region 1050 - 900 cm^{-1} *[see section 4.2 (iv)]*.

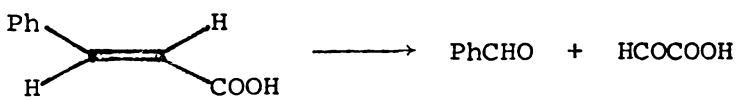
2'.3 Mechanism of Oxidation

An analysis of the products of the Jones reagent oxidation of hopane-7β,22-diol (62) and hopane-15α,22-diol (59) has demonstrated that oxidative cleavage of the isopropanol side chain occurred concurrently with oxidation of the secondary alcohol function. Such cleavage products have

frequently[169,170] been obtained from the chromic acid oxidation of α-hydroxyacids, for example:



Moreover, cyclopropanols[171] and the unsaturated acid *trans*-cinnamic acid[172] have also been reported to yield cleavage products. In contrast to the foregoing, α-hydroxyacids, *trans*-cinnamic acid was not decarboxylated but the double bond was oxidatively cleaved:



Notwithstanding, allylic alcohols[173] have been successfully oxidised to the α,β-unsaturated aldehydes with no evidence of over-oxidation or cleavage products.

The analogous nature of the oxidation products of *trans*-cinnamic acid suggested that, in the case in hand, oxidation proceeded immediately following acid catalysed dehydration to the 21-ene. The cleavage could then be considered to occur *via* the intermediacy of a chromium (v) complex analogous to that proposed by Krumholc and Rocek [170] (Figure 50).

FIGURE 50

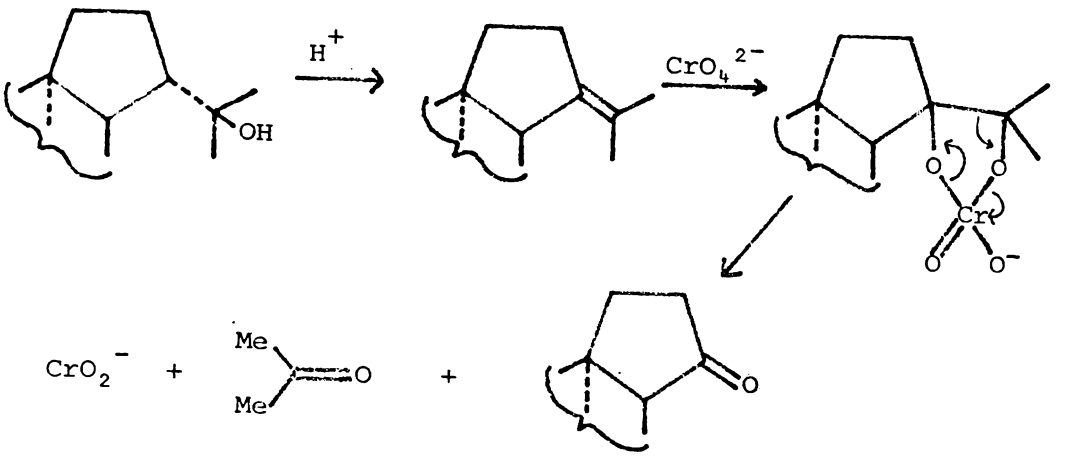
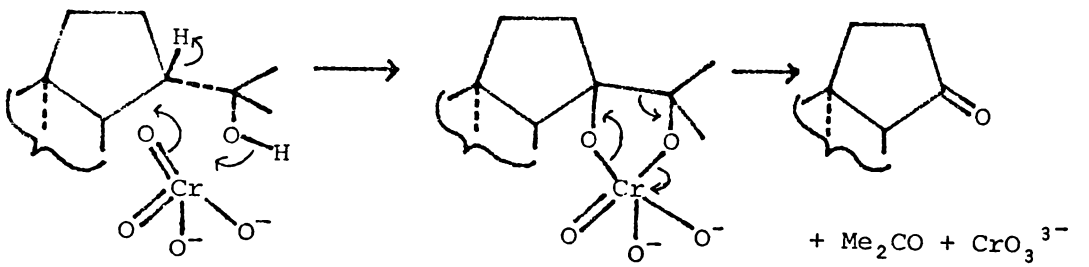


FIGURE 51



Perhaps a more likely mechanism is that suggested by the work of Ip and Rocek[169] and presented in Figure 51. By this process the chromate ion adds directly to the alcohol to form a Cr(VI) complex.

2'.4 Conclusion

Both mass spectrometry and ^{1}H nmr spectroscopy indicated that the products of the Jones reagent oxidations of hopane-7β,22-diol (62) and hopane-15α,22-diol (59) were trisnordiones, the former by the fragmentation pattern and the latter by giving evidence of only 6 methyl groups. Although both ^{1}H nmr and ir indicated that the 7,21-dione had isomerised to the 17αH-epimer, 7,21-dioxo-22,29,30-trisnor-17αH-hopane (18), yet ir suggested that the 15,21-dione had retained the 17βH-configuration, 15,21-dioxo-22,29,30-trisnorhopane (83), while ^{1}H nmr inferred a twisted conformation for rings D and/or E.

In addition a literature comparison demonstrated that the nature of the oxidative cleavage of the isopropanol side-chain was not unprecedented.

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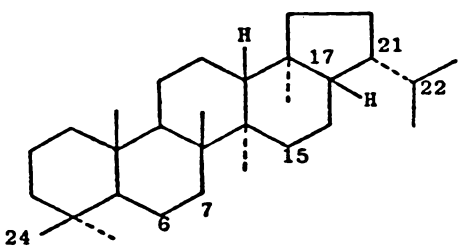
Since, by an analysis of ^{1}H n.m.r. and mass spectral data, it is not always possible[1–4] to assign unambiguously the substitution pattern of an oxygenated hopane triterpenoid (this difficulty arises, in part, from the local C_2 axis of symmetry existing between rings *B* and *D* about ring *C*) a study of the low frequency (900–450 cm^{-1}) i.r. spectra of a series of hopane derivatives variously substituted at the 6-, 7-, 15-, 22-, and 24-positions was undertaken.



Hopane skeleton

It emerged that 7-substitution was characterised by a medium intensity absorption in the region $\nu_{max.}$ 773–781 cm^{-1}, but derivatives possessing a 7β-substituent could be distinguished from those with a 7α-substituent by the additional intensity of the absorption at $\nu_{max.}$ 887 ± 2 cm^{-1} (see Figures 3B and 3C). Although hopane-6α,22-diol also absorbed very strongly at $\nu_{max.}$ 889 cm^{-1}, it was distinguished from the 7β-substituted derivatives by its lack of significant absorption in the region $\nu_{max.}$ 773–781 cm^{-1} and by the increased intensities of the absorptions at $\nu_{max.}$ 850 and 828 cm^{-1} (see Figure 3A).

Intense absorption in the $\nu_{max.}$ 640–500 cm^{-1} region of the spectra of the oxo-derivatives appeared to correspond to absorptions attributed by Weinmann[5] to the in-plane vibrations of the oxo-group of 20-oxo-steroids. Similarly the acetoxy absorptions ($\nu_{max.}$ 670–600 cm^{-1}) observed by Weinmann and Weinmann[6] were also present in the spectra of the acetoxyhopane derivatives, but were more numerous and of lower frequency.

