STUDIES   IN   NATURALLY-OCCURRING

PLANT   SESQUITERPENOIDS.

A thesis submitted to the University of Glasgow

for the degree of Ph.D.

by

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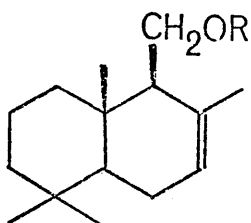


52, R=H
53, R=Ac
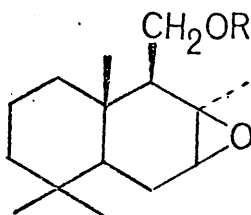


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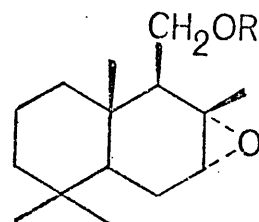


56, R=H
57, R=Ac

Part I of this thesis is concerned essentially with structure elucidations of naturally-occurring plant sesquiterpenoids. To this end, the utility of derivatization of certain functional groups has been investigated in Part II, in order that spectroscopic data may be supplemented and structure elucidation facilitated.

## Part I - Structure Elucidations

Several samples of the resin of Ocotea Caparrapi were compared, and the structures of the principal constituents elucidated. Nerolidol (2) was the principal constituent of certain samples. The structures of the new sesquiterpenoids caparrapidiol (10), caparrapitriol (12) and dihydroxycaparrapi acid (13) were elucidated mainly by spectroscopic considerations: infra-red, nuclear magnetic resonance and mass spectroscopy were successfully employed. Trimethylsilylation of these tertiary alcohols as a mass spectroscopy derivatization procedure was a critical factor in this work. Caparrapi oxide (16), which represents a novel sesquiterpenoid skeleton, was present as a major constituent of some samples. The structure elucidation was again dependent principally on spectroscopic techniques. Comparison of spectral data with those of the analogous monoterpenoid (23) and the diterpenoid manoyl oxide (28) provided corroboratory information. It is suggested that the biosynthesis of caparrapi oxide involves enzymatic 'cationic' cyclization of nerolidol. Attempted replication of this

process *in vitro* was unsuccessful. A detailed comparison of the constitution of the caparrapi oil samples is reported: the incidence of acyclic terpenoids in these exudates is unusual.

The essential oil from the heartwood of Brachylaena hutchinsii, an East African tree, was examined. Evidence is presented that the structures of the major constituents are given by (36a) and (36b). This evidence depends mainly on a detailed NMR analysis of the two epimers and a comparison with the monoterpenoid analogue myrtenal (40). The spectral characteristics of an oxidative degradation product were also in accord with the postulated structure. Conversion to the known hydrocarbons copaene (35a) and ylangene (35b) was not satisfactorily accomplished because of lack of time. Accordingly, the relative configuration at the isopropyl centre is indefinite.

In the appendix to Part I, the relative stereochemistries of the drimenol epoxides (54) and (56) are assigned on the basis of detailed NMR spin decoupling experiments. Examination of multiplicity of the epoxidic proton (which comprises one part of an AMNX system) permits unequivocal assignment. Variations in the spin pattern of the methylenic protons of the hydroxymethyl group of the series of compounds (52) - (57) are interpreted in terms of preferred orientations.

41 → 50 → 52

46a

66   68   70

80   81   82   83
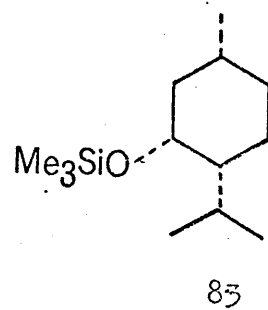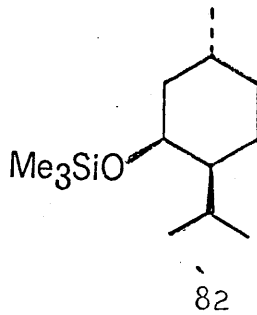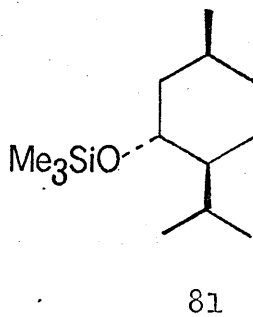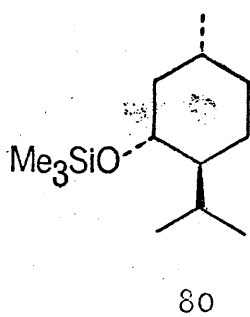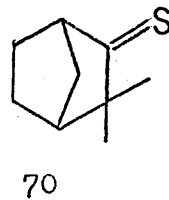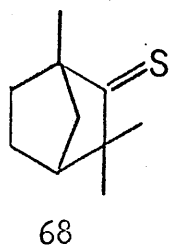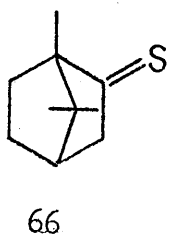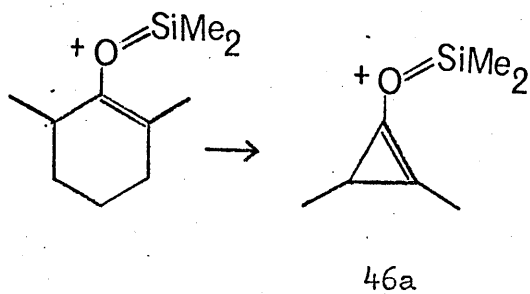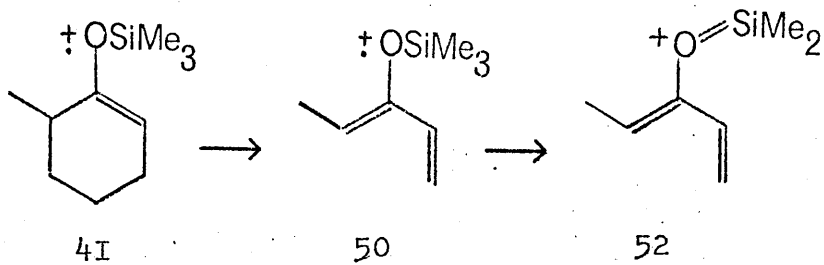
## Part II - The facilitation of structure elucidation by functional group derivatization

A review of derivatives which are potentially useful in combined gas chromatography - mass spectrometry (GC-MS) is presented. Two carbonyl group derivatives are investigated; firstly, enol trimethylsilyl ethers and secondly, thiones.

Conditions for the synthesis of enol trimethylsilyl ethers are reported. The mass spectra of a series of derivatives, e.g. (41), were examined and the fragmentation-directing properties of the functionality evaluated. When allowed, the combination of Retro-Diels Alder and loss of methyl radical is a predominant process, affording the highly-stabilized cation (52). Examination of metastable transitions indicates that the chronological sequence is as depicted opposite. An unusual extrusion, affording ions of possible structure (46a), also occurs.

The thiocarbonyl group was investigated in the related thiones (66), (68) and (70). The fragment ions in the mass spectrum were predominantly stabilized by sulphur, compared to the fragment ions from the corresponding ketones, which were predominantly hydrocarbon cations. The conclusion is that the thione function directs the fragmentation to a greater extent than does the ketone. Tentative mechanisms for the principal fragmentations are advanced.

The properties on GC-MS of epimeric trimethylsilyl ethers of the menthol series (80) - (83) was investigated. It was hoped that the epimeric differences would be manifested as significant differences

in ion abundances in the mass spectrum. The operational variables in GC-MS, however, resulted in ion abundance variations which masked the differences due to epimerism.

Two important aspects of the nuclear magnetic resonance characteristics of thiones have been examined; firstly, the magnetic anisotropic properties, and secondly, the aromatic-solvent-induced chemical shifts.

A qualitative assessment of the nuclear magnetic resonance characteristics of the thione group was obtained from a study of the monoterpenoid thiones (66), (68) and (70). The thione group was observed to have a greater magnetic anisotropic effect than the ketone group. A cone of shielding/deshielding similar to that postulated for the ketone group is proposed.

Solvent-induced chemical shifts in a wide variety of aromatic solvents have been measured. Distinct parallelisms in solvent shifts of corresponding methyl groups in ketone and thione were observed. A plane of reference is postulated for the thione group (similar to that reported for the carbonyl group) orthogonal to the C=S bond axis; methyl groups behind this plane experience a positive chemical shift in going from carbon tetrachloride (or chloroform) to benzene as solvent. The association constants for the complexing of benzene with camphor and thiocamphor have been measured and found to be 0.3-0.4 (mole fraction)$^{-1}$.

<u>PART I</u>

INTRODUCTION TO THE STUDY OF THE PRIMITIVE
SESQUITERPENOIDS FROM THE ESSENTIAL OIL OF
OCOTEA CAPARRAPI


Botanical and Background Aspects.

Ocotea caparrapi (Nates) Dugand, previously known as

Nectandra caparrapi or Laurus giganteus, is a large evergreen

forest tree belonging to the natural order Lauraceae. It grows

in humid regions of Colombia at an altitude of about 1300 metres

in a climate where the mean temperature is 21°C. According to

Bayon[1] the tree derives its name from the village of Caparrapi

in the province of Cudinamarca, Colombia. The essential oil is

collected by making a horizontal incision into the trunk and

collecting the viscous effluent*.

The first chemical study of the oil was that of Tapia[2] who

reported the presence of an alcohol, 'caparrapiol", $C_{15}H_{26}O$, and

a crystalline hydroxy-acid, mp. 85°, $[\alpha]_D \pm 3°$, $C_{15}H_{26}O_3$. The

properties reported for caparrapiol did not match those of any

known sesquiterpenoid. Moreover, in 1960, no sesquiterpenoid

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*The oil is allegedly of therapeutic value, and is used
by the natives for the relief of a wide variety of
complaints such as chronic bronchitis, laryngitis,
nervous asthma, and for chronic inflammation of the
urinary tract such as leucorrhoea and blenorrhagia.
It is applied either internally, or externally in
large doses.

hydroxy-acid had been described. At that time, Dr. Brooks of
this Department was prompted to obtain several samples of the
oil for re-investigation of Tapia's work. Subsequent
examination failed to confirm the presence of Tapia's acid,
but the presence of nerolidol, $C_{15}H_{26}O$, as a major constituent
together with other acyclic sesquiterpenoid alcohols and acids
was indicated. The comparative rarity of these skeletal types
encouraged further investigation.

## Biogenetic Aspects

Examples of naturally-occurring acyclic sesquiterpenoids
are farnesene (1), nerolidol (2), farnesol (3), and the $\alpha$ - and
$\beta$ -sinensals (4,5). Oil of caparrapi which was shown by
Dr. Brooks to consist mainly of acyclic sesquiterpenoids is
therefore of some intrinsic interest, since it appears to
represent an essential oil in a primitive biosynthetic state.

The mevalonic acid[3] pathway to acyclic sesquiterpenoids is
fairly well established. The focal point of sesquiterpene
biogenesis is in fact the naturally-occurring compound farnesol (3)
whose formation from Acetyl CoA has been demonstrated in yeast[4-6]
and is assumed to occur in higher plants[7]. Suitable cyclization
of either cis- or trans-farnesyl pyrophosphate provides possible
routes to the carbon skeletons of virtually all the sesquiterpenoids[8].

The initial step in these cyclizations is envisaged as removal
of the pyrophosphate anion accompanied by participation of either
the central or terminal double bonds leading to the intermediate
non-classical cations (7-9). The formal charge representation
is, of course, only a convenient symbolism and is not meant to
represent the enzymatic processes involved. It is possible that
in _Ocotea caparrapi_, farnesyl pyrophosphate, or a related species,
is enzymatically bound in such a fashion that processes other than
cyclization predominate. The nature of the acyclic constituents
of the essential oil was therefore of interest.

Fig. 2
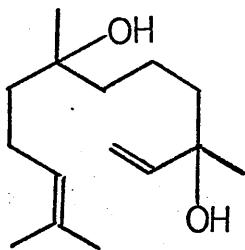
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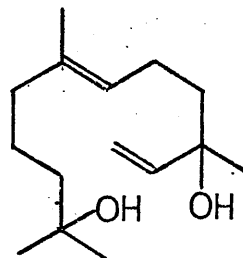
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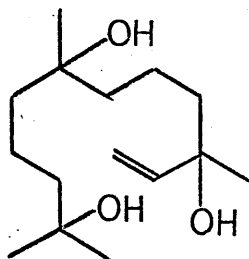
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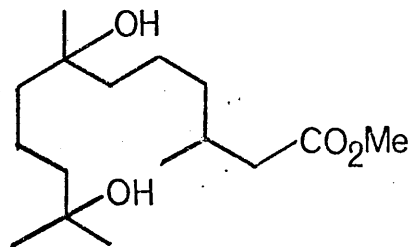
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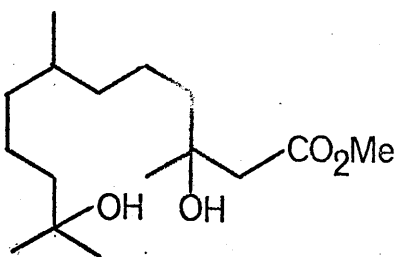
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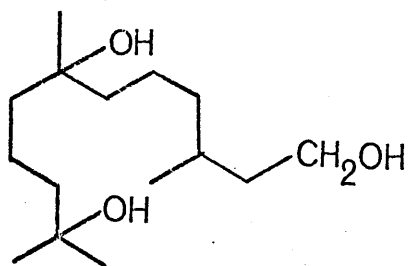
15

Preliminary examination of caparrapi oil (Sample 1; see
Table 3, p.26 ) using the techniques of GLC and TLC indicated
the presence of three constituents in the ratio 90 : 9 : 1
whose retention data were consistent with those of mono-, di-
and tri-hydroxylated sesquiterpenes.  The observed simplicity
of constitution of the oil, and the marked differences in polarities
of the components, prompted chromatographic separations, which
resulted in the isolation of nerolidol, caparrapidiol and
caparrapitriol.

## Isolation and Characterisation of Nerolidol.

Dr. C.J.W. Brooks and Dr. J. Borges del Castillo, performed
the initial investigations of sample 1.  They isolated nerolidol (2),
the major constituent, as an unstable, colourless oil.  Analysis
indicated the formula $C_{15}H_{26}O$.  Evidence for the correspondence
of this sample with (+)-S-nerolidol was obtained from its infra-red,
NMR and mass spectra, its physical constants (refractive index and
optical rotation) and GLC properties.  Caparrapi oil appears to be
the richest source of nerolidol so far described, and would therefore
appear to be potentially of value to the perfumery industry in
which the fragrance of nerolidol is widely utilized.

Fig. 3
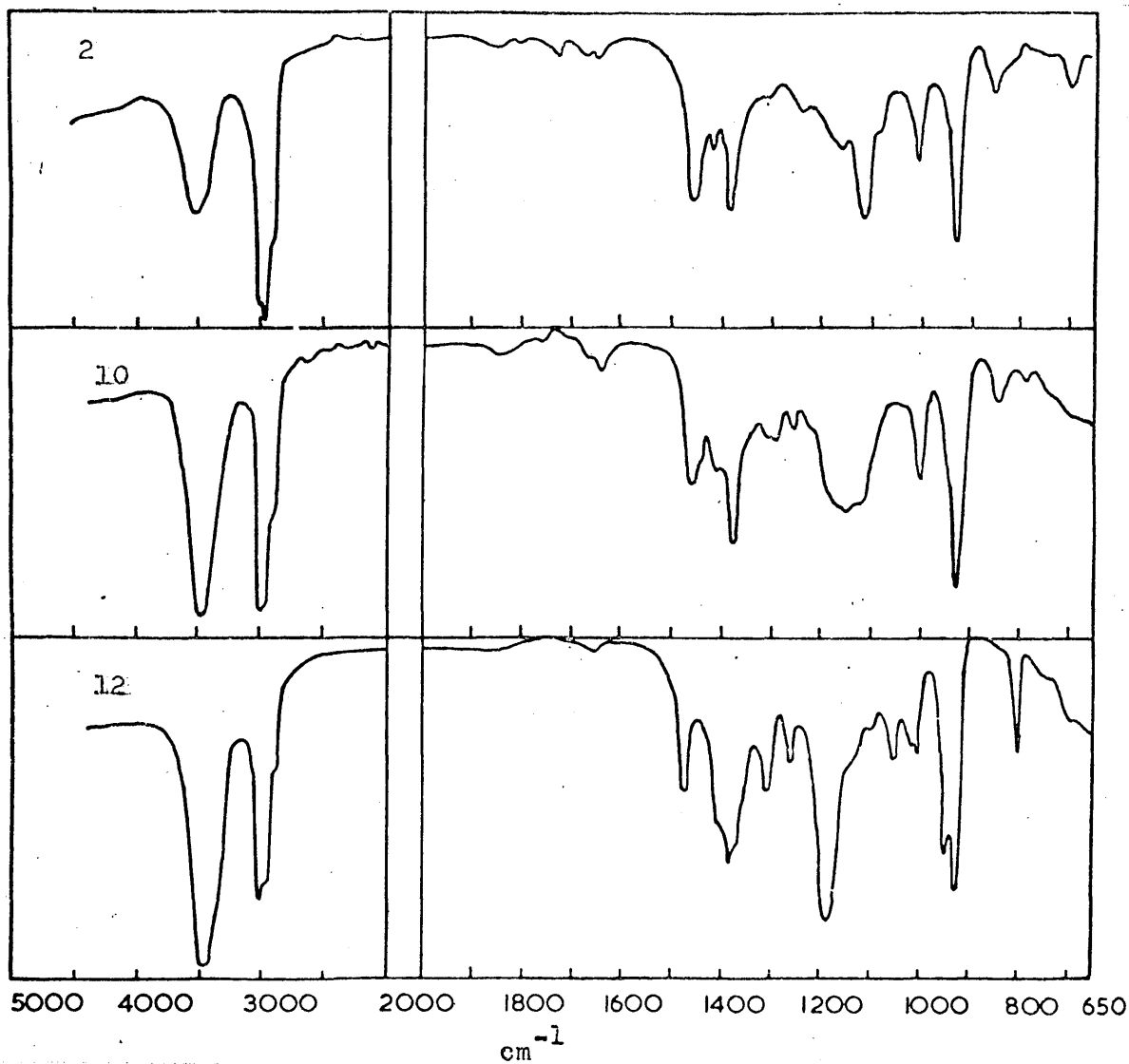


Liquid film IR spectra of nerolidol (2), caparrapidiol (IO),
and caparrapitriol(12).

The Structure of Caparrapidiol (3,11-dihydroxy-3,7,11-trimethyl-dodeca-1,10-diene).

We have subsequently isolated caparrapidiol (10), the secondary constituent of caparrapi oil (sample 1) as a clear, fragrant, viscous oil which decomposed rapidly on exposure to air, but which could be stored under ethanol at $0^{o}C$.

Analysis indicated the composition $C_{15}H_{28}O_2$. This formula was supported by the mass spectrum which suggested a sesquiterpene diol (molecular ion $^m/e$ 240, observed to dehydrate thermally in the MS9 probe over a period of five minutes with loss of 36 mass units). An acyclic structure was shown by hydrogenation: uptake of two moles of hydrogen was evidenced by mass spectrometry of the product. Difficulty was experienced in acetylation and trimethylsilylation of the diol under normal conditions, indicating the presence of tertiary, or hindered hydroxyl groups. The infrared spectra (liquid film and carbon tetrachloride solution) were found to be closely similar to those of nerolidol, except for increased absorption intensity in the hydroxyl region (Fig. 3). Strong bands near 920 and 1000 $cm^{-1}$ indicated the presence of a vinyl group. The NMR spectrum (60 Mc; Fig. 4) confirmed this structural feature, showing typical multiplets centred on $\tau$ 4.06 and 4.80. This latter multiplet also contained the olefinic proton on $C_{(10)}$. Singlets due to the isopropylidene grouping appeared at $\tau$ 8.30 and 8.36. Two unsplit peaks at $\tau$ 8.71 and 8.82 were assigned to methyl groups at tertiary alcoholic centres. A peak

Fig. 4
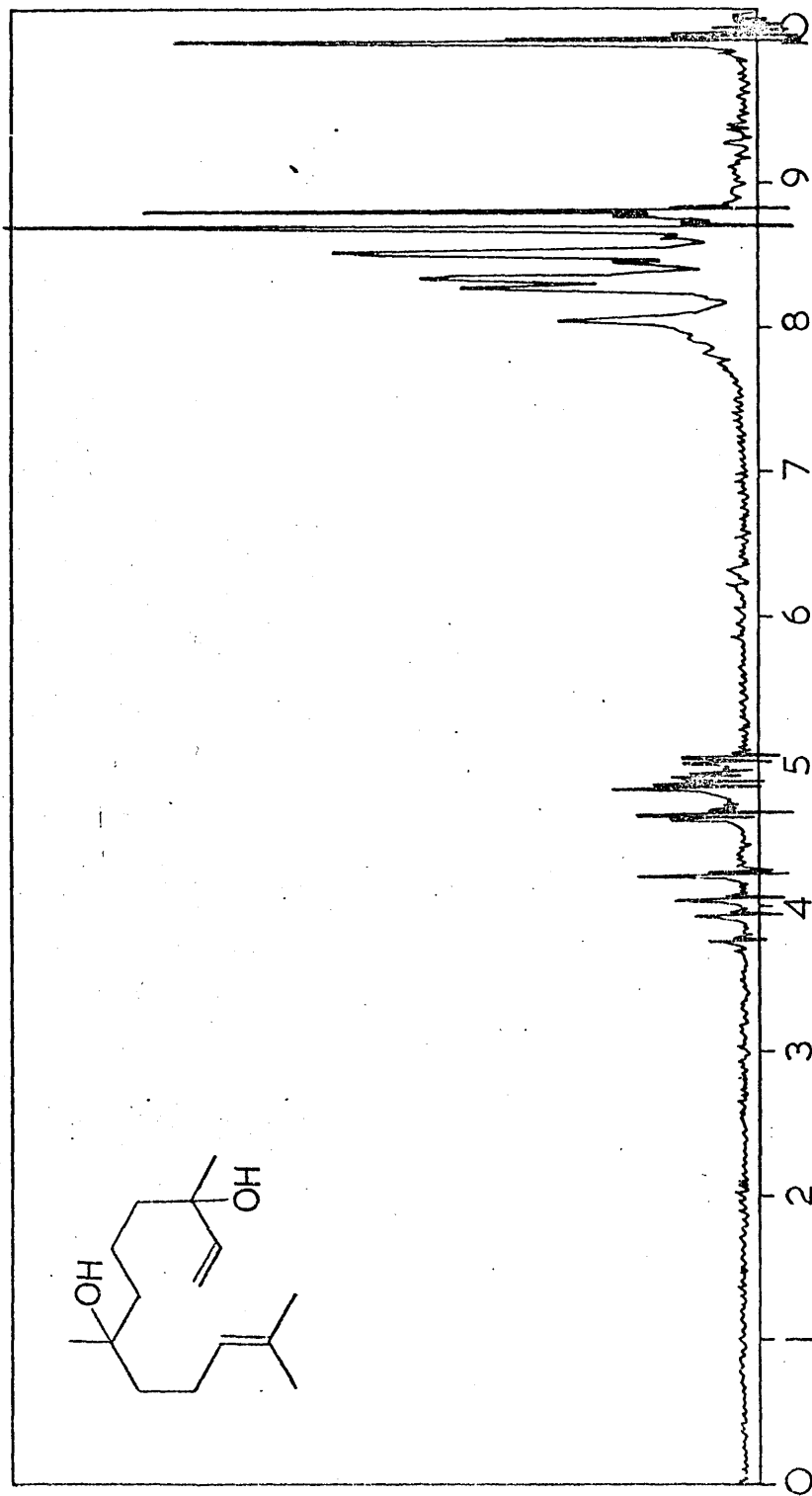


NMR spectrum ((60Mc/s.) of caparrapidiol (10) in CHCl$_3$

Fig. 5



m/e 199
(25%)

m/e 384 (0.5%)

+OR

m/e 143
(100%)

m/e 301
(0.5%)

R = Si(CH₃)₃
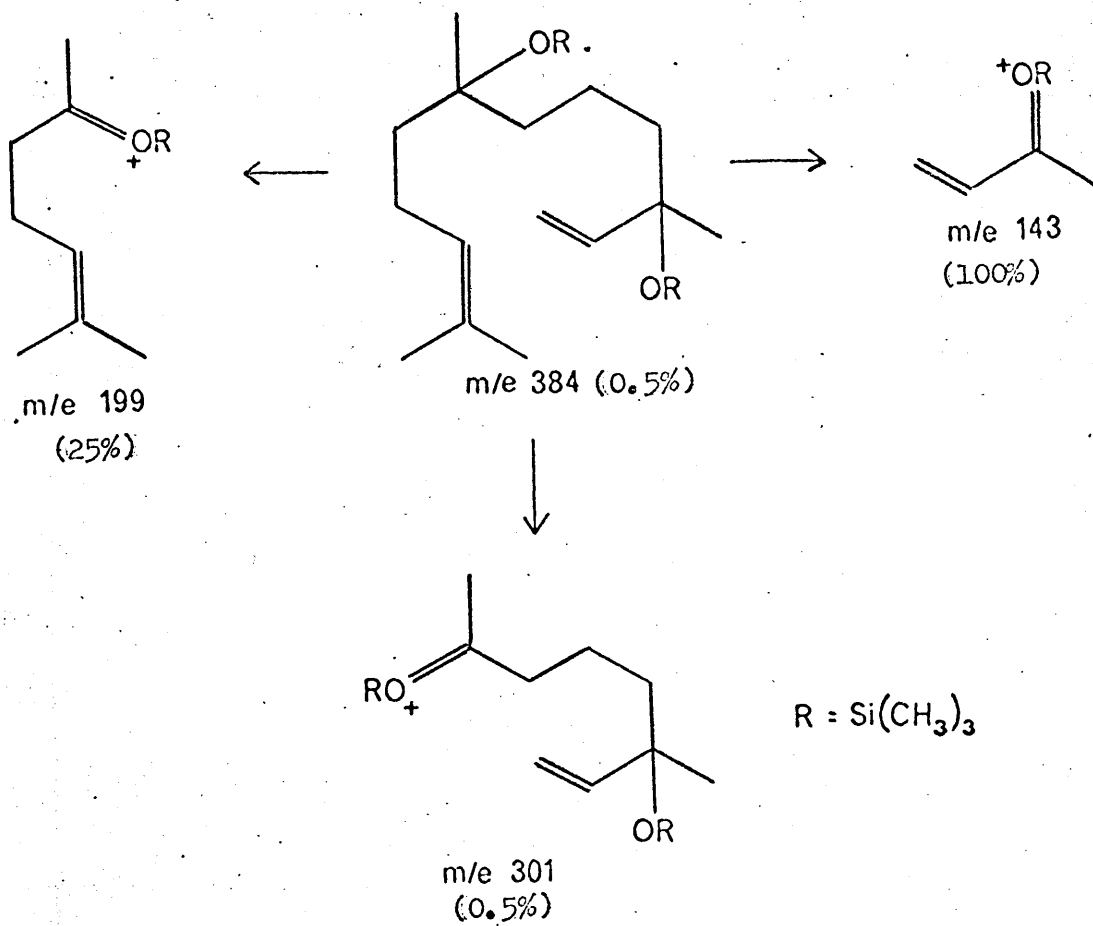
Mass spectral fragmentation of caparrapidiol bis-TMS ether.

ascribed to two hydroxyl protons at $\tau$ 7.95 disappeared on $D_2O$

exchange. All assignments were supported by integration.

Caparrapidiol was observed to dehydrate when left in ethanol

for several days on alumina. Nerolidol was identified by

comparative GLC as a major product. The skeletal affinity of

nerolidol and caparrapidiol was thus indicated.

The bis-trimethylsilyl derivative of caparrapidiol was

formed quantitatively by dissolution of the diol in hexamethyldisilazane

and a catalytic trace of trimethylchlorosilane, and heating in a

sealed tube at $160^\circ$ for several hours. Caparrapidiol bis-trimethylsilyl

ether was thus characterized by combined gas chromatography-mass

spectrometry (GC-MS). The following significant ions were observed:

$^m/e$ = 384 (0.5%)[*] (parent ion); $^m/e$ = 301 (0.5%) (loss of $C_6H_{11}$

by cleavage at $C_{(7)} - C_{(8)}$); $^m/e$ = 294 (1%) $(M-(CH_3)_3SiOH)$;

$^m/e$ = 204 (1%) $(M-2x(CH_3)_3SiOH)$; $^m/e$ = 199 (25%) $(C_{(6)} - C_{(7)}$ cleavage);

$^m/e$ = 143 (100%) $(C_{(3)} - C_{(4)}$ cleavage). The definitive fragmentations

are outlined in Fig. 5.

The above evidence confirms structure (10) for caparrapidiol,

rather than the alternative structure (11): the relative configuration

remains to be determined, but the co-occurrence of caparrapidiol with

(+)-S-nerolidol would suggest a common biogenetic origin and it

might therefore be expected that caparrapidiol will have the

_____

*Except as otherwise stated, mass spectra were recorded
using the LKB 9000 gas chromatograph-mass spectrometer.
Percentage ion abundances are cited with respect to the
base peak.

(+)-S-configuration at $C_{(3)}$. Proof would depend on introducing a suitable chromophore, and correlating the Cotton Curve with that of a model compound.

The biogenetic origin of caparrapidiol could conceivably involve the enzymatic Markovnikoff hydration of nerolidol. (An analogous hydration reported in the literature [9] involved the Markovnikoff addition of water to $\Psi$-ionone, using sulphuric acid in water). It is perhaps also possible that caparrapidiol is the biogenetic precursor of nerolidol in Ocotea caparrapi.

Caparrapidiol is the first reported naturally occurring acyclic sesquiterpene diol.

Fig.6
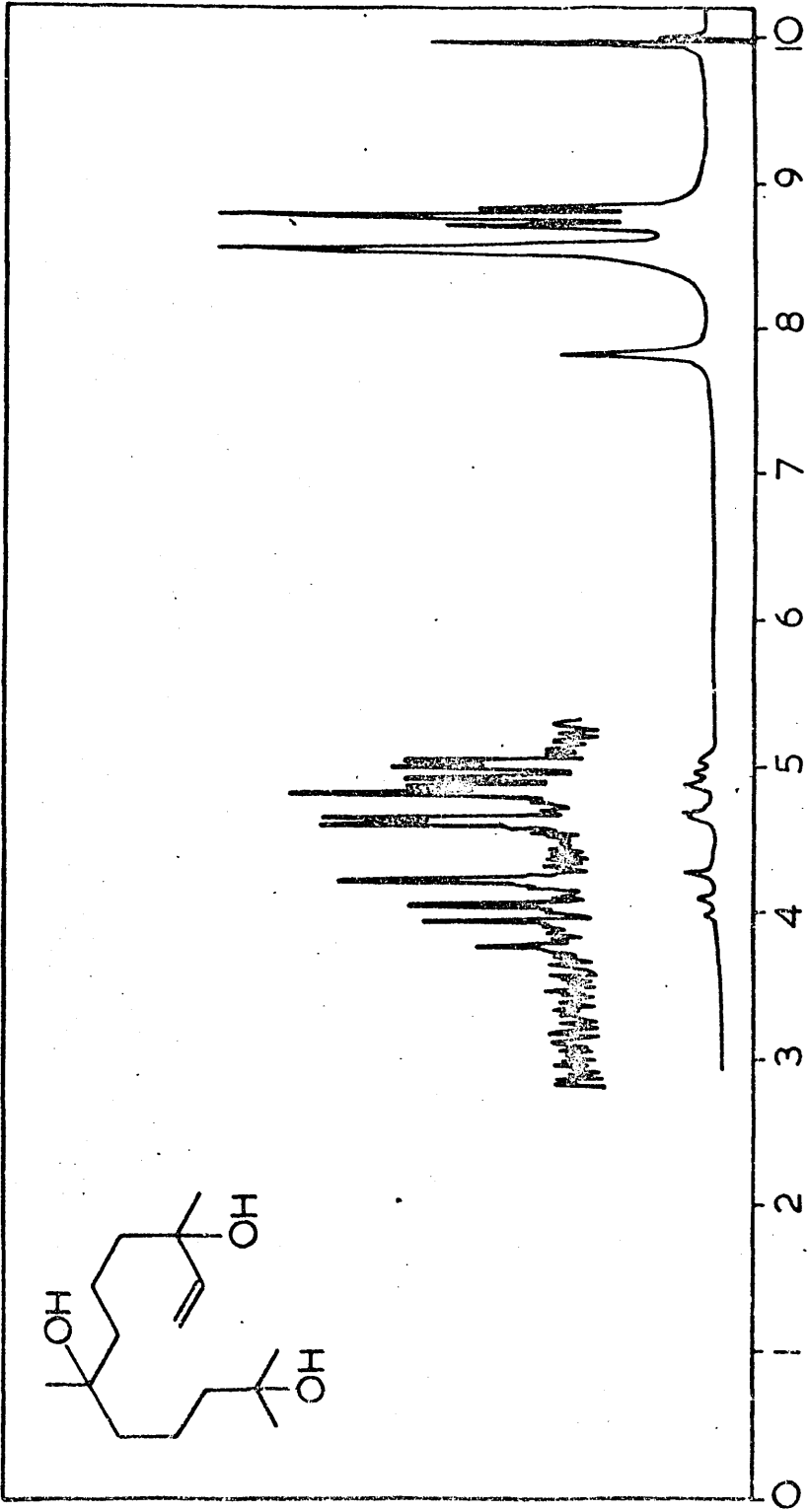


NMR spectrum (60Mc/s.) of caparrapitriol (12) in CHCl$_3$

Further elution of the chromatographic column from which

nerolidol and caparrapidiol were obtained, yielded "caparrapitriol"

(12) as a stable crystalline compound. Analysis indicated the

structure $C_{15}H_{30}O_3$. This compound was also observed to dehydrate

on an alumina column, affording principally nerolidol and

caparrapidiol, as shown by comparative TLC and GLC.

The infra-red spectrum of caparrapitriol (melted film:

Fig. 3, p. 9 ) closely resembled those of nerolidol and caparrapidiol,

but showed more intense hydroxyl absorption and diminished olefinic

absorption. These results confirmed the close relationship of the

three alcohols. Solutions of the triol in $CCl_4$ showed a sharp

peak at 3610 $cm^{-1}$ corresponding to non-bonded hydroxyls: a broad

peak at 3340 $cm^{-1}$ disappeared on dilution, indicating inter- molecular

hydrogen bonding.

The NMR spectrum (Fig. 6) confirmed structure (12), the

following assignments being supported by integration: two multiplets

centred at $\tau = 4.06$ and $\tau = 4.82$ (vinyl group); a sharp singlet

at $\tau = 7.87$ which corresponded to three hydroxyl protons and

disappeared on $D_2O$ exchange; a diffuse peak at $\tau$ 8.60 (methylene

protons); two singlets at $\tau = 8.74$ and 8.86 (two methyl groups at

tertiary alcoholic centres), and a sharp singlet at $\tau = 8.81$,

characteristic of a gem-dimethyl attached to a tertiary alcoholic

centre.