The correlations obtained in this work were successfully applied to the identification of two triol derivatives of hopane, which had uncertain substitution patterns. The first triol was originally considered[7] to be hopane-6α,7α,22-triol, but its low frequency i.r. spectrum, particularly in the region 900–800 cm^{-1} (see Figure 3F), suggested that it was hopane-6α,7β,22-triol. This assignment has since been confirmed.[3] The second triol had an absorption pattern almost identical with that of hopane-15α,22-diol in the definitive region 900–800 cm^{-1} (see Figure 3E). Since this triol had already been shown to be substituted at the 24-position it was considered to be hopane-15α,22,24-triol. The latter assignment was confirmed when the 15α,22,24-triol was degraded[4] to the 15α,22-diol.

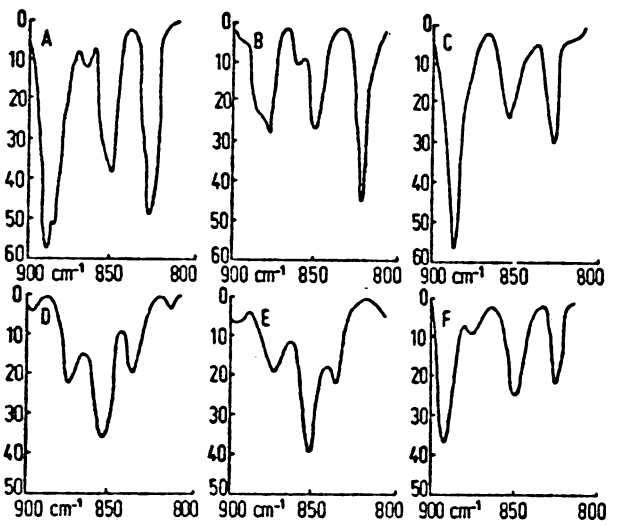


Figure 3 The i.r. spectra (900–800 cm^{-1}) of hopane-diols and -triols; the ordinate scale is percent absorbance relative to the δ(C–H) band at $\nu_{max.}$ *ca.* 1460 cm^{-1}
A: Hopane-6α,22-diol B: Hopane-7α,22-diol
C: Hopane-7β,22-diol D: Hopane-15α,22-diol
E: Hopane-15α,22,24-triol F: Hopane-6α,7β,22-triol

Techniques used: I.r., mass spec.

Tables 1–3: The low frequency (900–450 cm^{-1}) i.r. absorptions of various hopane derivatives

Figure 1: C_2 Rotation of a 7β-substituted hopane derivative about ring *C*
Figure 2: The i.r. spectra (1050–900 cm^{-1}) of the four isomeric 17ξ,21ξ-hopanes

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[2] R. E. Corbett and H. Young, *J. Chem. Soc. (C)*, 1966, 1564.
[3] R. E. Corbett and A. L. Wilkins, *Aust. J. Chem.*, 1977, 30, 2329.
[4] K. J. Ronaldson and A. L. Wilkins, *Aust. J. Chem.*, 1978, 31, 215.
[5] S. Weinmann, *C.R. Acad. Sci.*, 1962, 255, 2072.
[6] S. Weinmann and J. Weinmann, *C.R. Acad. Sci.*, 1963, 256, 2578.
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APPENDIX THREE

THE LOW FREQUENCY INFRARED SPECTRA OF SOME HOPANE TRITERPENOIDS

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ABSTRACT

Hopane triterpenoids identically substituted at the 7β- or 15α-position, which cannot be readily distinguished by ^{1}H n.m.r. spectroscopy or mass spectrometry, have been found to produce distinctive skeletal absorption patterns in the infrared spectrum. Accordingly a study of the low frequency (900 - 450 cm^{-1}) spectra of various 6-, 7-, 15-, and 22-substituted hopane triterpenoids has been undertaken, the correlations obtained being successfully applied to the determination of the substitution position of several trisubstituted hopane derivatives.

INTRODUCTION

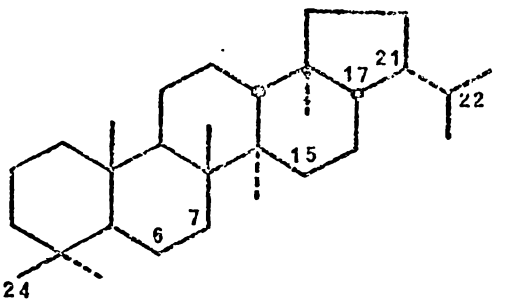
Whilst mass spectrometry is an effective means of distinguishing between hopane-7β,22-diol[1] (10) [*m/e* 444(M^+), 207 and 189], and hopane-15α,22-diol[2] (16) [*m/e* 444(M^+), 223, 205, 191 and 187], it fails to differentiate between hopane-6α,7β,22-triol[3] (22), and hopane-15α,22,24-triol[4] (23), each of these triols possessing mass spectral fragments of *m/e* 460(M^+), 442, 207, 205 and 189. Similarly by ^{1}H n.m.r. spectroscopy it is difficult to distinguish between a hopane triterpenoid substituted at the 7β-position, and one equivalently substituted at the 15α-position since in hopane (1) as also in hopan-22-ol (5) the 8β-and 14α-methyl groups have essentially identical resonance positions[1,2] and the substituent group effects associated with 7β- and 15α-substituents are such that the resonance positions of the 8β- and 14α-methyl groups are interchanged. This reversal of resonance position arises as a consequence of the local C_2 axis of symmetry existing about ring C (see figure 1). However, because a similar relationship does not exist between rings A and E, which, in particular, differ in ring size and hence steric strain, it was reasoned that

1. R.E.Corbett & H.Young, *J.Chem.Soc.(C)*, 1966, 1556.

2. R.E.Corbett & H.Young, *J.Chem.Soc.(C)*, 1966, 1564.

3. R.E.Corbett & A.L.Wilkins, *Austral.J.Chem.*, 1977, 30, 2329.

4. K.J.Ronaldson & A.L.Wilkins, *Austral.J.Chem.*, 1978, 31, 215.



1. Hopane
2. 21α-Hopane
3. 17α-Hopane
4. 17α,21α-Hopane
5. Hopan-22-ol
6. Hopan-7β-ol
7. Hopan-7α-ol
8. Hopan-15α-ol
9. Hopan-15β-ol
10. Hopane-7β,22-diol
11. 7β-Acetoxyhopan-22-ol
12. 7β-Trideuteroacetoxyhopan-22-ol
13. Hopane-7α,22-diol
14. 22-Acetoxyhopan-7α-ol
15. 7-Oxohopan-22-ol
16. Hopane-15α,22-diol
17. 15α-Acetoxyhopan-22-ol
18. 15α-Trideuteroacetoxyhopan-22-ol
19. 15-Oxohopan-22-ol
20. Hopane-6α,22-diol
21. 6-Oxohopan-22-ol
22. Hopane-6α,7β,22-triol
23. Hopane-15α,22,24-triol
24. 15α-Acetoxy-22-hydroxyhopan-24-oic acid
25. Methyl 15α-acetoxy-22-hydroxyhopan-24-oate
26. 15α,24-Diacetoxyhopan-22-ol
27. 15α,22-Dihydroxyhopan-24-yl tosylate

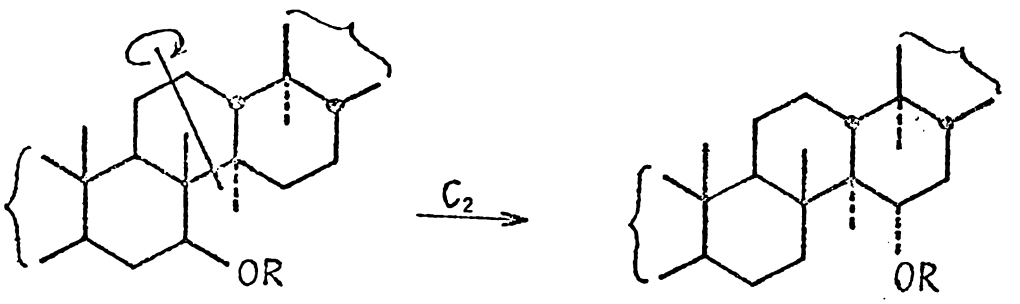


FIGURE 1: R = H or Ac.

the skeletal vibrations of a hopane triterpenoid substituted in ring B would differ from those of one equivalently substituted in ring D, and these differences might serve to distinguish derivatives possessing confusingly similar ^1H n.m.r. and/or mass spectral characteristics. Accordingly the infrared spectral features reported in this paper were investigated.