Fig. 7

m/e 301 (1%)

R = SiMe₃

⁺OR

m/e 131
(30%)

m/e 474 (1%)

⁺OR

m/e 143 (100%)

m/e 289 (3%)
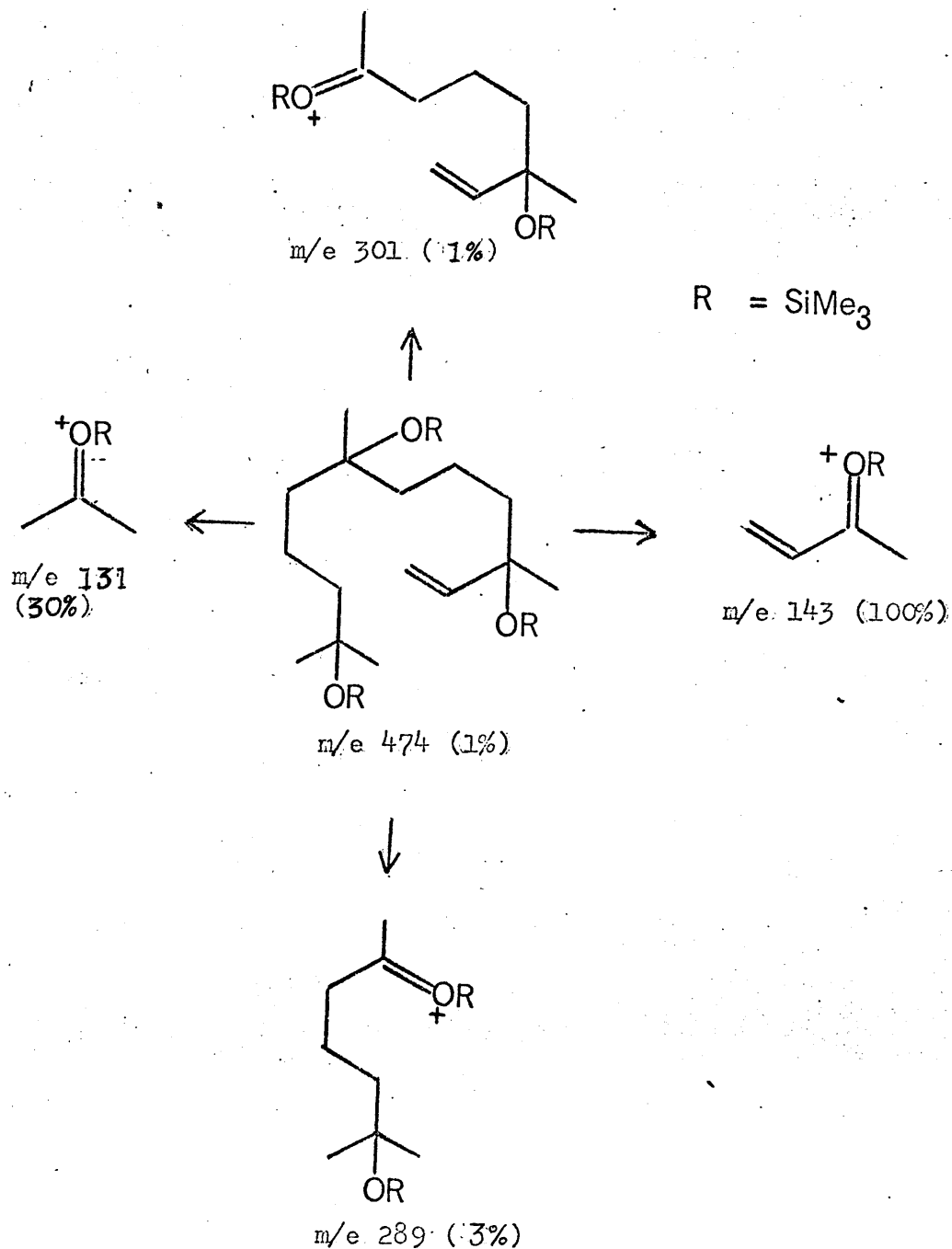
Mass spectral fragmentation of caparrapitriol tris-TMS ether

The three alcohols (2), (10) and (12) were found to give similar mass spectra as a result of initial thermal dehydration followed by complex fragmentation (Fig. 11, p.17 ).

Caparrapitriol tris-trimethylsilyl ether was prepared in the manner already described for tertiary alcohols, and characterised by its gas-chromatographic retention and by GC-MS, affording the following significant ions:

$m/e$ = 384 (0.5%) (M- $(CH_3)_3SiOH$); $m/e$ = 301 (1%) (cleavage at $C_{(7)}$ - $C_{(8)}$); $m/e$ = 289 (3%) (cleavage at $C_{(6)}$- $C_{(7)}$); $m/e$ = 211 (14%) (301 -$(CH_3)_3SiOH$); $m/e$ = 199 (8%) (289 -$(CH_3)_3SiOH$); $m/e$ = 143 (100%) (cleavage at $C_{(3)}$- $C_{(4)}$); $m/e$ = 131 (30%) (cleavage at $C_{(10)}$ - $C_{(11)}$). The most important fragmentations are illustrated in Fig. 7.

Caparrapitriol (12) therefore represents the first reported naturally-occurring acyclic sesquiterpene triol. It possibly corresponds to Tapia's acid (triol mp. 96-97°, $[\alpha]_D$ + 3°; Tapia's acid mp. 86°, $[\alpha]_D$ + 3°) since it is not inconceivable that caparrapitriol would be extracted during an aqueous bicarbonate treatment of the essential oil: on the other hand, Tapia described silver and calcium salts of his acid which are less easily reconciled with a non-acidic structure.

Again, the biosynthetic origin is a matter of speculation. The simplest explanation would invoke enzymatic hydration of a nerolidyl or farnesyl precursor.

Fig.8
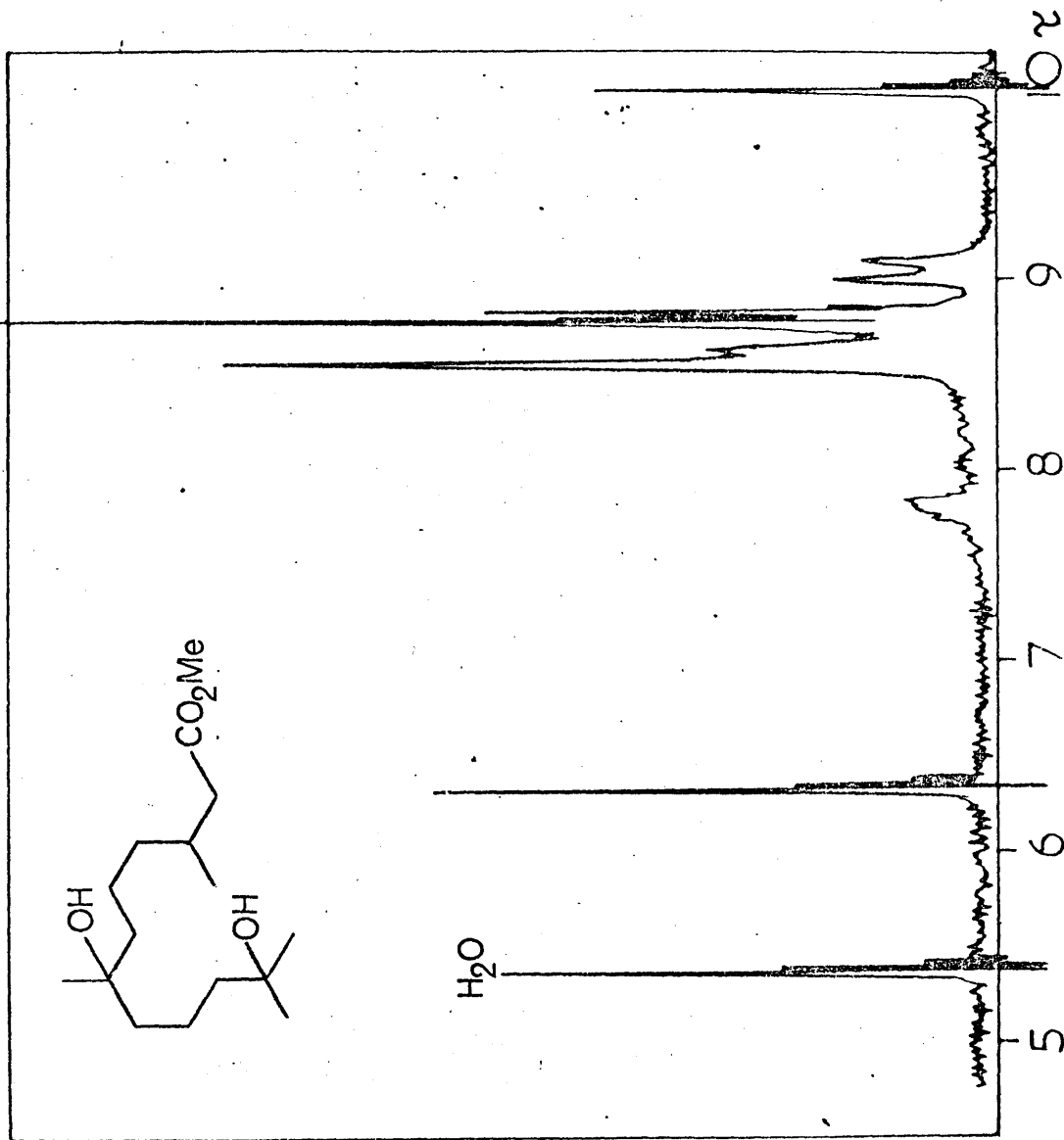
NMR. spectrum (60Mc/s.) of the methyl ester of di-hydroxy
caparrapi acid in CDCl$_3$

<u>The Structure of Dihydroxycaparrapi Acid.</u> (<u>Methyl-7,11-dihydroxy-3,7,11-trimethyl-dodecanoate</u>).

Following initial studies by Dr. C.J.W. Brooks, the acidic

fraction of Sample 4 (see Table 3, p.26 ) of caparrapi oil was

methylated and chromatographed, affording an inseparable mixture

of two isomeric, monocyclic hydroxy-esters, and a pure, though

non-crystalline dihydroxy-ester. Elemental analysis of this

compound was not satisfactorily achieved, analyses consistently

indicating the formula $C_{16}H_{32}O_4 \cdot {}^{1/2}H_2O$. Mass spectral analysis

of the dihydroxyester and its corresponding bis-trimethylsilyl

derivative were in accord with the formulae $C_{16}H_{32}O_4$ and

$C_{16}H_{30}O_4$ $(SiMe_3)_2$, however.

The infra-red data (liquid film and $CCl_4$ solution) suggested

the presence of two hydroxyl groups and confirmed the presence of

an ester. The following assignments could be made in the NMR

spectrum (Fig. 8): $\tau$ 6.32 (3H, singlet; $CO_2$Me); $\tau$ 7.8 (2H, multiplet;

C$\underline{H}_2CO_2$Me); $\tau$ 8.57 (multiplet, methylenic protons); $\tau$ 8.78 (6H, singlet;

2x C$\underline{H}_3$-CR$_2$CH); $\tau$ 8.83 (3H, singlet; C$\underline{H}_3$-CR$_2$OH); $\tau$ 9.05 (3H, doublet;

C$\underline{H}_3$-CHR$_2$). Preliminary examination of these spectral data suggested

(13) and (14) as possible structures.

Definitive structural data were obtained by GC-MS examination

of the bis-trimethylsilyl ether, and of the tris-trimethylsilyl

ether of the triol obtained by lithium aluminium hydride reduction

of the dihydroxy-ester. The fragmentations outlined in Figs. 9 and 10

were characteristic and strongly supported structure (13).

Fig. 9

m/e 259 (100%)

R = Si (Me)$_3$

m/e 289 (10%)

m/e 432 (<1%)

m/e 373 (1%)

m/e 173 (10%)

m/e 131 (80%)
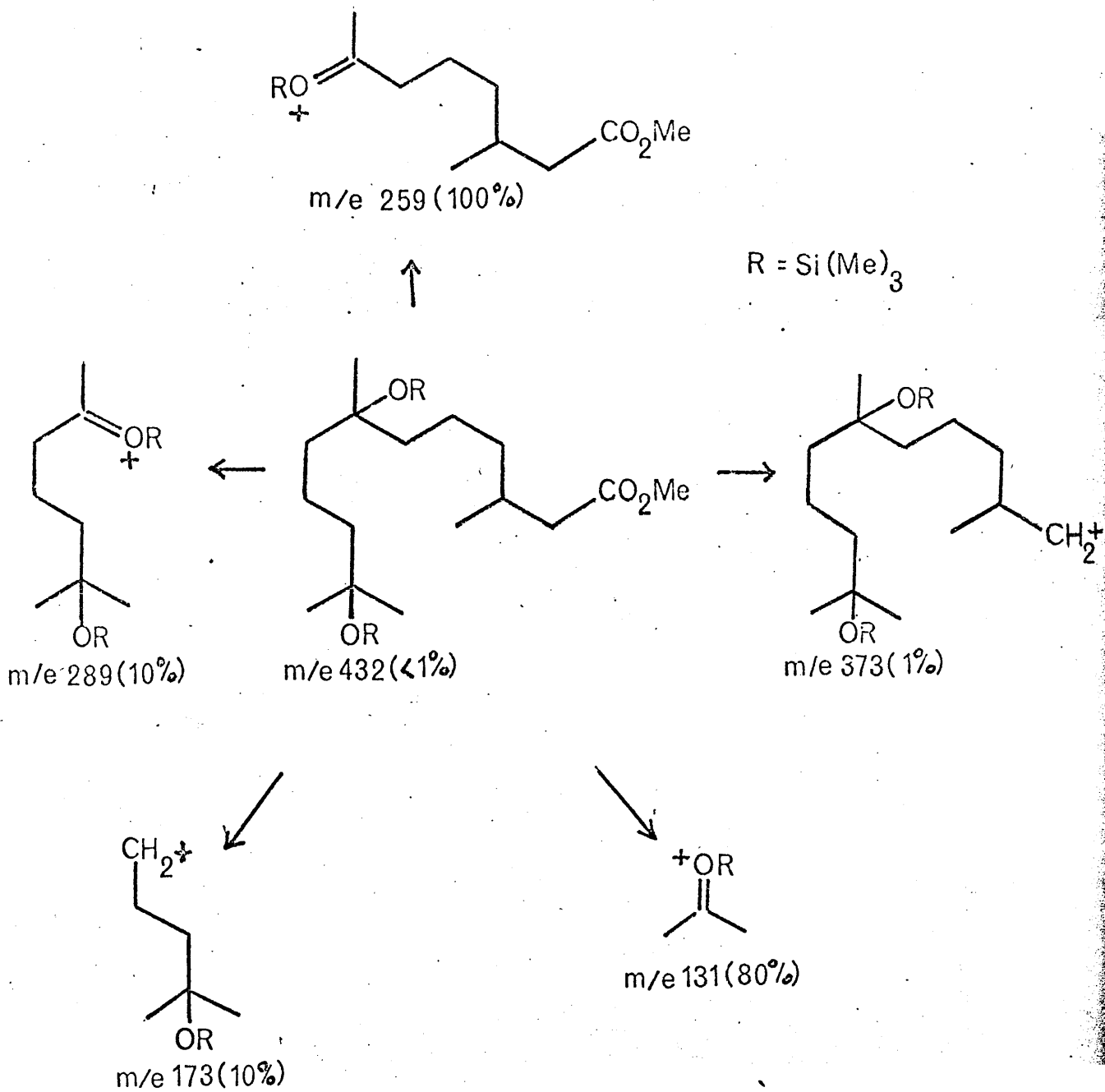
Mass spectral fragmentation of bis - TMS ether of di -hydroxy-ester.

Fig. 10



m/e 303 (25%)

m/e 145 (6%)

m/e 289
(5%)

m/e 476 (0.5%)

CH$_2$=$\overset{+}{\text{O}}$R

m/e 103 (10%)

R = SiMe$_3$

m/e 131 (62%)
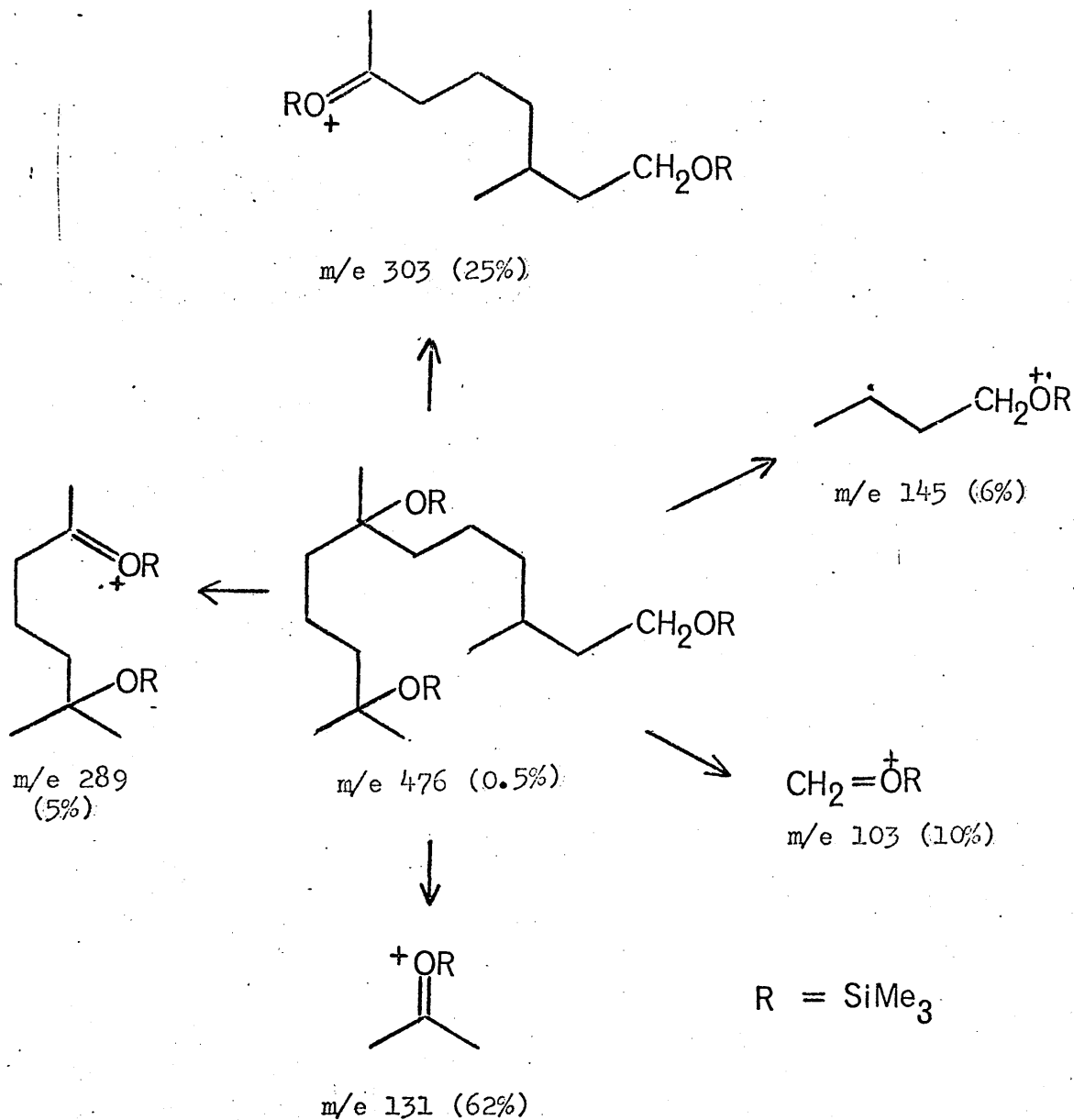
Mass spectral fragmentation of tris-TMS ether of triol (15)

On comparison of the NMR spectrum of (13) (see Fig.8) with that of methyl isovalerate it was found that the methylene protons adjacent to the ester function appeared as a multiplet, whereas in methyl isovalerate a doublet was apparent. This is caused by the pro-chiral nature of the $\alpha$-methylene group in (13): as a consequence of the asymmetric centres in the acyclic skeleton the methylene protons are necessarily non-equivalent so that the $AB_2$ system in methyl isovalerate becomes an $ABB^1$ system in the dihydroxy ester.

The benzene-induced solvent shifts of the various groups in (13) have been compared with those of methyl isovalerate.

## TABLE 1.

| Dihydroxy Ester (13) | | | Methyl isovalerate | | |
|---|---|---|---|---|---|
| $CDCl_3$ $\tau$ | $C_6H_6$ $\tau$ | $\Delta$(ppm.) | $CDCl_3$ $\tau$ | $C_6H_6$ $\tau$ | $\Delta$(ppm.) |
| 6.32 (3H) | 6.60 | + 0.28 | 6.33 (3H) | 6.64 | + 0.31 |
| 7.80 (2H) | 7.87 | + 0.07 | 7.81 (2H) | 7.98 | + 0.17 |
| 8.56 ⎫ (12H) | 8.60 | + 0.04 | - | - | - |
| 8.64 ⎬ | 8.70 | + 0.06 | - | - | - |
| 8.78 (6H) | 8.89 | + 0.11* | - | - | - |
| 8.83 (3H) | 8.93 | + 0.10* | - | - | - |
| 9.00 ⎫ (3H) | 9.05 | + 0.05 | 8.99 ⎫ (3H) | 9.10 | + 0.10 |
| 9.10 ⎬ | 9.15 | + 0.05 | 9.10 ⎬ | 9.20 | + 0.10 |

---

\* The benzene-induced solvent shifts for t-butanol were also measured, and $\Delta$ ($\tau C_6H_6 - \tau CDCl_3$) observed to be + 0.09 ppm. The shifts observed for the tertiary methyl groups in (13) are therefore due to complexing of the tertiary hydroxyls with benzene, rather than complexing of the ester function with benzene.

Parallelisms were observed (Table 1) although the shifts for (13) were all slightly lower. This may be due to greater steric inhibition of the benzene complex by the acyclic chain.

The spectroscopic data outlined (vide supra) are therefore consistent with the hitherto unreported structure methyl-7,11-dihydroxy-3,7,11-trimethyl-dodecanoate (13). Elemental analyses of this ester and its reduction product (15) (see Fig. 2) were in accordance with a 2:1 complex with water. This may be due to hygroscopic absorption of atmospheric moisture, rather than the occurrence of a discrete hydrogen-bonded complex.

The biogenesis of this compound, if it is formed from a farnesyl or nerolidyl precursor, must involve hydration, reduction and oxidation.
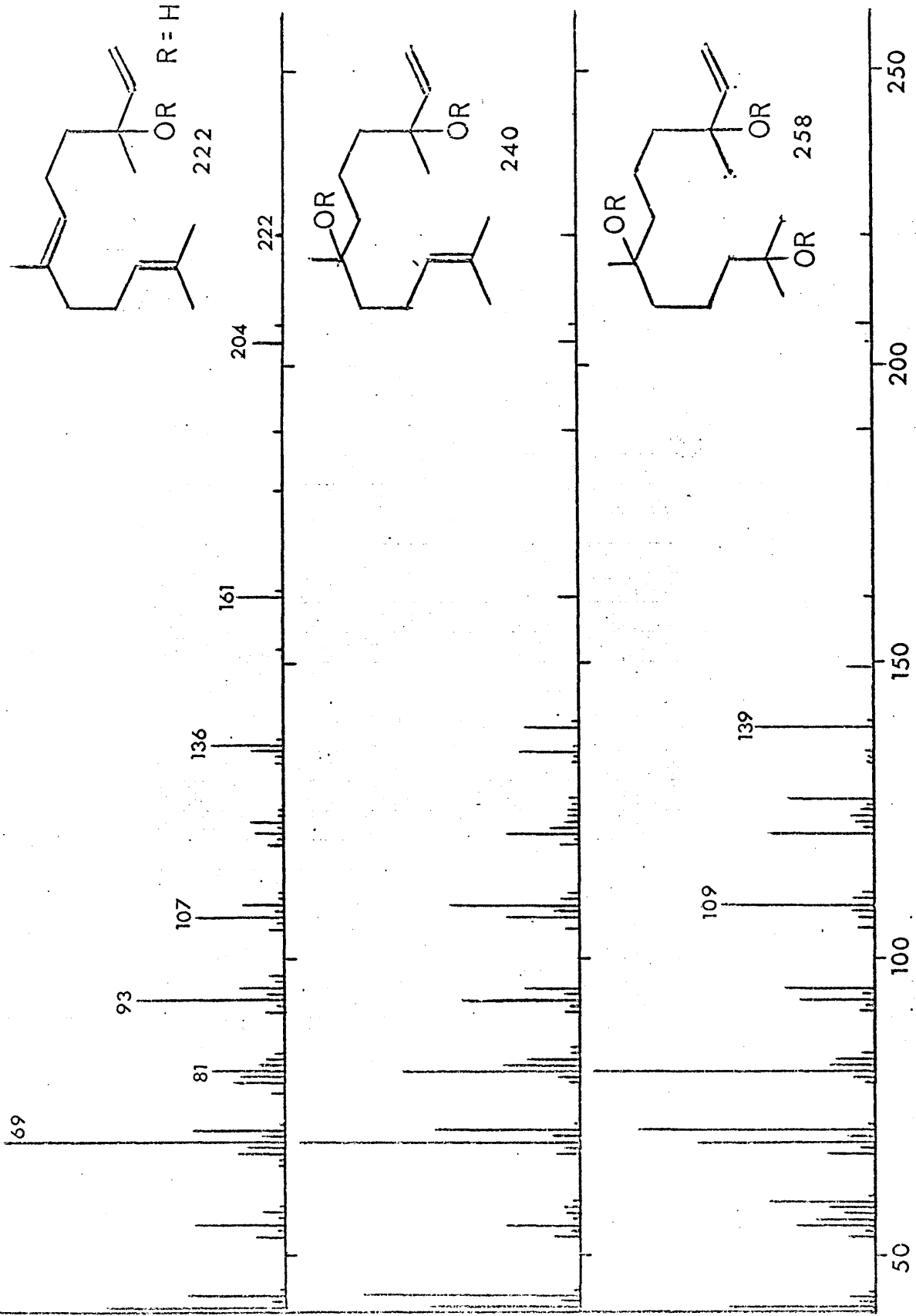
Mass spectra of nerolidol, caparrapidiol and caparrapitriol illustrating dehydration in the spectrometer

## Analytical Utility of Trimethylsilyl Ethers.

Trimethylsilyl derivatives [10] are of widespread value in analysis. The utility of trimethylsilyl ethers for micro-separation and characterization of hydroxylic compounds is increasingly recognised. They are readily preparable, [10, 11] even from many tertiary alcohols, they are of low polarity, and the range of polarities from mono-ethers to the derivatives of polyols is small: these properties render them highly suitable for gas chromatography, [12] and for group separations by TLC or column chromatography. [13, 14] Separations of individual derivatives may also be achieved by using solvent systems of low polarity. Moreover, they undergo characteristic mass spectrometric fragmentations, frequently involving fission adjacent to the ether group [15, 16, 17, 18, 19]: due consideration must be given to the possible occurrence of rearrangements in certain examples (see p.98 ).

The mass spectra of nerolidol, caparrapidiol and caparrapitriol, measured during the initial studies of the oil, were of limited value owing to dehydration to hydrocarbons (probably both thermally, and by 1,3 and 1,4 elimination processes) which gave similar mass spectra (Fig. 11). The methyl ester of dihydroxycaparrapi acid also suffered extensive dehydration in the mass spectrometer. The corresponding trimethylsilyl ethers, however, gave significant, though weak molecular ions, and gave well-defined fragments conveying useful structural information (Figs. 5 and 7, p. 10,13)

The application of combined GC-MS to these derivatives was a
decisive factor in the elucidation of the structures of the
parent hydroxy-compounds.  The procedure clearly has general utility.

Fig. 12
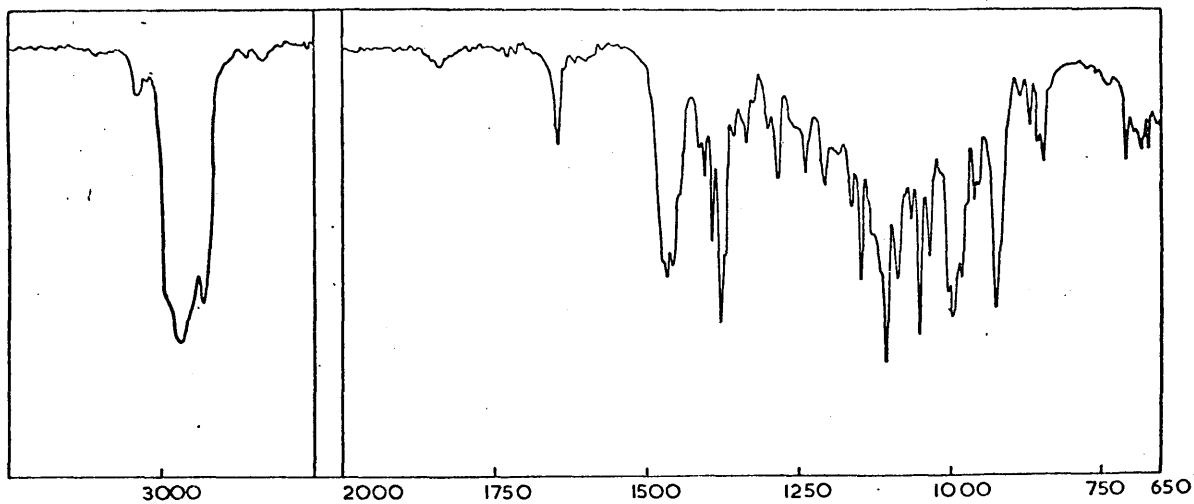


IR spectrum (liquid film) of caparrapi oxide
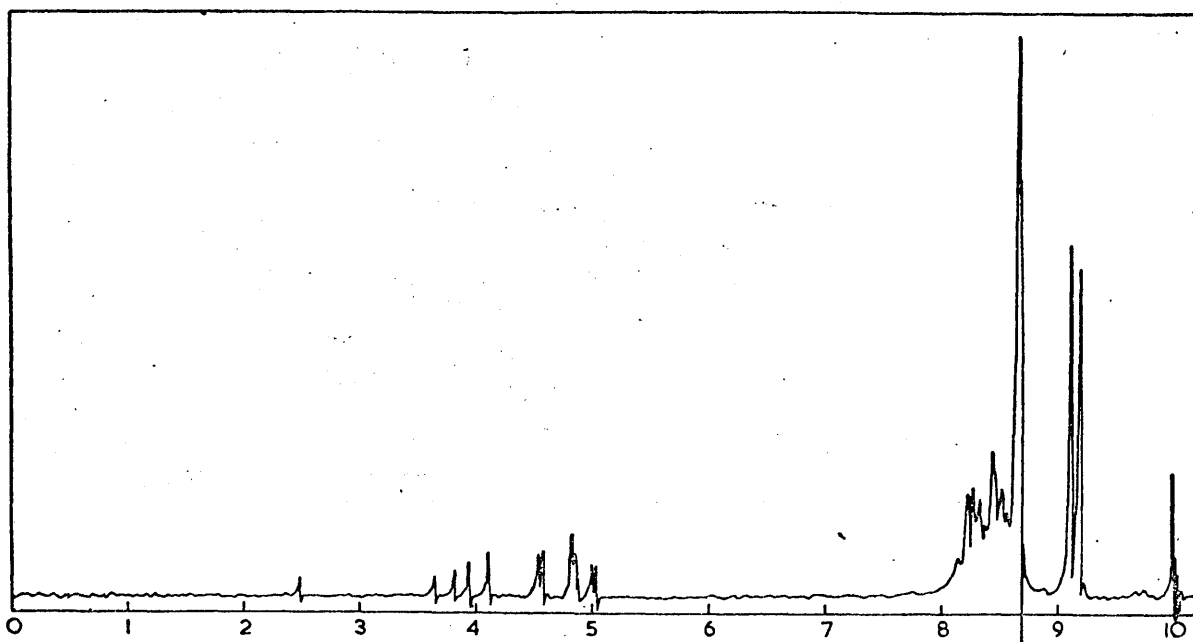


NMR spectrum (60Mc/s.) of caparrapi oxide in $CDCl_3$

## The Gross Structure of Caparrapi Oxide. (1,3,7,7-tetramethyl-3-vinyl-2-oxabicyclo-(4,4,0)-decane)

Certain samples of caparrapi oil (3 and 4, Table 3, p.26 ) contained, as a relatively abundant constituent, a compound, unaffected by mild acetylation, having a retention index $I_{SE-30}^{100°}$ 1420, and yielding in its mass spectrum main peaks at $m/e$ 222 ($M^+$), 207, 189, 137, 124, and 109 (Fig. 20). Accurate mass measurement of the ion at $m/e$ 124 (MS 9 mass spectrometer) gave a value 124.1205 : calcd. for $C_9H_{16}$, 124.1252 : for $C_8H_{12}O$, 124.0888. The unusually short retention time in relation to molecular weight together with the mass spectral data (reminiscent of bicyclofarnesyl derivatives) prompted further investigation of this component, which we have named caparrapi oxide.

Caparrapi oxide was readily isolated as an oil from the neutral fraction by column chromatography, and shown to be homogeneous by GLC on three phases of graded polarity (1% OV-1, 1% OV-17 and 3% OV-22) which have been noted [20] for their utility in separating double bond isomers.