RESULTS AND DISCUSSION

In each of the twenty seven hopane triterpenoids investigated in this study the relatively intense C-H, C=O, C-O and O-H stretching and bending vibrations, absorbing in the region $\nu_{max.}$ 4000 - 900 cm^{-1}, were found to be useful only in confirming the presence of a particular functional group (*viz.* hydroxyl, acetoxyl functions *etc.*). By contrast however the skeletal absorption patterns in the region below $\nu_{max.}$ 900 cm^{-1} (1050 cm^{-1} in the case of the isomeric hydrocarbons) were found to be sensitive not only

to the type of substituent group, but also its location within the hopane skeleton. Since, in steroids and triterpenoids, skeletal vibrations often couple to the extent of embracing the whole molecule, it was not possible, except in the case of certain acetate and ketone vibrations, to precisely assign the vibrations. Nevertheless some structurally significant relationships were empirically determined, particularly in respect to the pattern of a series of re-occurring skeletal absorptions in the region $\nu_{max.}$ 900 - 450 cm^{-1}. To facilitate comparisons between compounds the intensities of these absorptions were assessed relative to the percentage absorbance of the intense δC-H band occurring at *c.* $\nu_{max.}$ 1460 cm^{-1} in the respective substances (see Tables 1-3 and Figures 2 and 3).

(a) Hydrocarbons and Monosubstituted Derivatives

Each of the four isomeric 17ξ,21ξ-hydrocarbons studied was found to possess a series of related skeletal absorptions below $\nu_{max.}$ 900 cm^{-1}. (see Table 1), the more intense of these absorptions occurring at *c.* $\nu_{max.}$ 854 and 610 cm^{-1}. In the isomeric hydrocarbons, but not the substituted compounds, the region $\nu_{max.}$ 1050 - 900 cm^{-1} also possesses structural significance. In this region hopane (1) and 21α-hopane (2) have their most intense absorptions at $\nu_{max.}$ 988 and 985 cm^{-1} respectively, and they also absorb strongly at $\nu_{max.}$ 1032 ± 1 cm^{-1} (see Figure 2). However the most intense absorptions of 17α-hopane (3) and 17α,21α-hopane (4) occur at the lower frequencies

TABLE 1: The low frequency (900 - 450 cm^{-1}) infrared absorptions of the isomeric hopanes and mono-alcohol derivatives of hopane[+].

1	2	3	4	5	6	7	8	9
893w	893w	894vw	892w	890vw	893s	895w		
		880w				890w	888m	
870w				876vw	871w	867w	870w	866m
855s	853s*	853m	854m*	855m	857s	857m	853m	852m
844vw	833m	847w		845vw	845w	843w	837w	
		840w		827s*			822m	
815w	818w	816w	817w	820m	817m	822w	803m	816w
800w	799w	784vw	793w		801w	800w	785w	803w
771w	770vw	770vw	774-1w	771vw	777s	776m	770m	769s
751vw	757-9w	740m	745w	759vw	748w	753w	748m	747vw
730w	731w	730w	730w	729w	729s	742m	739s	732w
				726w	722s	730w	729w	
720vw				713w			720m	
					694m	699m	696w	693m
		650w	645w		654m	646w		674vw
		632-0w			633w	631w		630w
					625vw			
611s*	618m	615m*	604m	607m	603vs*	602vs*	614vs*	606vs *
560w	571m	553w	555w		556vw	553m	575m	559s
546w					552vw	533w	545m	
531w							528m	
513w						517w	525m	510m
						500w	501m	500m

* Strongest absorption.

+ The intensities of the absorptions have been standardised relative to the δ C-H band at *c.* $\nu_{max.}$ 1460 cm^{-1} such that; vs>30%, 30%>s>20%, 20%>m>10%, 10%>w>3% and 3%>vw>o, while sh = shoulder peak.