Analysis of caparrapi oxide indicated the elemental composition $C_{15}H_{26}O$ which was in agreement with the mass spectral molecular ion. The infra-red spectrum (liquid film, Fig. 12) indicated the presence of a vinyl group ($v_{max}$ 3080, 1840, 930 and 920 cm$^{-1}$), while bands in the C-O stretching region ($v_{max}$ 1047, 1080, and 1120 cm$^{-1}$) were attributable to a tetrahydropyran ring. [21, 22]  Hydrogenation (Pd-C)

Fig. 13
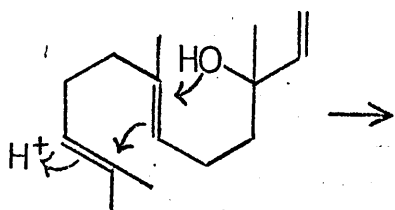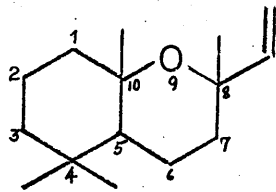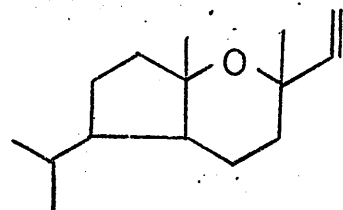


2          16          17
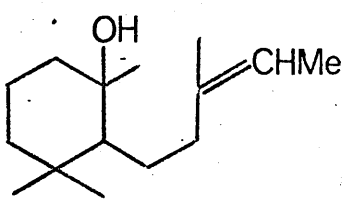
18
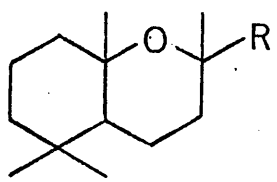
19, R=CHO
20, R=CH$_2$OH
21, R=CH$_2$OTMS
22, R=CH$_2$CH$_3$
23, R=CH$_2$OH$^3$CH$_2$OH
24, R=CHOTMSCH$_2$OTMS
25, R=CHNOCH$_3$
26, R=CHBrCH$_2$Br
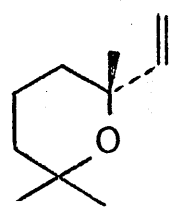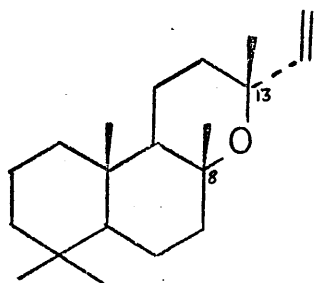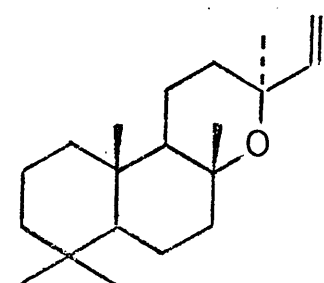
27          28          29

Fig. 14

# Characteristic fragmentations of caparrapi oxide



m/e 222

M⁺-15



allylic stabilization    M⁺-(15+18)    1,4-elimination
(6-membered
transition state)



Retro-Diels Alder    m/e 124    m/e 109



alternative RDA    m/e 68
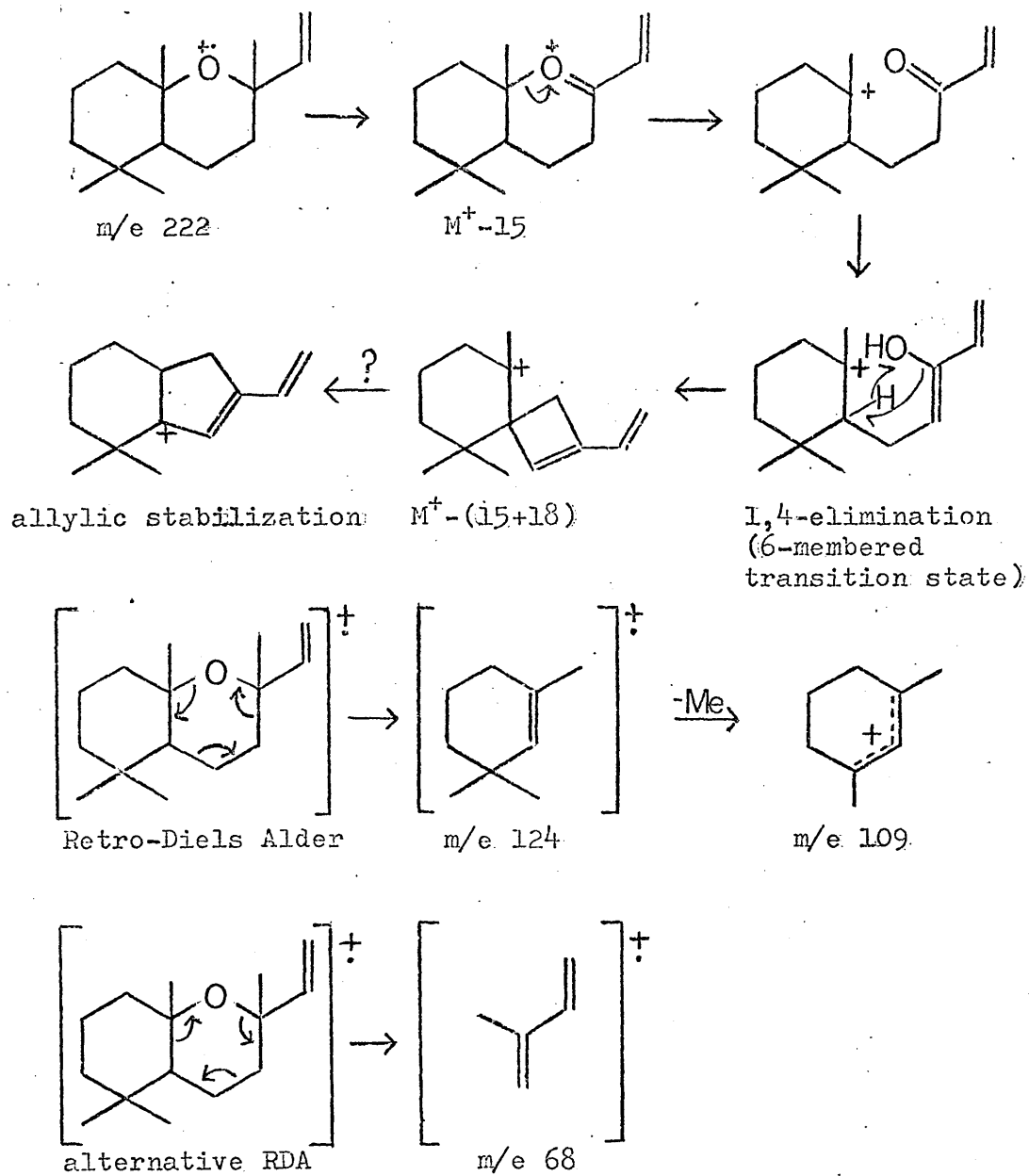
# Ring-opened product of caparrapi oxide



1,4-elimination    M⁺-18    m/e 82

m/e 124

afforded a dihydro-compound (M.W. 224 from mass spectrometry) showing no vinyl absorption.

The NMR spectrum (60 Mc/sec; Fig. 12) showed a typical vinyl multiplet, which was not further coupled, indicating an adjacent tertiary carbon atom. Four methyl groups could be distinguished by singlet peaks at $\tau$ 9.10 and 9.20 and (two superimposed) at 8.70. The absence of methylenic signals below $\tau$ 8 indicated that the ethereal oxygen was flanked by tertiary carbon atoms.

The above spectral data suggested that caparrapi oxide possessed the gross structure (16), formally derivable by cationic cyclisation of nerolidol. The alternative structure (17) is inconsistent with NMR data: the separation (cps) of peaks ascribed to the geminal methyl groups was proportional to the applied frequency, and double irradiation confirmed the absence of an isopropyl group. Mass spectroscopic data (Fig. 14) were also in better accord with (16).

Reduction of caparrapi oxide with lithium in liquid ammonia gave mainly (ca. 85% as judged by GLC) the expected product (18). This was not isolated in a pure state, but was characterised by NMR, which indicated five methyl groups (peaks at $\tau$ 8.40 (broad), 8.44 (centre of doublet), 8.84, 9.05 and 9.19 (singlets) ), a single olefinic proton ( $\tau$ ca. 4.75, multiplet) and two allylic protons of a methylene group ( $\tau$ ca. 7.9, multiplet). The IR spectrum included $\nu_{max}$ 830 and 1660 cm$^{-1}$ ( $\geq C = C \diagdown^{H}$). The mass spectrum (Fig. 14) was also consistent with structure (18). A possible rationalization

afforded a dihydro-compound (M.W. 224 from mass spectrometry) showing no vinyl absorption.

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of the fragmentation is shown in Fig. 14. Hydrogenation resulted

in formation of the dihydro-compound, the IR spectrum of which showed

no olefinic absorption. The mass spectrum was consistent with the

proposed structure.

Caparrapi oxide was converted smoothly by $OsO_4/NaIO_4$ cleavage

to the aldehyde (19). NMR showed singlet peaks for the gem-dimethyl

group ( $\tau$ 9.15, 9.20) and for the methyl groups flanking the ether

oxygen ( $\tau$ 8.70 and 8.80, consistent with the expected small change

in shielding). The aldehyde proton appeared as a sharp singlet at

$\tau$ 0.42. The infra-red spectrum ($CCl_4$ solution) showed <u>two</u> carbonyl

absorptions at 1740 cm$^{-1}$ ($\varepsilon$a, <u>ca.</u> 320) and 1732 cm$^{-1}$ ($\varepsilon$a, <u>ca.</u> 200) which

could perhaps be attributable to aldehyde rotamers.

Reduction of the aldehyde afforded alcohol (20). The infra-red

spectrum ($CCl_4$) showed a strongly intra-molecularly bound hydroxyl

group ($v_{max}$ 3570 cm$^{-1}$, $\varepsilon$a 40, unaffected by dilution). This bonded

form was also obvious in the NMR spectrum, in which two well defined

doublets centred on $\tau$ 6.72 and 6.90 ( J~10 c/s) could be attributed

to the hydrogen-bonded hydroxymethylene function.

Structure (16) was strongly supported by comparings its spectral

data (IR, MS and NMR) with those of manoyl and epimanoyl oxides

(28 and 29) and the monoterpenoid oxide (27). The IR spectrum of

caparrapi oxide (Fig. 12) closely resembled those of (27) (28) and

(29) [23, 24, 25] in accordance with the proposed correspondence of the

tetrahydropyran ring system and vinylic group. In the mass spectrum,

many of the abundant ions from caparrapi oxide had their counterparts

# Fig. 15



13-Epimanoyl oxide.

4.10  5.09  5.22  8.80 8.89 9.16 9.23 9.31  10.00



Manoyl oxide.

4.28  5.03  5.27  8.78 8.90 9.18 9.25  10.00
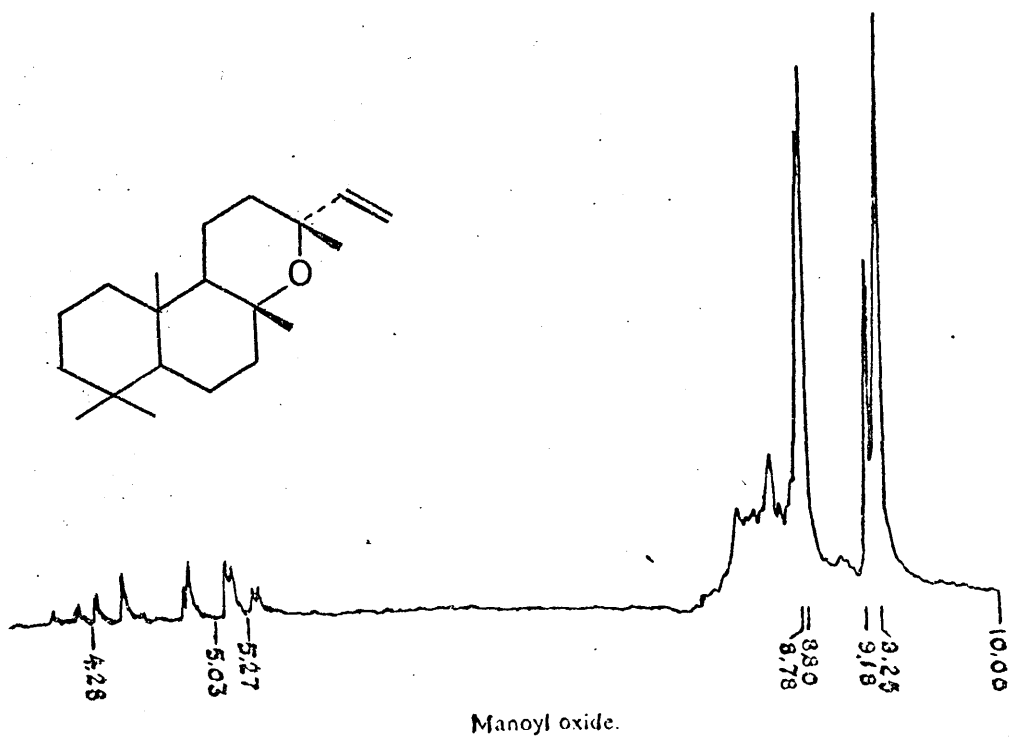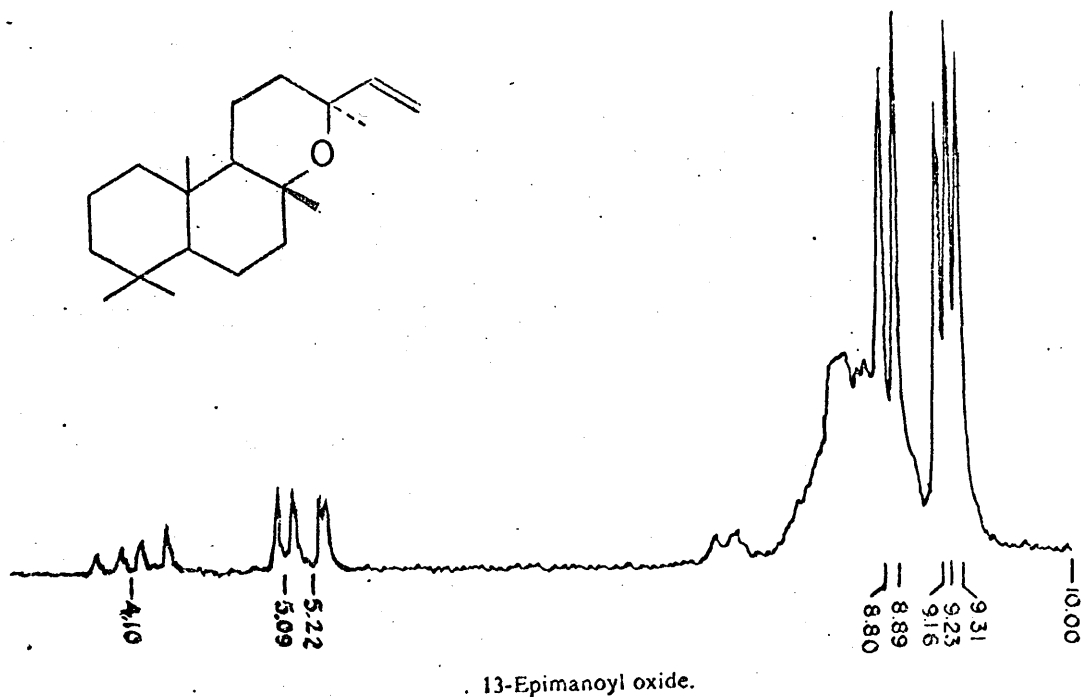
(Reprinted from Tetrahedron, 18, 173, 1962)

in the spectra [26] of (28) and (29). The abundant $M^+$-15 ion is a consequence of the stabilized oxonium ion obtained by the fragmentation depicted in Fig. 14. The authors of the manoyl oxide mass spectral publication offer no explanation for the loss of water from the oxonium ion. We suggest a plausible pathway for the corresponding loss in caparrapi oxide as being that depicted. Caparrapi oxide also exhibit the _two_ Retro-Diels Alder processes which can theoretically occur in the tetrahydropyran ring. The mass spectra of a series of derivatives of the oxide (19 - 26) were unremarkable, apart from the common presence of abundant ions at $m/e$ 177 and 195. The latter is the stabilized oxonium ion formed by loss of alkyl radical (19, CHO; 20, $CH_2OH$, etc.) rather than the methyl radical. The ion at $m/e$ 177 represents loss of water from the resultant oxonium ion (corroborated by a metastable peak at $m/e$ 160.9).

## TABLE 2.
### Chemical Shifts of Methyl Groups ($CDCL_3$)

| Manoyl oxides | | | | Caparrapi oxide and derivatives | | | |
|---|---|---|---|---|---|---|---|
| Oxide | $C_{(8)}$ | $C_{(13)}$ | Ref. | Oxide | $C_{(8)}$ | $C_{(10)}$ | $C_{(4)}$ |
| 28 | 8.73 | 8.73 | (27) | 16 | 8.70 | 8.70 | 9.10 | 9.20 |
| | 8.78 | 8.80 | (28) | | | | |
| 29 | 8.92 | 8.83 | (27) | 19 | 8.70 | 8.80 | 9.15 | 9.20 |
| | 8.89 | 8.80 | (28) | 20 | 8.72 | 8.84 | 9.12 | 9.24 |

The published NMR spectrum of (28)[27,28] (Fig. 15) is closely similar to that of (16), particularly in the vinylic region.

Fig. 16
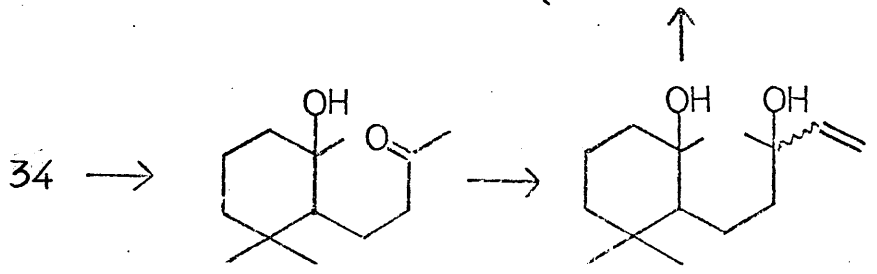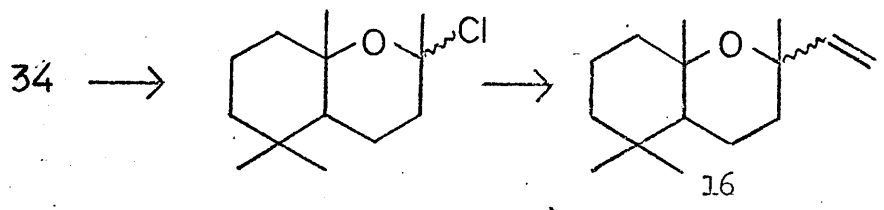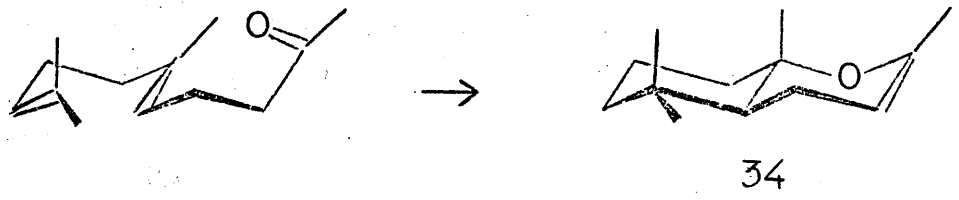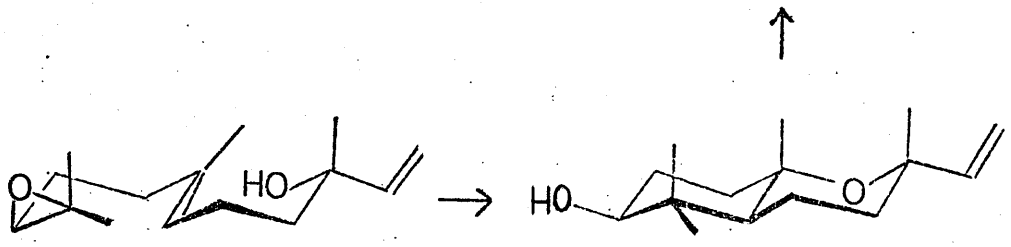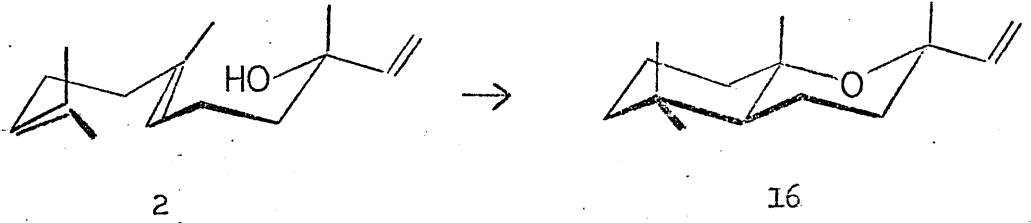
Moreover, the chemical shifts of the $C_{(8)}$ and $C_{(10)}$ methyl groups of caparrapi oxide (Table 2) are almost identical to those of the $C_{(8)}$ and $C_{(13)}$ methyl groups of manoyl oxide. The implied cis-configuration of the methyl groups in caparrapi oxide must be treated with some reserve in the absence of confirmatory evidence.