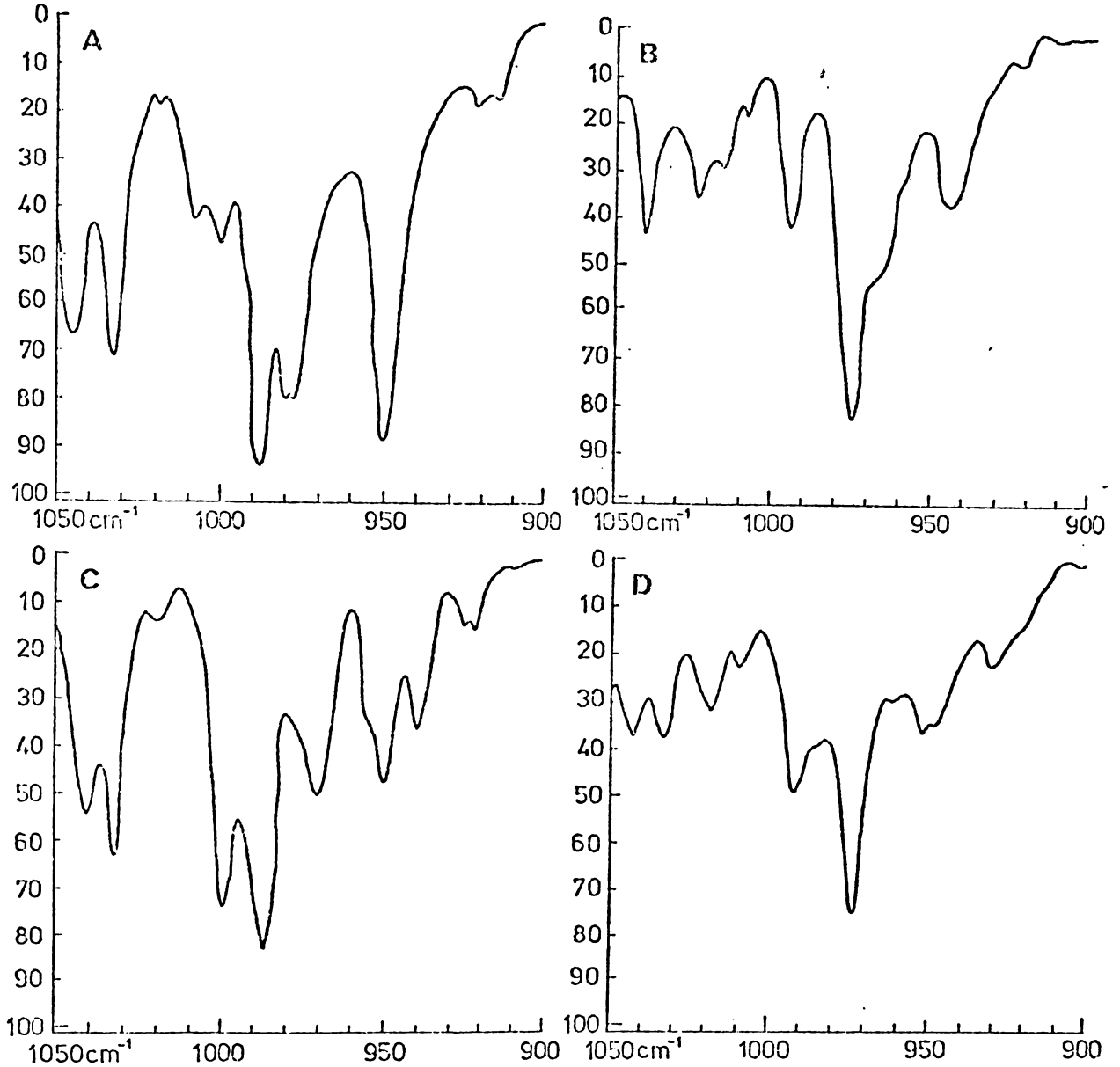


FIGURE 2: The infrared spectra (1050 - 900 cm^{-1}) of the four isomeric hydrocarbons. The ordinate scale is percent absorbance relative to the δC-H band *c.* $\nu_{max.}$ 1460 cm^{-1}.

A. Hopane (1). B. 17α-Hopane (3).

C. 21α-Hopane (2). D. 17α,21α-Hopane (4).

of $\nu_{max.}$ 973 and 971 cm^{-1} respectively, and only weak absorptions are to be found in the vicinity of $\nu_{max.}$ 1030 cm^{-1}.

In oxygenated derivatives a similar analysis of the region $\nu_{max.}$ 1100 - 900 cm^{-1} is complicated by the presence of additional δC-O vibrations which, because of their greater intensity invariably mask the less intense skeletal vibrations. However, below $\nu_{max.}$ 900 cm^{-1} the spectrum is less complex, so that most of the absorptions of hopane (1) can also be located in the spectra of the 7α-, 7β-, 15α-, 15β- and 22-alcohols (see Table 1). In each of these mono-ols the absorptions occurring at $\nu_{max.}$ 854 ± 3 and 610 ± 8 cm^{-1} retained their intensity whilst, with the exception of hopan-22-ol (5), the absorption at $\nu_{max.}$ 773 ± 4 cm^{-1} was of increased intensity. Additional absorptions were also present at $\nu_{max.}$ 827 cm^{-1} in hopan-22-ol (5); at $\nu_{max.}$ 722 cm^{-1} in hopan-7β-ol (6); and at $\nu_{max.}$ 559 cm^{-1} in hopan-15β-ol (9), whilst several of the re-occurring absorptions [*e.g.* those appearing at $\nu_{max.}$ 777 and 729 cm^{-1} in hopan-7β-ol (6)] were of increased intensity in some of the mono-ols.

(b) Disubstituted Derivatives

The majority of the skeletal absorptions occurring in the low frequency infrared spectra of the hydrocarbons and mono-substituted derivatives can also be located in the infrared spectra of the disubstituted derivatives. Those possessing a C-7 substituent are characterized by a medium intensity absorption occurring in the region

$\nu_{max.}$ 773 - 781 cm^{-1}, whereas the C-6 and C-15 substituted derivatives absorb weakly, if at all, in this region. The variations in the shape and relative intensity of the medium to strong absorptions in the region $\nu_{max.}$ 900 - 800 cm^{-1} of the spectra of the 6α,22-, 7α,22-, 7β,22- and 15α,22-disubstituted derivatives (see Figure 3 and Table 2) are such that they can be profitably correlated with substitution pattern. In particular hopane-7β,22-diol (10) and its 7-acetate (11) absorb very strongly at $\nu_{max.}$ 886-8 cm^{-1}, but to a lesser extent at $\nu_{max.}$ 854-5 and 831-29 cm^{-1}, whereas the equivalently substituted 15α,22-derivatives (16) and (17) absorb strongly at $\nu_{max.}$ 853-6 cm^{-1}, albeit to a lesser extent at $\nu_{max.}$ 873 and 836-8 cm^{-1}. The intensity of the absorption occurring at $\nu_{max.}$ 886 - 888 cm^{-1} also serves to distinguish 7β-substitution from 7α-substitution for this absorption is of reduced intensity in the 7α-alcohol (7) and 7α,22-diol (13). Although hopane-6α,22-diol (20) also absorbs strongly at $\nu_{max.}$ 889 cm^{-1} it is adequately distinguished from the 7β,22-diol (10) by its shoulder peak ($\nu_{max.}$ 884 cm^{-1}), together with the increased intensity of the absorptions at $\nu_{max.}$ 850 and 828 cm^{-1}, and another weak absorption at $\nu_{max.}$ 864 cm^{-1}. Additionally the medium intensity absorption occurring at *c.* $\nu_{max.}$ 777 ± 4 cm^{-1} in all of the C-7 substituted derivatives is absent from the 6α,22-diol (20).

Intense absorptions occurring in the region $\nu_{max.}$ 660 - 580 cm^{-1} of the infrared spectra of