The co-occurrence of (+)-S-nerolidol and caparrapi oxide would suggest a biosynthetic link, involving the cationic cyclization of nerolidol as depicted in Fig. 16. In accordance with the postulate of anti-periplanar cyclization [29], the energetically most favourable product will result from the incipient carbonium ion which affords a trans – anti – trans 'decalin-type' system. The mechanistic requirements for this anti-periplanar cyclization to occur are assumed to be (a) synchronous bond formation taking place with essentially no conformational reorganization during the reaction; or (b) a stepwise reaction proceeding by formation of configurationally stable cation intermediates (e.g. bridged carbonium ions), followed by sufficiently-concerted addition steps. Examination of a model of (+)-S-nerolidol indicates the expected product to be caparrapi oxide with a trans ring-junction, and cis-oriented methyl groups at the $C_{(8)}$ and $C_{(10)}$ positions, as suggested by comparative NMR studies.

In vitro replication of this process has not yet succeeded. Reactions of nerolidol with a variety of cationic cyclizing reagents such as mineral acids, formic acid, boron trifluoride etherate and p-toluenesulphonic acid have yielded complex mixtures, none of which contained demonstrable quantities of caparrapi oxide. It is clear

from these results, that caparrapi oxide is not an acid-produced
artefact.

In retrospect, this failure to achieve cyclization may perhaps
be explained by the fact [30] that alcohols are generally stronger
bases than olefins, with the result that preferential protonation
at the hydroxyl group prevents the desired cyclization process.
Possible methods of achieving cyclization could involve either
(a) formation of nerolidol terminal epoxide, followed by boron
trifluoride-catalysed cyclization (Fig. 16), or (b) derivatizing
the hydroxyl function in such a way that the oxygen is rendered
less basic, e.g. by formation of the trifluoroacetate. Other
potential synthetic routes (Fig. 16) involve the precursor
trans - 1,3,7,7-tetramethyl-2-oxa-3,4-dehydro-bicyclo-(4,4,0) decane
(34) which has been prepared [31] in a high yield by the acid-
catalysed cyclization of geranyl acetone.

Relevant cyclizations have been described in the literature,
notably that [32] of geraniolene (30) which on treatment with dilute
sulphuric acid formed the tetrahydropyran (31) by a reversible
process (Fig.17). The possibility that caparrapi oxide may
therefore arise in vivo from farnesene cannot be precluded, even
though no trace of farnesene was present in the caparrapi oil samples
examined. Dehydrolinalool has been cyclized [25] under similar
circumstances to the acetylenic analogue (32), but linalool affords
hydrocarbon products. The pertinent acid-catalysed cyclization [33]

Fig. 17
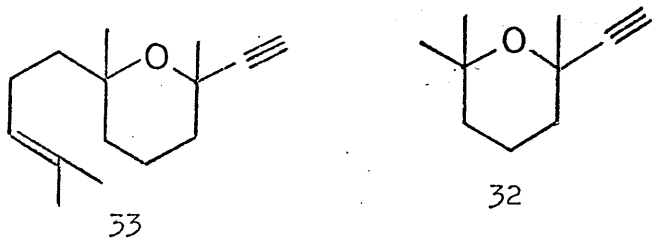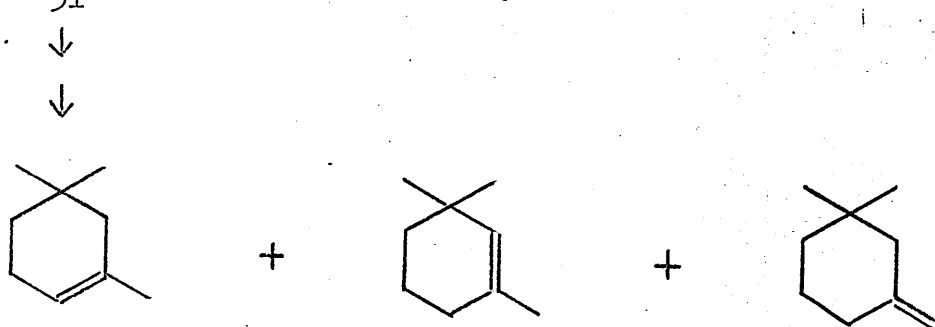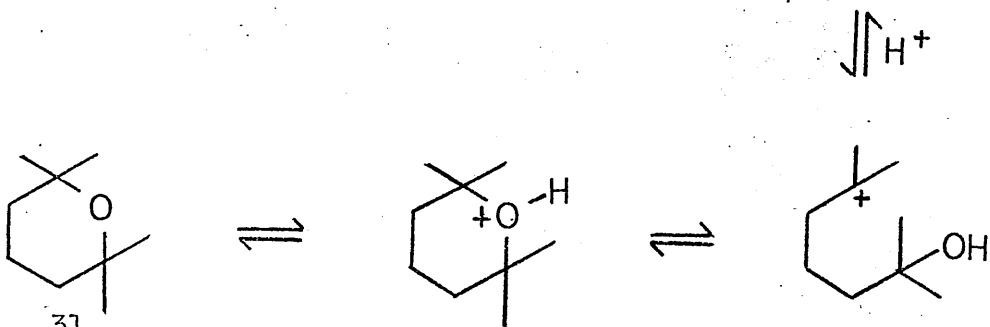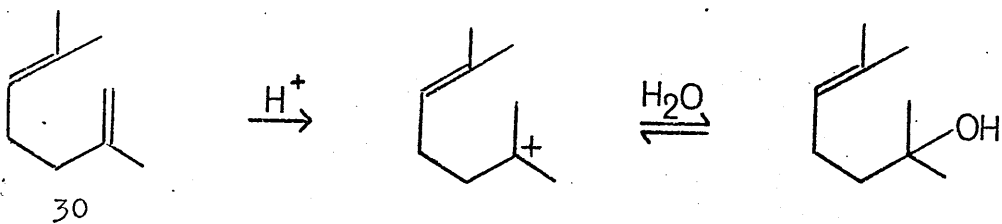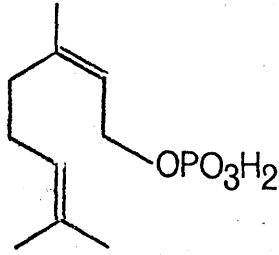
neryl phosphate
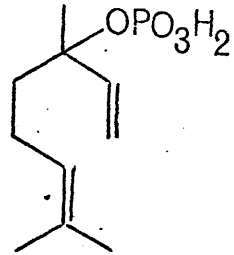
linalooyl phosphate
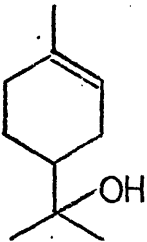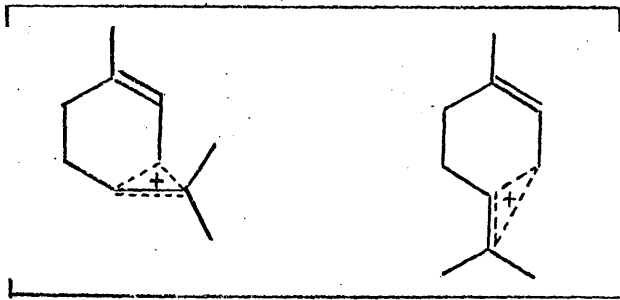
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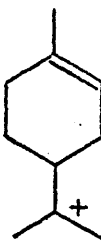
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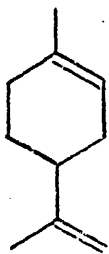
of the phosphate esters of nerol and linalool occurs via anchimerically-assisted attack (Fig. 17) of the $C_{(2)}-C_{(3)}$ double bond on $C_{(8)}$ of the terpene. The non-classical ion thus formed can either hydrate, or eliminate a proton. The acid-catalysed cyclization of nerolidol and farnesol has recently been the subject of a careful study.[33] Complex mixtures of monocyclic and bicyclic polyenes and monoalcohols were isolated in each case, the pathway presumably involving the reaction sequence; farnesol (nerolidol) → bisabolyl cation → rearranged bisabolyl cation → cadinyl cation → cadinyl derivatives. A private communication from Dr. Klein (Dragoco, Holzminden) describes the cyclization of dehydronerolidol in formic acid, the principal product of which appears to be the monocyclic oxide (33).

In conclusion, caparrapi oxide is therefore the isoprenologue of the monoterpene oxide (23) and the diterpene oxides (21, 22). Its biogenesis possibly involves cationic cyclization of either nerolidol or farnesene.

TABLE 3.

COMPARATIVE ANALYTICAL DATA FOR FIVE SAMPLES OF CAPARRAPI OIL

| Sample source and date | Insol. in NaHCO$_3$ | Sol. in NaHCO$_3$ | Characteristic constituents of neutral fraction | Approx. percentage | Retention index, 1% SE-30 100° |
|---|---|---|---|---|---|
| 1. Tropical Products Institute, 1962 | 100% | - | D Nerolidol. G. Caparrapidiol J Caparrapitriol | 90 9 1 | 1540 1680 |
| 2. Alberto Jimines, Botico Popular, Bogota. Dec. 1964 | 97.2% | 2.0% | D Nerolidol G Caparrapidiol | 90 8 | 1540 1680 |
| 3. Bought in Bogota, 1963. "Collected in 1943". | 96.8% | 3.2% | B Caparrapi oxide D Nerolidol | 25 75 | 1420 1540 |

TABLE 3 (Cont'd.)

| Sample source and date | Insol. in NaHCO$_3$ | Sol. in NaHCO$_3$ | Characteristic constituents of neutral fraction | Approx. percentage | Retention index, 1% SE-30 100° |
|---|---|---|---|---|---|
| 4. Mr. Aldo Albertini, 1961. | 83.2% | 10.8% | A (MW 194*) | — | 1380 |
| | | | B Caparrapi oxide | 43 | 1420 |
| | | | C (MW 206*) | 16 | 1490 |
| | | | D Nerolidol | 21 | 1540 |
| | | | E (MW 222*) | 7 | 1610 |
| | | | F (MW 240*) | 11 | 1620 |
| | | | G Caparrapidiol | — | 1680 |
| | | | H Isomeric diol ? | — | 1680 |
| 5. Bought in Bogota, Dec., 1964 | 97.6% | 2.4% | No constituent in common with other samples. | Data not determined | |

* Assignments of molecular ions are tentative partly because of the prevalence of eliminations, and partly as a result of incomplete separation.

TABLE 4.

| Examination of total neutral fraction | | | | Examination of separated components | | Tentative identification or designation |
|---|---|---|---|---|---|---|
| Retention | | Molecular ion | TLC Retention Zone; $R_f$ (Benzene) | Infra-red characteristics | Effect of Acetylation on GLC behaviour | |
| Time (min.) | I SE-30 100° | | | | | |
| 3.2 | 1380 | 194 loses 15 + 18 | Not detected | – | Peak removed, not located (GLC)b | Alcohol, A |
| 6.3 | 1420 | 222 | 0.60 | Corresponds to a vinylic tetrahydropyran | Unchanged (GLC, TLC) | Caparrapi oxide, B |
| 9.5 | 1490 | 206a | 0.80 | 970 vs, 910 w 850 diffuse | Unchanged (GLC, TLC) | Hydrocarbon C |
| 12.8 | 1540 | 222 | 0.40 | 910 s 980 w | Acetate 125° 1690 (I SE-30) | Nerolidol D |

## TABLE 4 (Cont'd).

| Retention Time $l^{100°}_{SE-30}$ (min.) | | Examination of total neutral fraction | | Examination of separated components | | Tentative identification or designation |
|---|---|---|---|---|---|---|
| | | Molecular ion | TLC Retention Zone; $R_f$ (Benzene) | Infra-red characteristics | Effect of Acetylation on GLC behaviour | |
| 20.0 | 1610 | 222 | Not detected | — | | Alcohol E |
| 20.5 | 1620 | 222 | 0.22 | Similar to nerolidol but v. intense 980 cm$^{-1}$ | Acetate ($I^{125°}_{SE-30}$ 1755) | Alcohol F |
| 28.5 | 1680 | no parent obs. but $M^+$–36 corresp. to mol wt.240 | 0.06 | 910 s $^c$ 980 w | bis-acetate $^c$ ($I^{125°}_{SE-30}$ 1900) | Caparrapidiol |
| 30.0 | 1685 | Similar to caparrapidiol | Presumed to be <u>ca.</u> 0.06 | — | — | Isomeric diol? H |

a   A peak at m/e 220 is ascribed to an impurity

b   Observation made on total neutral fraction

c   Data from pure diol isolated from sample No. 1.

Comparisons of Caparrapi Oil Samples.

Five samples of caparrapi oil were examined. A summary of
their origins, together with an indication of the principal
constituents found, is given in Table 3. Figs. 18 and 19, illustrate
respectively the results of comparative TLC and GLC analyses of the
neutral fractions derived from the samples, whilst Fig. 20 shows
mass spectra recorded for peaks obtained by GLC of the neutral
components of the most complex sample (No.4). Fig. 21 depicts
comparative TLC for the acidic fractions.

### Sample No. 1

The composition of this sample, which contained no acidic
material, was extremely simple as shown in Figs. 18 and 19. Separation
of the constituents was readily achieved by preparative TLC or on a
larger scale, by column chromatography. Nerolidol (90%),
caparrapidiol (9%) and caparrapitriol (0.8%) were isolated and
characterised.

### Sample No. 2

The neutral constituents of this sample accounted for 96%
of the oil, and were shown by comparative TLC and GC-MS to
correspond with those of sample 1.

### Sample No. 3

TLC and GC-MS studies on the neutral constituents (96%

# Fig. 18
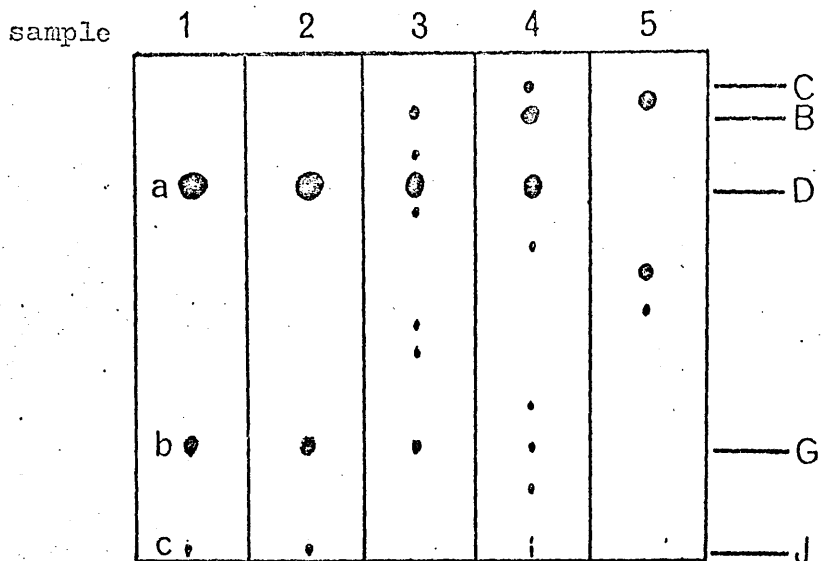


TLC comparison of caparrapi oils
   eluent benzene/ether (1/1)

B   caparrapi oxide

C   unidentified hydrocarbon

D   nerolidol

G   caparrapidiol

J   caparrapitriol

Fig. 19
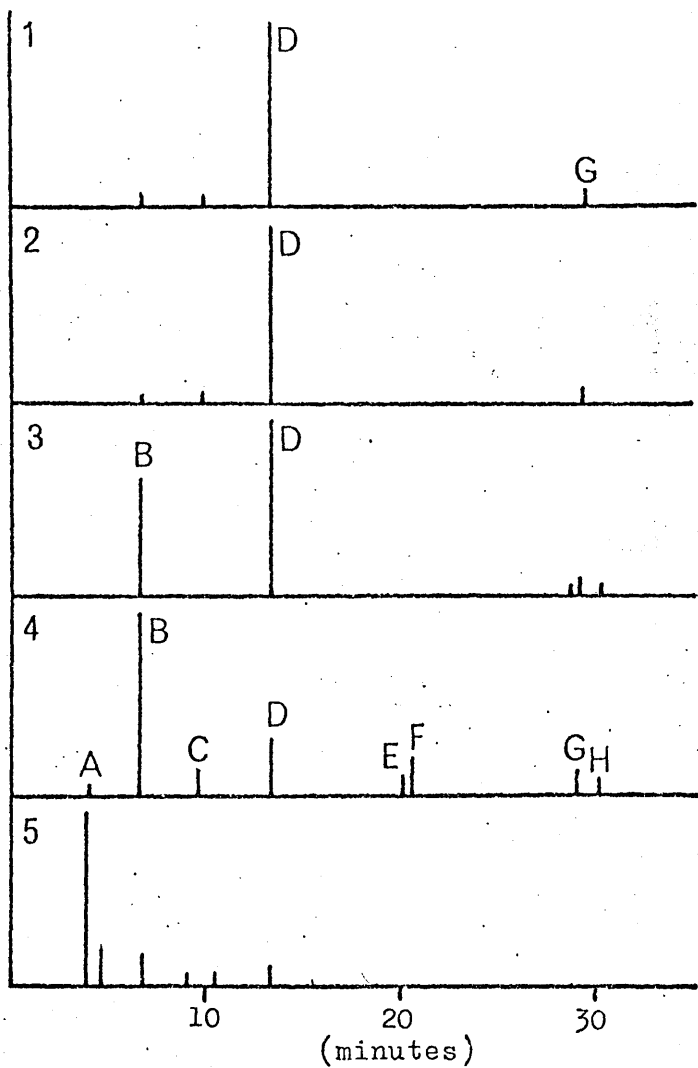


GLC comparison of caparrapi oils.
4' column, 1% SE-30, 100°C, 50 ml/min.

Fig. 20
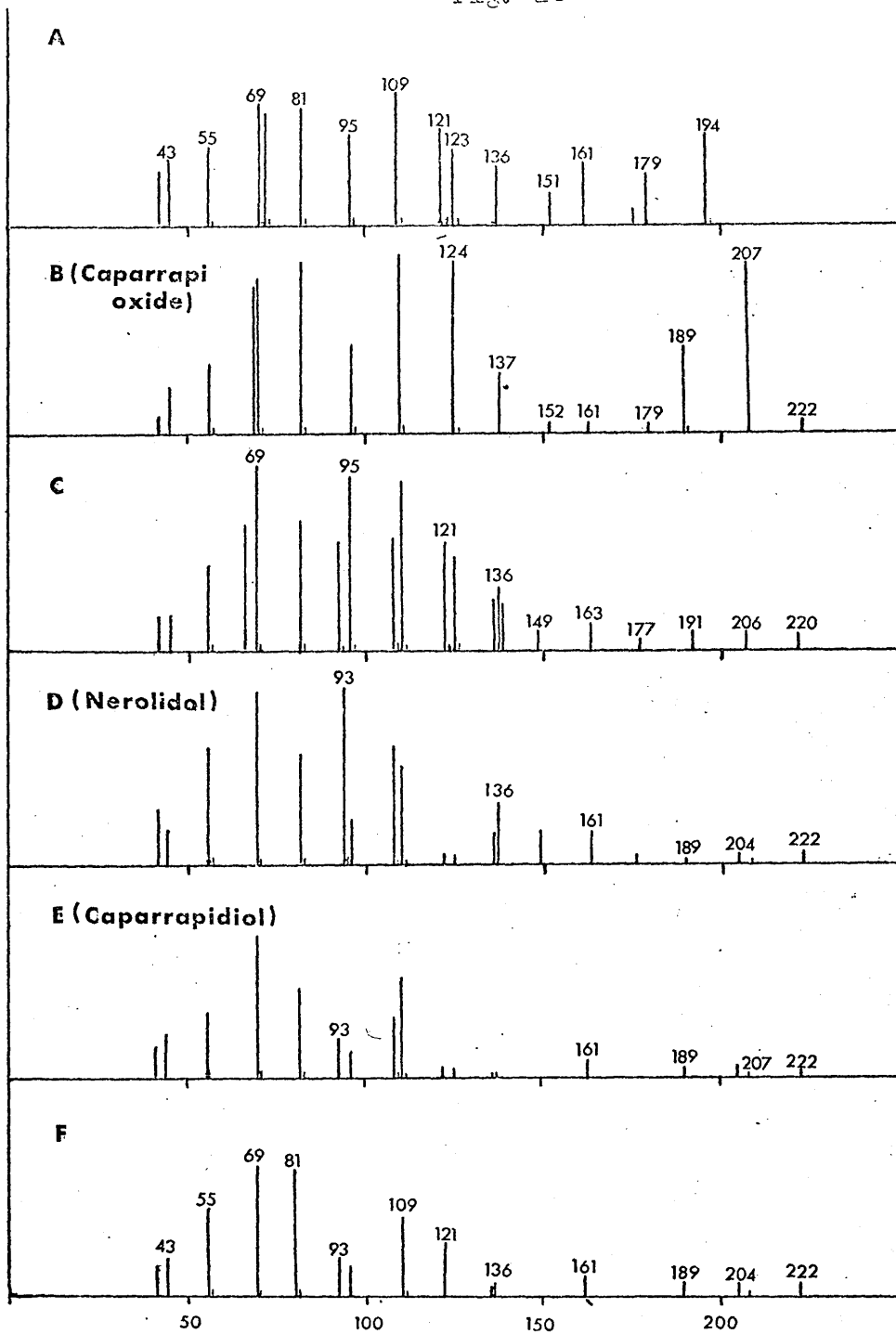
Mass spectra of constituents of sample 4.

Fig. 21

Sample



a   Isomeric mono-hydroxy
    acids

b   Di-hydroxy acid

c   Probably mono-hydroxy
    acids
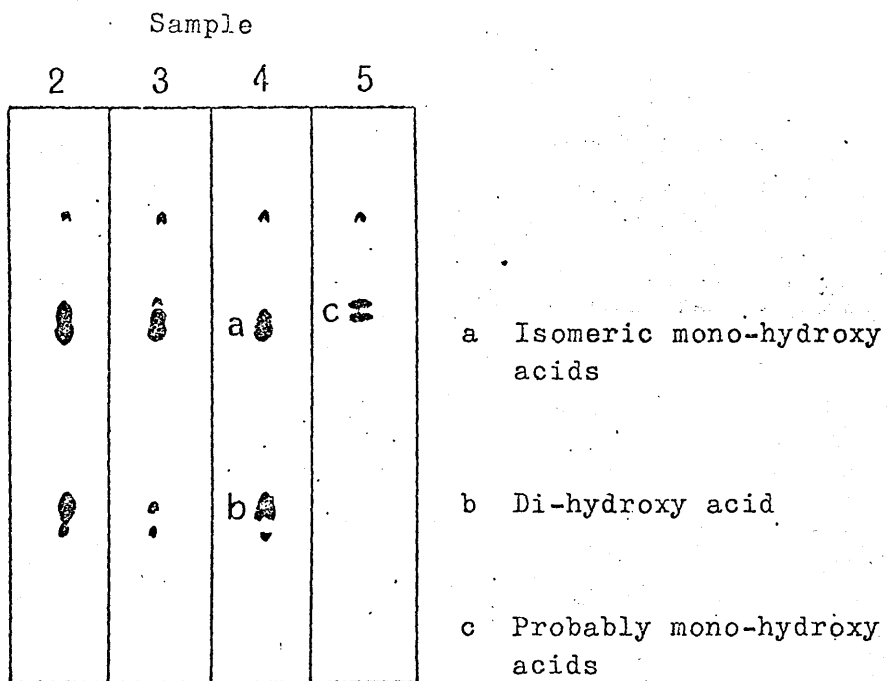
TLC comparison of acids, eluent-
benzene/dioxan/acetic acid (95/25/4)

by weight) showed the principal component to be nerolidol, together
with a high proportion of caparrapi oxide. GLC data indicated the
presence of trace amounts of several other components, including
caparrapidiol. Saponification had no significant effect on the
GLC behaviour of the mixture. The most notable features of this
sample, as compared with Nos. 1 and 2, were the slightly lower
content of nerolidol, and the presence of caparrapi oxide.

## Sample No. 4

Sample No. 4 proved to be the most complex of the caparrapi
oils (Figs. 18 and 19). Examination of the neutral fraction by
TLC disclosed a high proportion of relatively non-polar constituents.
This sample was also the richest in acidic material (10% by weight),
yielding two inseparable isomeric monohydroxy acids (a, Fig. 21)
and a dihydroxy acid (b, Fig. 21), all apparently of sesquiterpenoid
type. The neutral fraction was shown by comparative TLC, GLC and
GC-MS to consist of a mixture of several components, as indicated
in Table 4. The principal constituent was caparrapi oxide,
identical with the minor component of sample No. 3. Nerolidol was
present in smaller proportion than in samples 1-3. A sample of the
material was treated with acetic anhydride - pyridine in a sealed
tube at 160°C and the total product examined by GLC. The results
showed that peaks B (caparrapi oxide) and C were unchanged, whereas
the other components had evidently reacted. Combined GC-MS of the
neutral fraction yielded the mass spectra shown in Fig. 20: peaks

E and F, and peaks G and H were poorly resolved in this instance, and the corresponding mass spectra, though recorded at the appropriate retention times, probably represent mixtures. Fig. 20 includes only those representing E and G respectively. The assignment of molecular ions is uncertain for hydroxylic compounds because of the prevalence of thermal dehydration.

Separation of the main constituents of the neutral fraction was readily achieved by preparative TLC (benzene). Five zones were observed, four of which were eluted for further examination. GLC indicated that each contained one major component: correlations of retention data and $R_f$ values are indicated in Table 4. Infra-red spectra were also recorded.

Compound A, a minor constituent, was not detected in the preparative TLC separation. The mass spectrum was consistent with a formula $C_{13}H_{21}OH$ but no further data have been obtained.

Compound B, isomeric with nerolidol (molecular ion 222) was the most interesting constituent: its markedly distinct mass spectrum prompted a more detailed study, and the structure (16) caparrapi oxide, was elucidated.

Compound C gave an apparent molecular ion at $m/e$ 206; a small peak at $m/e$ 220 seemed to be an impurity. The compound is presumed to be a hydrocarbon $C_{15}H_{26}$, but has not been identified.

Compound D was identified as nerolidol by comparative TLC, GLC, IR and mass spectrometry. Compounds E and F appear to be isomeric with nerolidol.

Compounds G and H appear to comprise an isomeric pair:
G corresponded to caparrapidiol, already described as isolated
from sample No. 1.

### Sample No. 5

TLC and GC-MS examination of the neutral fraction (94% by
weight) of this oil showed such gross differences from the other
samples that its origin must be in doubt. The acidic fraction was
also quite different in composition (Fig. 21). The constituents
were not conveniently separable by preparative TLC. Saponification
yielded an acidic fraction (0.64 g from 1.70 g of neutral oil) which
could not be crystallised. Treatment with diazomethane gave a
product yielding two peaks on GLC but remaining unresolved on TLC.
GC-MS indicated molecular weights of 318 and 332. In view of the
apparently non-sesquiterpenoid nature of these compounds they have
not been examined further at the present time.

TABLE 5
## Antibacterial activity of samples of oil

Four samples of oil were tested for antibacterial activity on a range of 8 species of bacterium. The oils were dissolved in ether and spots of the ethereal solutions made on a nutrient agar plate. Stroke inocula of the bacteria were made across the spots and any inhibition of growth noted. The results are given below.

|  | Oil 1 | Oil 3 | Oil 4 | Oil 5 |
|---|---|---|---|---|
| Escherichia coli | - | - | - | - |
| Klebsiella aerogenes | - | - | - | - |
| Pseudomonas aeruginosa | - | - | - | - |
| Proteus vulgaris | - | - | - | - |
| Staphylococcus aureus | ? | - | - | + |
| Bacillus cereus | ? | - | - | + |
| Cornybacterium xerose | ? | + | - | + |
| Mycobacterium phlei | + | - | - | + |

- = no inbibition
+ = inhibition
? = doubtful

None of the oils had any inhibitory effect on the gram-negative bacteria (the first four on the list). Oil No. 5 was inhibitory to all the gram-positive bacteria, oil No. 3 to C.xerose, and oil No. 1 was slightly inhibitory to the organism M. phlei.

The acid-fast organism M. phlei was included because of its relationship to other acid-fast bacteria such as M. tuberculosis and M. leprae.

## Conclusions

Of the five samples examined[*], one (No. 5) appeared to be of totally different composition from the others and we accordingly believe that it is unlikely to be a genuine sample of caparrapi oil. Samples 1-4 were sufficiently similar in qualitative composition to suggest a common botanical origin, although certain interesting differences were observed: these are perhaps attributable to species variations or seasonal variations. We have observed no appreciable changes in composition during storage of the oils for periods up to six years. The stability of nerolidol and caparrapidiol in the oil is noteworthy, because once these constituents have been isolated they are extremely susceptible to decomposition in the presence of air.

The most characteristic constituent is nerolidol: indeed, two of the samples (Nos. 1 and 2) appear to be the richest sources of this compound so far described. The oil is further characterised by the presence of acyclic sesquiterpenoids such as caparrapidiol, caparrapitriol and dihydroxy-caparrapi acid. Inhibition of the cyclization and oxidation processes which normally take place in plants has apparently occurred. Cyclization, when it does occur,

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[*]Antibacterial screening of the various samples was performed by Dr. R.B. Morrison (Bacteriology Dept., University of Glasgow) whose results are tabulated in Table 5. They indicate that the 'medicinal properties' of caparrapi oil are probably not due to anti-bacterial activity.

is exemplified by the 'abnormal' product caparrapi oxide.  The

essential oil is therefore primitive in that it comprises mainly

sesquiterpenoids in an early stage of biosynthesis.

It is interesting to speculate as to how much phylogenetic

insight would be provided by a knowledge of the steps of

biosynthesis of all known secondary constituents.  Present

knowledge in this field is fragmentary.

# Experimental Procedure

Conventional techniques were used for column chromatography, thin layer chromatography (TLC) and gas-liquid chromatography (GLC). Materials used included Kieselgel G (Merck) for analytical TLC and Kieselgel $HF_{254}$ (Merck) on 0.1 cm thick 20 x 20 cm preparative TLC plates. Spots were detected by charring with ceric sulphate – sulphuric acid reagent or spraying with iodine vapour. Bands were detected by the suppression of fluorescence of "HF 254" or by the method of staining separate lanes with a destructive reagent. The column packing (1% SE-30 on 100-120 mesh silanised Gas-Chrom P) most commonly used for GLC was prepared according to the method of Horning et al. [34] Analytical gas chromatograms were run on either Pye Argon chromatographs or the Perkin Elmer F-11.

Melting points were recorded on a Kofler block and are uncorrected. Rotations were measured in chloroform at room temperature unless otherwise stated. Light petroleum refers to the fraction of b.p. $40\text{-}60^\circ C$.

Ultraviolet spectra were measured on an automatic-recording instrument (Unicam SP800). Routine infra-red spectra were measured on a Unicam SP200 model and high resolution spectra on the SP100 double beam spectrophotometer. Mass spectra were measured on an AEI MS9 spectrometer and gas chromatography– mass spectrometry (GC-MS) was effected on an LKB 9000 instrument. Nuclear magnetic resonance (NMR) spectra were determined on a Perkin Elmer 60 Mc/s. instrument

or on a Varian HA 100 model equipped with a spin decoupler.

Several of the reactions described in the experimental section were performed on the micro-scale and consequently, characterisation of the products relies primarily on GC-MS data, rather than elemental analysis.

Chromatographic Separation of the Constituents of Sample 1.

A.

Sample 1 (3.1 g) was adsorbed on to neutral alumina
(150 g Woelm, grade III). A gradient elution using petroleum
(2 l) / diethyl ether (2 l), with collection of aliquots of
40 ml yielded, in fractions 17-38, a clear, fragrant oil (2.76 g).
Analysis, following preparative TLC separation and vacuum diffusion
purification, indicated the formula $C_{15}H_{26}O$. This fraction was
shown to be (+)-S-nerolidol from its IR, NMR, mass spectra,
its physical constants ($n^{20}$, 1.4807; $[\alpha]_D$ + $18^o$ in ethanol) and
GLC properties.

Fractions 82-97 yielded caparrapidiol (0.29 g) as a clear,
faintly fragrant, viscous oil $[\alpha]_D$ + $8^o$ (C, 1.0 in chloroform),
which decomposed readily on exposure to air, but which could be stored
in ethanol at $0^oC$. Following preparative TLC and vacuum diffusion
purification ($80^o/0.03$ mm Hg), analysis indicated the composition
$C_{15}H_{28}O_2$ (Found, C, 74.67; H, 11.82%; Calcd., C, 74.95; H, 11.74%).

The column was stripped (after standing at room temperature
for several days) by elution with methanol (250 ml) which on
evaporation at $100^oC$ on a Büchi Rotary Evaporator yielded a pink
liquid residue containing organic material and silicates. Extraction
of this residue with ethyl acetate afforded 60 mg of a brownish oil,
which was adsorbed on a preparative TLC plate and eluted with ether.
The principal constituent, caparrapitriol (25 mg, $R_f$ 0.1) was

extracted with ethyl acetate, as a clear oil which crystallised

on standing. Sublimation at $115^{\circ}/0.03$ mm Hg yielded a white

crystalline solid mp $96-97^{\circ}$, $[\alpha]_D + 3^{\circ}$ (c 1.4 in chloroform).