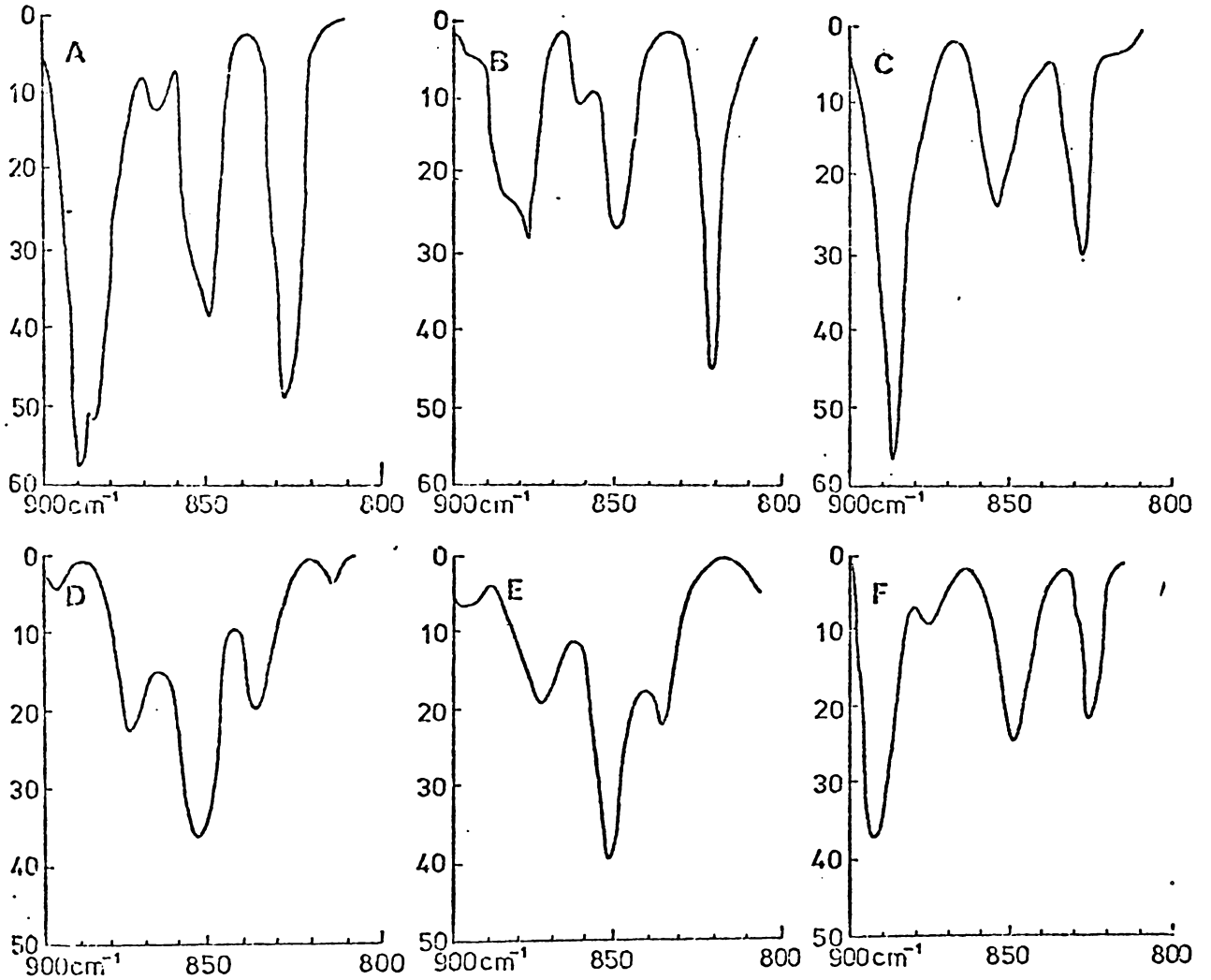


FIGURE 3: The infrared spectra (900 - 800 cm^{-1}) of the diols and triols. The ordinate scale is percent absorbance relative to the δC-H band *c.* $\nu_{max.}$ 1460 cm^{-1}.

A. Hopane-6α,22-diol (20).
B. Hopane-7α,22-diol (13).
C. Hopane-7β,22-diol (10).
D. Hopane-15α,22-diol (16).
E. Hopane-15α,22,24-triol (23).
F. Hopane-6α,7β,22-triol (22).

TABLE 2: The low frequency (900 - 450 cm^{-1}) infrared absorptions of the disubstituted hopane derivatives[+].

10	11	12	13	14	15
886vs*	888vs	890vs	886sh	895w	891m
			880s	870m	
	862w	866w	863-0w		856sh
855m	854w	855w	852s	852vw	845sh
				843w	839s
829s	831s	831vs	824vs*	836w	827m
814vw		813m	802vw	809w	
798vw		802vw	796vw	790s	798w
779m	781m	781m	777m	778m	773m
750vw		761-57m	758w	754m	741w
728m	729m	729m	752m	738w	726m
725m				727w	710m
713w			716-3w	717vw	693-84w
661w	647m		700w	700w	649m
622s	624vs	620vs		654w	632vs
615s	610s	611m		648w	610w
	601m	603w	605w	606vs*	599w
581w		580m			587vs
558w	562m	563s	553w		567w
542w		536vs		544w	529s
522w			525-19w	516m	516s
506w	509vs*†	510vs*†		511m	510vs*

+ See note below Table 1 regarding intensities.

* Strongest absorption. † Broad.

TABLE 2 Cont.

16	17	18	19	20	21
896vw	898m	895s		889vs*	
	886w		889s	884vs	884m
873s	873w	873sh	877w		879m
		865vs		864vw	
853vs*	856vs	855vs	855m	850s	854vs*
838s	836m	837m			
817w		823m	820m	828vs	822vw
804w	819w	818m	815m		
786w	802w	802w	804w	785w	791vw
771vw	773w		774vw	774vw	771w
760w	745m	744m	748w	751m	749w
730m	730m	729m	728vw		736w
725m			710w		
694vw	719w	720-17w	690vw	718vw	711w
	685w	663w	655m		681w
630w	639s	627vw	634vw		632s
611m	617vs*	612vs	610m	610w	619w
	606vs	600w			
575w	572vs	578vs*	585vw	591m	587vw
561w	560vw		560m	549w	559m
538w	538w	524w	550s*	525w	
			539m	515w	
	502s	501m	520-15m		
	485vs	467s	493w		

+ See note below Table 1 regarding intensities.

* Strongest absorption. † Broad.

20-oxosteroids have been attributed by Weinmann[5] to an in plane vibration of the keto-group. The strong absorptions which in the 6-oxo-, 7-oxo- and 15-oxo-derivatives, (21), (15) and (19) respectively, occur in the region $\nu_{max.}$ 640 - 500 cm^{-1} appear to have similar origins. The absorption patterns, which, in the region $\nu_{max.}$ 900 - 800 cm^{-1}, characterize 6α-, 7α-, 7β- and 15α-substitution respectively (see Table 2 and Figure 3) are modified in the foregoing oxo-derivatives. This difference in the skeletal vibrations may be associated with the additional steric strain introduced into rings B or D by an sp^2 hydridized carbon atom.

(c) Acetoxyl Absorptions in the Region $\nu_{max.}$ 650 - 450 cm^{-1}

Weinmann and Weinmann[6] have associated two relatively intense absorptions of acetoxyl steroids in the region $\nu_{max.}$ 670 - 600 cm^{-1} with vibrations of the carbonyl segment of the acetoxyl group. They observed that the more intense of the absorptions (that which occurred at greater wave number), was configuration dependent ($\nu_{max.}$ 670 - 660 cm^{-1} for equatorial acetoxyl groups, $\nu_{max.}$ 640 - 620 cm^{-1} for axial acetoxyl groups) whereas the other absorption located at $\nu_{max.}$ 613 ± 5 cm^{-1} was relatively constant in both its shape and position. Both of the 7β- and 15α-acetates (11) and (17) respectively were found to absorb strongly in the regions $\nu_{max.}$ 624 - 617 cm^{-1} and $\nu_{max.}$ 510 - 485 cm^{-1}. That, in the hopane skeleton, the configuration dependent absorption

5. S. Weinmann, *Compt. Rend.*, 1962, 255, 2072.

6. S. Weinmann & J. Weinmann, *Compt. Rend.*, 1963, 256, 2578.

of the equatorial 7β- and 15α-acetates occur at frequencies which in the steroid examples cited by Weinmann would be associated with an axial acetoxyl group can be attributed to the highly hindered nature of the 7β- and 15α-positions. Attempts to prepare axially substituted 7β- and 15α-acetates were unsuccessful. For example reaction of the 7α,22-diol (13), even under the most forcing conditions[1], resulted only in acetylation at the tertiary 22-position, rather than the highly hindered secondary 7α-position. The resultant 22-acetate (14) possesses only a single strong (configuration independent) absorption, at $\nu_{max.}$ 606 cm^{-1}, in the region $\nu_{max.}$ 640 - 450 cm^{-1}, compared with the larger number for the 7β- and 15α-acetates, where the acetoxyl group is attached to a ring carbon atom.

Significantly, in the 7β- and 15α-trideuteroacetates (12) and (18) the configuration dependent absorptions of the respective acetoxyl groups, occur at $\nu_{max.}$ 620 and 612 cm^{-1} respectively (reductions of 4 and 5 cm^{-1} respectively) whereas the majority of the other absorptions above $\nu_{max.}$ 650 cm^{-1} were not sensitive to the increased mass of the deuterated acetoxyl substituents. In the region below $\nu_{max.}$ 650 cm^{-1} the absorptions of the 15α-acetate (17) appear to have been shifted to a lower wavenumber in the 15α-trideuteroacetate (18) (*viz.* the absorptions at $\nu_{max.}$ 606, 538 and 485 cm^{-1}), whilst that at $\nu_{max.}$ 572 cm^{-1} appears to have been shifted to a higher frequency.

(d) Trisubstituted Derivatives

The utility of the foregoing spectral correlations was exemplified in the structural elucidation of two hopane-triols. The skeletal absorptions in the region $\nu_{max.}$ 900 - 800 cm^{-1} of a triol isolated from *Pseudocyphellaria mougeotiana* and considered[7] to be hopane-6α,7α,22-triol, are depicted in Figure 3f. Notwithstanding the location of two hydroxyl groups on adjacent carbons and the possibility that the skeletal vibrations of the triol could be related in a non-additive fashion to those of other hopane triterpenoids substituted in ring B at the 6- or 7-positions it was apparent that the infrared spectral features of the triol were more in keeping with 6α,7β,22-substitution than 6α,7α,22-substitution. It was this observation which prompted the recently reported[3] re-investigation of the *P. mougeotiana* extractives in which 6α,7β,22-substitution was confirmed.

Similarly the skeletal absorptions appearing in the infrared spectra of amphistictinic acid* (24) and its derivatives (23), (25), (26) and (27) also served to elucidate their structure. For example, reduction of methyl amphistictin (25) afforded a triol which was established from an analysis of ^1H n.m.r. and mass spectral data to be either hopane-7β,22,24-triol or hopane-15α,22,24-triol. Skeletal absorptions in the region $\nu_{max.}$ 900 - 800 cm^{-1} (see Figure 3) clearly

7. R.E. Corbett & S.D. Cumming, *J.Chem.Soc. (C)*, 1971, 955.

TABLE 3: The low frequency (900 - 450 cm^{-1}) infrared absorptions of the trisubstituted hopane derivatives[+].

22	23	24	25	26	27
892vs*	897vw				895vw
875vw	875m	878w	872w	880m	877m
852m	855vs*	860s	859vs	861vs	854vs
		855sh		853m	
	840m	843vw	837w	835m	845vs
829m	810-8w	823vw	820s	823w	818vs
790w	792vw	795s	810m		794vw
772-3m	770-60w		775vs	777m	774vw
		757vs*	755w	766m	
	724s	732sh	743m	750w	724m†
702w			730m	730w	705m
				721vw	685m
		667m	678vs*	674w	673vs
643m		647m	642m	646s	663sh
				636sh	
621m		625m	627m	631vs*	635m
619m	614m			617m	609-15w
		610vs	610vs	610s	
603m		588w	599sh	603vs	599s
	574w	572w	570m	577m	576m
		531m	528w		563s
543-38w	538w	527m	513w		554vs*
515w		486m	484m	485s	500m

* Strongest absorption. † Broad.

[+] See note below Table 1 regarding intensities.

differentiated in favour of 15α- rather than 7β-substitution, and this was subsequently confirmed by the degradation of the triol[4] to hopane-15α,22-diol (16).

In accord with 15-substitution, each of the other derivatives prepared from amphistictinic acid absorbed strongly at *c.* $\nu_{max.}$ 857 ± 4 cm^{-1}. Additional absorptions occurring at $\nu_{max.}$ 795 and 757 cm^{-1} in the acid (24), at $\nu_{max.}$ 775 and 678 cm^{-1} in the methyl ester (25), and at $\nu_{max.}$ 631 cm^{-1} in the diacetate (26), appear to be associated with the respective C-24 substituents. In the case of the tosylate (27) absorptions attributable to a *para*-substituted aryl ring system (*viz.* those occurring at $\nu_{max.}$ 563 and 554 cm^{-1}) also appear in Table 3.

CONCLUSION

The skeletal absorption pattern summarized in the Tables appears to be uniquely characteristic of the hopane skeleton, for differing absorption patterns are to be found in other groups of triterpenoids. The utility of the data in the structural elucidation of polyfunctional derivatives, especially in circumstances where ^1H n.m.r. and mass spectral data is not structurally definitive, has been clearly established.

EXPERIMENTAL

Infrared spectra were determined as KBr discs (*c.* 2.5 mg triterpenoid/50 mg KBr) on a Perkin-Elmer 180 spectrometer. Spectra in the region $\nu_{max.}$ 900 - 450 cm^{-1}

were recorded with an abscissa expansion of 5 and with an increased ordinate expansion. Calibration and reproducibility was demonstrated to be better than $\pm 0.5\ cm^{-1}$.

Preparation of trideuteroacetates - Solutions of the 7β,22- and 15α,22-diols (10) (300 mg) and (16) (300 mg) respectively in pyridine (4 ml) were stirred with acetic anhydride-D_6 (0.5 ml) for 96 hr at 20°. Work up and purification by p.l.c. on silica gel with ether-hexane (3:2) gave the trideuteroacetates (12) (200 mg) and (18) (190 mg) respectively.

7β-Trideuteroacetoxyhopan-22-ol (12) had *m/e* 489 (M^+), 471, 456, 408, 393, 368, 207 and 189.

15α-Trideuteroacetoxyhopan-22-ol (18) had *m/e* 489 (M^+), 471, 427, 408, 393, 368, 204, 191 and 187.

We wish to thank Prof. R.E. Corbett for providing some of the samples and the University of Waikato for granting a postgraduate study award (to K.J.R.)

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APPENDIX FOUR

Galley-proof

DEPSIDONE CONSTITUENTS FROM THE QUINTARIA GROUP OF *NEPHROMA* SPECIES

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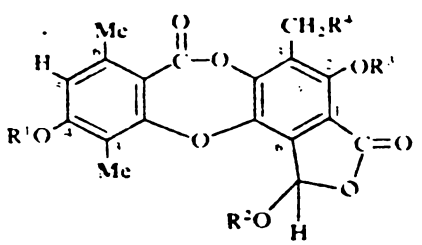
(*Revised received* 6 *August* 1980)

Key Word Index *Nephroma antarcticum*; *N. australe*; Nephromataceae; lichens; β-orcinol depsidones; hypostictic acid; hyposalazinic acid; hypoconstictic acid.

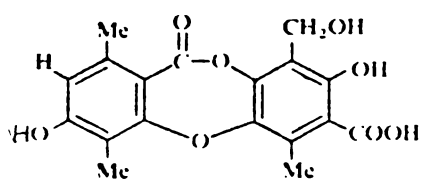
Abstract A new lichen depsidone was isolated, in the form of its triacetate derivative from the acetylated extracts of *Nephroma antarcticum* and has been demonstrated to be hypoconstictic acid-triacetate. Two related depsidones, hypostictic acid and hyposalazinic acid, were isolated from *N. australe*.

INTRODUCTION

Hale [1, 2] has reported the presence in collections of *Xanthoparmelia quintaria*, *Pseudoparmelia neoquintaria*, *Relicina abstrusa*, and some brown *Parmelia* species, of four substances which Culberson [3] designated PQ-1, PQ-2, PQ-3 and PQ-4. Subsequently Keogh [4] reported some of these substances in a new species of *Thelotrema*, and showed PQ-1 and PQ-2 to be hypostictic acid (**1a**) and hyposalazinic acid [5] (**1b**), respectively. More recently Culberson has intimated to us in a personal communication that another of these substances (PQ-3) is identical with hypoprotocetraric acid (2), and that the structure of PQ-4 has not yet been defined.



1a R^1 = Me, R^2 = R^3 = R^4 = H
1b R^1 = R^2 = R^3 = R^4 = H
1c R^1 = Me, R^2 = R^3 = H, R^4 = OH
1d R^1 = Me, R^2 = R^3 = Ac, R^4 = H
1e R^1 = R^2 = R^3 = Ac, R^4 = H
1f R^1 = Me, R^2 = R^3 = Ac, R^4 = OAc
1g R^1 = Me, R^2 = R^3 = CD_3CO, R^4 = H



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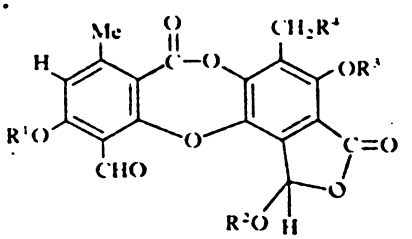


3a R^1 = Me, R^2 = R^3 = R^4 = H
3b R^1 = R^2 = R^3 = R^4 = H
3c R^1 = Me, R^2 = R^3 = H, R^4 = OH

RESULTS AND DISCUSSION

In the course of a chemotaxonomic survey of the genus *Nephroma* two of us (P. W. J. and A. L. W.) noted the presence of some of the foregoing substances in one of the two chemical races of *N. australe* (PQ-1 and PQ-2), and in *N. lobuligerum* and *N. antarcticum* (PQ-1 and PQ-4). Since the most polar of these substances (PQ-4) could not be satisfactorily isolated directly from the acetone extracts of *N. antarcticum*, which also contained hypostictic acid (**1a**), stictic acid (**3a**), constictic acid (**3c**), and traces of hyposalazinic acid (**1b**) and norstictic acid (**3b**), the extracts were acetylated and the major constituents were isolated as the corresponding acetates. By this means a modest quantity of PQ-4 triacetate was secured, and we now report spectral and synthetic correlations which reveal PQ-4-triacetate to be the 3-methyl analogue of constictic acid-triacetate. The designation hypoconstictic acid-triacetate is proposed for this compound.

A preliminary examination of the ^{1}H NMR spectra determined for hypostictic acid-diacetate (**1d**) and PQ-4-triacetate suggested the latter substance to differ from the former only to the extent that an aryl Me group signal at δ 2.32 had been replaced by aryl acetoxymethyl group signals at δ 5.30 (CH_2OAc, AB*q*) and 1.97 (CH_2OAc). This conclusion was also supported by the molecular formulations ($C_{23}H_{20}O_{10}$ and $C_{25}H_{22}O_{12}$) established for the respective substances, and by the presence, in the

mass spectra of the respective acetates, of fragment ions corresponding to the loss of up to two and three acetoxyl groups respectively. In each case the acetoxyl groups were lost mainly as ketene entities.

A total of five aryl Me group and acetate Me group signals appear in the ^{1}H NMR spectrum of hypostictic acid-diacetate (**1d**). On expansion, these signals were found to be of unequal half-band width and height. That the tallest and sharpest of these signals (δ 2.24 and 2.44) originated from the two acetoxyl groups was established by their absence from the ^{1}H NMR spectrum of the corresponding $^2D_6$-diacetate (**1g**).

Jackman *et al.* [6], and others [7, 8], have demonstrated that in depsidones such as granulatin and physciosporin a long range coupling of *ca* J = 0.5 Hz exists between a pair of aryl Me groups which are located *para* with respect to each other. Additionally, an *ortho*-aryl proton also couples [6] with one of the aryl Me groups and this results in a further broadening of the latter signal. In a series of decoupling experiments analogous to those described by Jackman *et al.* [6], we have demonstrated that similar couplings exist in the ring A portion of the hypostictic acid-diacetate molecule. For example, irradiation at δ 6.67 sharpened the signal at δ 2.53 (but not that at δ 2.17) and vice versa. The C-3 and C-6 Me group signals can therefore be assigned with confidence. The remaining Me group signal (δ 2.32) is of intermediate height and half-band width and must, by elimination, originate from the isolated C-3′ Me group.

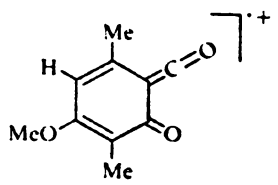
A similar analysis of the ^{1}H NMR spectrum determined for PQ-4-triacetate indicated the presence of an additional sharp acetate Me group signal at δ 1.97, and the absence of the C-3′ Me group signal. Signals corresponding to a pair of *para*-coupled aryl Me groups (one of which was coupled with an *ortho*-aryl proton) and two other acetate Me groups were also present. The chemical shift values of these signals, and also of the other lower field proton signals (see Table 1) correspond almost exactly to those determined for hypostictic acid-diacetate (**1d**). These observations suggested PQ-4 triacetate to be

Table 1. ^{1}H NMR assignments [δ (ppm) in $CDCl_3$]

Signal	Compound (1d)	(1f)	(1g)
3-Me	2.17 (1.8)	2.18 (1.8)	2.18 (1.8)
6-Me	2.53 (1.9)	2.56 (1.9)	2.53 (1.8)
3′-Me	2.31 (1.5)	--	2.32 (1.5)
3′-CH_2OAc	--	1.94 (1.1)	--
3′-CH_2OAc	--	5.30 (ABq)*	--
2′-OAc	2.24 (1.1)	2.25 (1.2)	--
6′-CH(OAc)O	2.44 (1.0)	2.43 (1.1)	--
6′-CH(OAc)O-	7.51 (1.3)	7.50 (1.2)	7.50 (1.2)
4-OMe	3.91 (1.0)	3.92 (1.0)	3.91 (1.0)
5-H	6.67 (1.9)	6.67 (1.9)	6.67 (2.0)

* ABq, J = 11.5 Hz (doublets centred at δ 5.45 and 5.15).

Unless otherwise stated all signals are singlets, the half-band widths (in Hz) of which appear in parentheses after the chemical shift values.



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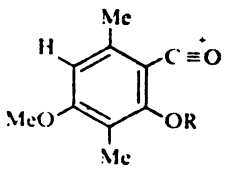

5a R = H
5b R = D

hypoconstictic acid-triacetate (**1f**). This conclusion is supported by the following observations. In both hypostictic acid-diacetate (**1d**) and PQ-4-triacetate the C-5 aryl proton signal resonates at δ 6.67. Thus, it can be inferred that a OMe group, rather than an acetoxyl group is located at C-4, since a OMe group typically shields an adjacent aryl proton to a greater extent (*ca* 0.21 ppm [7]). In hyposalazinic acid-triacetate the C-5 aryl proton signal appears [4] at δ 6.87. The structural significance of the ions of m/e 178 and 179 ~~which appear~~ in the mass spectrum of hypoprotocetraric acid and its fully methylated analogue have been discussed elsewhere [9]. Equivalent ions, to which structures **4** and **5a**, respectively, have been assigned [9], appear in the mass spectra of hypostictic acid-diacetate (**1d**) and of PQ-4-triacetate. In the case of hypostictic acid-$^2D_6$-diacetate (**1g**) the latter ion has m/e 180, hence, structure **5b** can be assigned.

Confirmation of the foregoing structural conclusions was obtained when a mixture of stictic acid (**3a**) and constictic acid (**3c**) was acetylated with acetic anhydride in pyridine, and subsequently hydrogenolysed over Pd-C to afford a mixture of hypostictic acid-diacetate (**1d**) and hypoconstictic acid-triacetate (**1f**). The selectivity of the acetylation reagent employed in this study is noteworthy in that the aldehyde group is not derivatized to a $CH(OAc)_2$ entity, as is the case with, for example, acetic acid in the presence of sulphuric acid [10].

Since PQ-4 is more polar than either PQ-1 or PQ-2, it can be inferred that PQ-4 is hypoconstictic acid (**1c**).

EXPERIMENTAL

Nephroma australe was collected in December 1977 and May 1978 in the vicinity of Lakes Waikaremoana and Waikareiti, Urewera National Park, New Zealand. Fragments of *N. antarcticum* were detached from a collection in the Herbarium of the British Museum (Natural History) London (J. D. Hooker, Cape Horn).

Extraction of N. australe. The finely ground lichen material (4.8 g) was extracted in a Soxhlet apparatus with petrol for 17 hr and then with Me_2CO for 2.5 hr. The petrol extracts consisted largely of usnic acid and zeorin (hopane-6α,22-diol). Separation of the Me_2CO extracts (490 mg) by prep. TLC on Si gel with toluene dioxane HOAc acid (TDA) (90:25:4) gave two

fractions which were subjected to further prep. TLC on Si gel with petrol Et_2O HCO_2H (12:13:2) (PQ-1 fraction, faster moving band) or with TDA (PQ-2 fraction, slower moving band) to give hypostictic acid (**1a**) (PQ-1) (44 mg) and hyposalazinic acid (**1b**) (PQ-2) (16 mg), respectively.

Hypostictic acid (**1a**) had mp 260–262° with decomposition from 220° (lit. [4] 264° with decomposition); ν_{max}^{KBr} 1750, 1695, 1605 and 1560 cm^{-1}; δ 60 MHz (C_5D_5N) 2.43 (6-Me), 2.62 (3'-Me), 2.67 (3-Me), 3.83 (4-OMe), 6.82 (5-H), and 7.52 (6'-CH(OH)O); MS (probe) 70 eV *m e* (rel. int.): 372 (M^+, 65), 354 (100), 328 (88), 327 (60), 326 (77), 300 (24), 299 (28), 298 (40), 272 (18), 271 (29), 270 (29), 244 (18), 243 (22), 242 (28), 216 (40) and 179 (21).

Hyposalazinic acid (**1b**) had mp 274° with decomposition from 249° (lit. [4] 280° with decomposition); ν_{max}^{KBr} 1720, 1695, 1610 and 1580 cm^{-1}; δ 60 MHz (C_5D_5N) 2.46 (6-Me), 2.63 (3'-Me), 2.81 (3-Me), 7.03 (5-H), and 7.93 (6'-CH(OH)O); MS (probe) 70 eV *m e* (rel. int.): (M^+, 76), 340 (100), 314 (72), 313 (36), 312 (51), 286 (26), 285 (28), 284 (34), 258 (16), 257 (29), 256 (29), 230 (18), 229 (31), 228 (22) and 165 (68).

Hypostictic acid-diacetate (**1d**) *and hyposalazinic acid-triacetate* (**1e**). The Me_2CO extracts of *N. australe* (203 mg from 1.8 g of lichen) were dissolved in C_5H_5N $(MeCO)_2O$ (1:1) (3 ml) and stirred for 16 hr at room temp. Work-up and purification by prep. TLC on Si gel with C_6H_6–Et_2O (93:7) gave hypostictic acid-diacetate (**1d**) (21 mg) mp 240° (lit. [4] 244°) and hyposalazinic acid triacetate (**1e**) (8 mg) mp 201° (lit. [4] 203–205°).

Hypostictic acid-$^2D_6$*-diacetate* (**1g**). Repetition of the acetylation exp described above with a portion of the Me_2CO extracts from *N. australe* (170 mg from 1.46 g of lichen) and $(CD_3CO)_2O$ (0.5 ml) in C_5H_5N (2 ml) gave, as the major product, hypostictic acid-$^2D_6$ diacetate (1 g) (17 mg). MS (probe) 70 eV *m e* (rel. int.): 462 (M^+, 15), 418 (100), 374 (72), 354 (96), 345 (16), 335 (23), 326 (71), 322 (33), 301 (25), 298 (36), 285 (31), 271 (39), 245 (23), 180 (14) and 178 (18).

Extraction of N. antarcticum. The finely ground lichen material (0.9 g) was extracted in a Soxhlet apparatus with petrol for 24 hr and then with Me_2CO for 6 hr. The petrol extracts consisted largely of usnic acid and zeorin. TLC (TDA) established the presence in the Me_2CO extracts of 3 major components, two of which gave distinctive red colourations when charred with H_2SO_4 [4]. Small scale expts established that the more polar of these substances could not be satisfactorily recovered from prep. TLC plates developed by the procedures employed for the separation of the *N. australe* extracts.

Reaction of the Me_2CO extracts (120 mg) with C_5H_5N–$(MeCO)_2O$ (1:1) (5 ml) for 24 hr at room temp. gave a gummy residue which was shown by TLC (petrol–Et_2O (3:2)) to consist of 3 major components, two of which gave red colourations when charred with H_2SO_4. Separation of the latter components by prep. TLC on Si gel with petrol–Et_2O (3:2) gave hypostictic acid-diacetate (**1d**) (22 mg) (higher R_f value). Hypoconstictic acid-triacetate (**1f**) had mp 190–192°; ν_{max}^{KBr} 1780, 1743, 1710, 1634, 1610, 1564, 1456, 1343, 1185, 1152, 1137, 1056, 986 and 913 cm^{-1}; MS (probe) 70 eV (rel. int.): *m e* 514 (M^+, 10), 472 (15), 454 (11), 412 (40), 370 (100), 342 (32), 314 (19), 221 (24), 179 (18) and 178 (12). (Found: *m e* 514.1129. $^{12}C_{25}{}^1H_{22}{}^{16}O_{12}$ requires: 514.1111.)

Synthesis of hypostictic acid-diacetate (**1d**) *and hypoconstictic acid-triacetate* (**1f**). A mixture of stictic acid (**3a**) and constictic acid (**3c**) (*ca* 1:1) (280 mg), isolated by co-crystallization from the Et_2O extract of *Pseudocyphellaria homeophylla* [11], was dissolved in a 1:1 soln of C_5H_5N–$(MeCO)_2O$ (10 ml) and stood for 2 hr at room temp. Work-up gave material which was demonstrated by ^1H NMR to be a mixture (*ca* 1:1) of stictic acid-diacetate, δ 60 MHz ($CDCl_3$) 2.22 (2'-OAc), 2.32 (3'-Me), 2.45 (6'-CH(O<u>Ac</u>)O), 2.58 (6-Me), 4.05 (4-OMe), 6.88 (5-H), 7.40 (6'-C<u>H</u>(OAc)O), and 10.47 (3-CHO) and constictic acid-triacetate, 1.95 (3'-CH_2O<u>Ac</u>), 2.25 (2'-OAc), 2.45 (6'-CH(O<u>Ac</u>)O), 2.63 (6-Me), 4.05 (4-OMe), 5.45 (d), 5.15 (d), (3'-<u>C</u>H_2OAc, AB*q*, J = 11.5 Hz), 6.88 (5-H), 7.40 (6'-<u>CH</u>(OAc)O), 10.47 (3-CHO).

Hydrogenolysis of the foregoing mixture over Pd-on-C prepared using the method of Keogh [4], afforded material which, when separated by prep. TLC on Si gel with C_6H_6–Et_2O (9:1) gave hypostictic acid-diacetate (50 mg) and hypoconstictic acid-triacetate (52 mg). The latter substance was identical with PQ-4-triacetate.

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