Analysis indicated the formula $C_{15}H_{30}O_3$ (Found, C, 69.52;

H, 11.45%; Calcd., C, 69.72; H, 11.70%).

The relatively non-polar minor constituents isolated from

the preparative TLC were artefacts formed by the alumina-catalysed

dehydration of caparrapitriol. Comparative TLC and GLC showed

these compounds to correspond to nerolidol (3 mg) and

caparrapidiol (2 mg).

B.

Sample 1 (100 mg) was adsorbed onto a preparative TLC plate

and eluted with ether. Nerolidol (90 mg, $R_f$ 0.8), diol (9 mg,

$R_f$ 0.5) and triol ($\sim 1$ mg, $R_f$ 0.1) were isolated in a pure state on

extraction of the appropriate bands with polar solvents such as

ethyl acetate.

## Hydrogenation of Caparrapidiol (10)

Caparrapidiol (5 mg) was dissolved in ethyl acetate and

hydrogenated over 10% palladium-charcoal overnight. Comparative

TLC and GLC showed one main product together with a less-polar,

minor product (possibly from hydrogenolysis). The principal

product (3 mg) was isolated as an oil by preparative TLC, eluting

with ether. Mass spectrometry indicated a molecular weight increase

of four mass units, corresponding to uptake of two moles of hydrogen.

Fig. 22
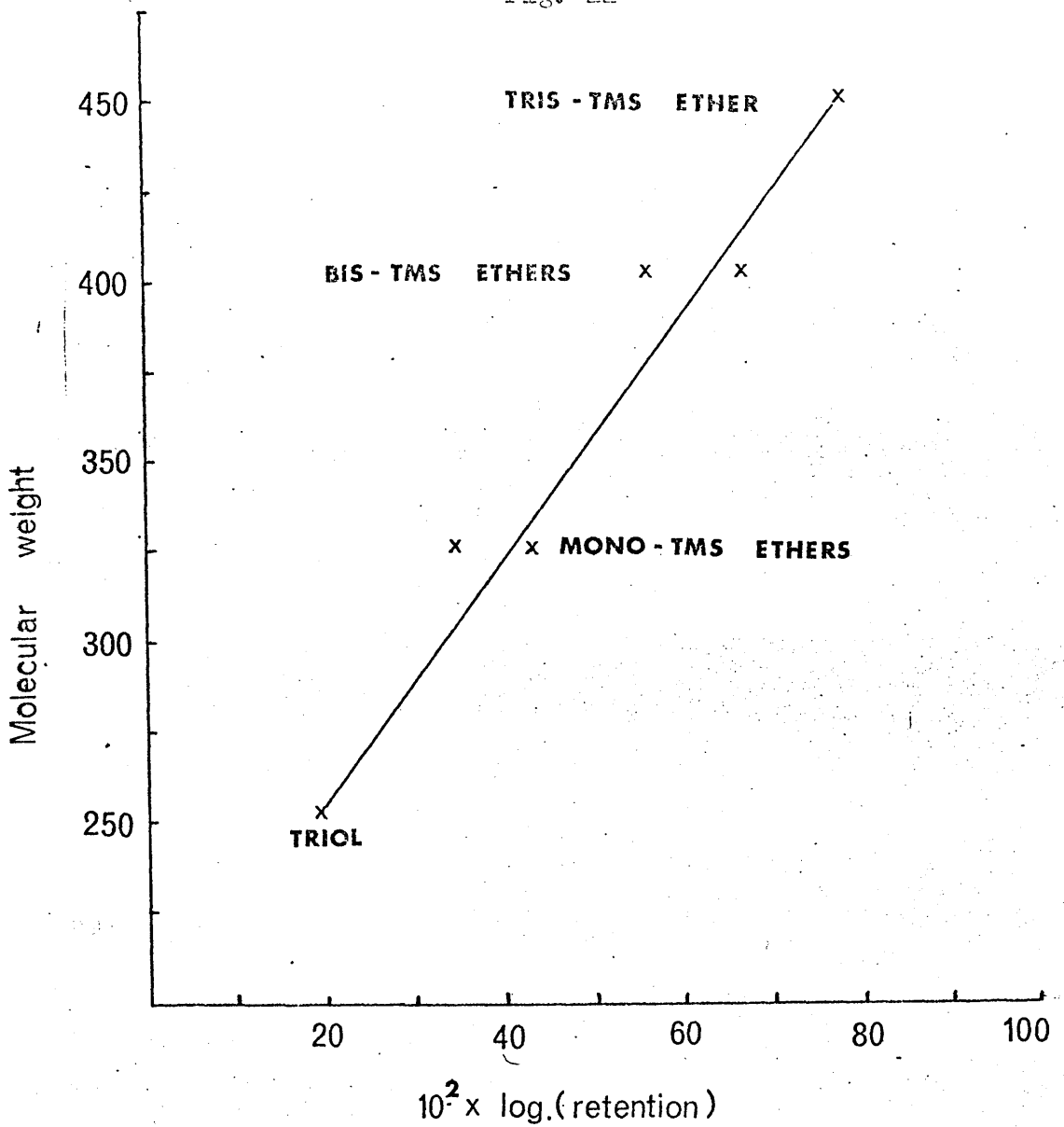
## Trimethylsilylation Procedures

A.  Incomplete trimethylsilylation.

Polyol (1 mg) was dissolved in hexamethyldisilazane (200 $\mu$l) and trimethylchlorosilane (catalytic trace) added. The solution was heated at 60-70$^{\circ}$ for 6 hours, evaporated in a stream of dry nitrogen and the derivative(s) extracted from the resultant white residue in dry ether. Thus, caparrapidiol (10) afforded two products as shown by GC-MS; $I_{SE-30}^{125}$ 1750, molecular ion 312 and $I_{SE-30}^{125}$ 1775, molecular ion 312. Caparrapitriol (12) afforded four products; $I_{SE-30}^{150}$ 1850 (M$^+$330), $I_{SE-30}^{150}$ 1900 (M$^+$330), $I_{SE-30}^{150}$ 1960 (M$^+$402) and $I_{SE-30}^{150}$ 2005 (M$^+$402). The relationship between the retention times of the triol trimethylsilyl ethers and their molecular weights is shown in Fig. 22.

B. Complete trimethylsilylation.

To polyol (2 mg) in a melting-point capillary tube was added hexamethyldisilazane (200 $\mu$l) and a catalytic trace of trimethylchlorosilane. The tube was sealed and heated at 160$^{\circ}$ for 12 hr. The derivatives were isolated as above, and characterized by GC-MS:

Bis-trimethylsilyl ether from caparrapidiol (10), $I_{SE-30}^{125}$ 1770 (M$^+$384)

Tris-trimethylsilyl ether from caparrapitriol (12) $I_{SE-30}^{140}$ 2055 (M$^+$474);

Bis-trimethylsilyl ether from dihydroxy methyl ester (13), $I_{SE-30}^{140}$ 2040 (M+432)

Tris-trimethylsilyl ether from triol (15), $I_{SE-30}^{140}$ 2100 (M$^+$476).

Extraction of Acids from Caparrapi Oil

Each sample of caparrapi oil (approximately 5 g in 25 ml ether) was extracted twice with saturated aqueous sodium bicarbonate (100 ml). Acidification with dilute hydrochloric acid was followed by two extractions with ether and one with ethyl acetate. The organic extracts were combined, dried over anhydrous magnesium sulphate and evaporated _in vacuo_, yielding a clear viscous oil. Analytical data are presented in Table 3, p. 26.

Methylation of Acids from Sample 4

30% sodium hydroxide (0.2 ml) was added to a solution of bis-(N-methyl-N-nitroso)-terephthalimide (0.6 g) in diethylene glycol (1.5 ml) and ether (10 ml). The gaseous diazomethane evolved on warming the resultant suspension was distilled into a solution of the acids (226 mg) in ether (20 ml). Unreacted nitrosan was neutralized by the careful addition of glacial acetic acid. The diazomethane solution was left at room temperature for 2 hours and concentrated _in vacuo_, yielding methyl esters (240 mg) as an oil. GLC retention data and molecular ions (GC-MS) of products:

(A) $I_{SE-30}^{150}1800$ ($M^{+}270$); (B) $I_{SE-30}^{150}$ 1830 ($M^{+}270$); (C) $I_{SE-30}^{150}1920$ (M+288).

Separation of Monohydroxy and Dihydroxy Methyl Esters

Methyl esters (70 mg) were adsorbed on to a preparative TLC plate and developed with diethyl ether. Extraction of the two principal bands afforded 30 mg oil from each. Band 1 ($R_f$ 0.6)

contained two inseparable monohydroxy esters (A) and (B). Band 2

($R_f$ 0.2) afforded a dihydroxy ester (C) as an oil (25 mg) shown by

GLC to be homogeneous. $v_{max}$ 1710 cm$^{-1}$ (liquid film); $[\alpha]_D$ 0$^o$

(c, 1.0 in chloroform): found, C, 64.63; H, 10.91% Calcd. for

$C_{16}H_{32}O_4$, C, 66.63; H, 11.18%: Calcd for $C_{16}H_{32}O_4 . ^{1/2}H_2O$, C, 64.7;

H, 11.1%: Calcd for $C_{16}H_{32}O_4 . H_2O$, C, 70.1; H, 12.4%

## Attempted Separation of Monohydroxy Esters

(i) Differential vacuum diffusion.

The monohydric ester mixture (54 mg) was subjected to

careful vacuum diffusion using a 'cold-finger' condensation

unit at 0.03 mm Hg. The temperature was slowly raised from

60-100$^o$C. Fractions were collected at 60-70$^o$, 70-80$^o$ and

80-100$^o$ ranges. GLC showed no separation under these conditions.

(ii) Preparative TLC.

Careful chromatography of 32.8 mg oil on a preparative

TLC plate (20 cm x 20 cm x 0.1 cm), eluting with 50% light

petroleum-ether, failed to separate the constituents.

(iii) Silver nitrate chromatography.

TLC performed as above using silver nitrate impregnated

kieselgel as solid support failed to separate the constituents.

(iv) Column chromatography.

Chromatography on neutral alumina (Woelm, Grade III)

using a 50:1 weight ratio of alumina to esters, performing a

careful diethyl ether - light petroleum gradient elution failed to separate the constituents.

### Treatment of Monohydroxy Esters with Osmium Tetroxide

The monohydroxy ester mixture (20 mg, 0.07 mM) was dissolved in ether (2 ml) and added dropwise to osmium tetroxide (20 mg, 0.08 mM) in ether (5 ml). 4 drops of pyridine were added and the solution left standing at ambient temperature overnight. The osmate complex was decomposed by adding saturated aqueous sodium metabisulphite (10 ml) and stirring for 30 minutes. TLC of the ethereal extract showed only starting material.

### Reduction of Dihydroxy Ester (13) to Triol (15)

Dihydroxy ester (26 mg) was dissolved in ether (2ml) and a slurry of lithium aluminium hydride ($\sim$15 mg) in ether added slowly with stirring at room temperature. After stirring overnight, water was added dropwise and the mixture extracted several times with ether. The ethereal extract was dried over anhydrous magnesium sulphate and evaporated under reduced pressure, yielding an oil (18 mg). Preparative TLC, eluting with ether, yielded a clear oil (12 mg). Analysis, following vacuum diffusion purification at $130^{\circ}/0.08$ mm Hg; found, C, 67.62; H, 12.08%; Calcd. for $C_{15}H_{32}O_3$, C. 69.18; H, 12.59% . Calcd. for $C_{15}H_{32}O_3 \cdot \frac{1}{2} H_2O$, C, 67.0; H, 12.5%: Calcd. for $C_{15}H_{32}O_3 \cdot H_2O$, C, 64.8; H, 11.5%

## Reduction of Monohydric Esters to Diols

50 mg of a mixture of the two monohydric esters was reduced and purified as above, yielding an oil (40 mg) which, although apparently homogeneous by TLC, was shown to consist of a mixture of two compounds ($I_{SE-30}^{150}$ 1785 and 1810) by GLC. Attempted resolution of the diol mixture by conventional separation techniques failed.

## Chromatographic Separation of Caparrapi Oxide (16)

The neutral fraction of sample 4 of caparrapi oil (1 g) was adsorbed on to neutral alumina (40 g Woelm Grade III) and subjected to gradient elution, using petroleum (500 ml b.p. 60-80°) and diethyl ether (500 ml), collecting 50 ml aliquots. Fractions 2-4 eluted with 1% ether/petrol afforded caparrapi oxide (140 mg). Preparative TLC and vacuum diffusion (40°/5 mm Hg) yielded pure caparrapi oxide $[\propto]_D$ -18° (c, 1.0 in chloroform). Found, C, 80.97; H, 11.87%; Calcd. for $C_{15}H_{26}O$; C, 81.02; H, 11.97% $I_{SE-30}^{100°}$ 1420. Further GLC on three phases of graded polarity showed no indication of inhomogeneity (retention indices (100°C) were respectively 1430, 1540 and 1570 on 1%OV-1, 1% OV-17 and 3% OV-22).

## Hydrogenation of Caparrapi Oxide (16)

Caparrapi oxide (5 mg) was dissolved in ethyl acetate (3 ml) and 10% palladium-charcoal (5 mg) added. The solution was hydrogenated for 2 hours, filtered through Celite and concentrated, yielding an oil (4 mg). GC-MS indicated one hydrogenation product

(molecular ion $m/e$ 224, $I^{120}_{SE-30}$ 1430).

## Effect of Lithium Aluminium Hydride on Caparrapi Oxide (16)

Oxide (18.2 mg) was dissolved in sodium-dried ether (2 ml) and lithium aluminium hydride (~10 mg) added slowly with magnetic stirring. After 12 hours, slightly acid water was added dropwise and the solution extracted twice with ether. Comparative TLC showed no reaction to have occurred.

## Ring-Opening of Caparrapi Oxide (16)

Oxide (50 mg) in ether (2 ml) was added dropwise to lithium (200 mg) in ammonia (50 ml) in a 'cold finger' reaction vessel. The deep blue solution was maintained at liquid ammonia temperature for 1 hour; ammonium chloride (500 mg) and ethanol (10 ml) was added. The liquid ammonia was allowed to boil off, and the residue partitioned between water and ether. The ethereal extract on evaporation under reduced pressure, afforded a clear oil (50 mg). Preparative TLC (benzene) afforded the principal constituent as an oil (43 mg), the infra-red characteristics of which corresponded to the expected ring-opening product ($v_{max}$ 3600, 1660, 830 cm$^{-1}$). GC-MS revealed the presence of isomeric constituents in the ratio 85: 15 ($I^{125}_{SE-30}$ 1545 and 1555 respectively.) This mixture was unresolvable in several TLC solvent systems, and consequently was not submitted for elemental analysis.

## Hydrogenation of Ring-Opening Product (18)

Ring-opening product (4 mg) was dissolved in ethyl acetate

(3 ml) and 10% palladium-charcoal (~5 mg) added. Following 30 minutes hydrogenation, the solution was filtered through celite and concentrated under vacuum at room temperature, yielding an oil (3 mg). Infra-red (no $V_{max}$ 830 cm$^{-1}$) and GC-MS (molecular ion $m/e$ 226, $I_{SE-30}^{120^{o}}$ 1555) indicated complete hydrogenation of the olefinic function. Minor constituents (less than 10% of the total) were not characterised.

## Cis-Hydroxylation of Caparrapi Oxide (16)

Caparrapi oxide (15 mg, 0.06 mM) was dissolved in dry tetrahydrofuran (3 ml). Osmium tetroxide (20 mg, 0.08 mM) in dry tetrahydrofuran (0.5 ml) was added with stirring. One drop of pyridine was added as catalyst. The solution, which darkened immediately, was left stirring overnight at room temperature. Saturated aqueous sodium metabisulphite (5 ml) was added and the solution stirred for one hour, gradually becoming red. The solution was extracted twice with ether, which, after drying over anhydrous sodium sulphate and evaporation under reduced pressure afforded a diastereoisomeric diol mixture as an oil (14 mg). ($I_{SE-30}^{140^{o}}$ 1800 and 1820).

## Periodate Cleavage of Diol Mixture (23)

The mixture of diastereoisomeric diols (13 mg) was dissolved in ethanol (2 ml) and saturated aqueous sodium periodate (2 ml) added. A white precipitate formed after several minutes stirring at room temperature. After 4 hours the solution was extracted with ether, which was then dried over anhydrous magnesium sulphate and

concentrated at room temperature under reduced pressure, yielding

an oil (10 mg) purified by vacuum diffusion ($40°/5$ mm Hg).

(Found; C, 74.70; H. 10.63% Calcd. for $C_{14}H_{24}O_2$; C, 74.95; H, 10.78%)

$v_{max}$ ($CCl_4$) 1740 cm$^{-1}$ ($\varepsilon_a$, ca 320); shoulder at 1732 cm$^{-1}$ ($\varepsilon_a$ ca 200):

$I^{125}_{SE-30}$ 1550.

### Reduction of Aldehyde (19)

Aldehyde (38 mg, 0.14 mM) was dissolved in dry ether (4 ml)

and lithium aluminium hydride (8 mg, 0.2 mM) added slowly with

stirring. After one hour, methanol (2 ml) was added dropwise,

followed by water (2 ml). The ether extract, dried over anhydrous

sodium sulphate and concentrated under vacuum afforded an oil (36 mg),

which after preparative TLC and vacuum diffusion purification

($40°/5$ mm Hg) gave pure alcohol (23 mg): $[\alpha]_D$-5.5° (c, 1.0 in

chloroform). Found, C, 74.59; H, 11.81%; Calcd. for $C_{14}H_{26}O_2$,

C, 74.29; H, 11.58%: $v_{max}$ ($CCl_4$) 3570 cm$^{-1}$ ($\varepsilon_a$ 40) unaffected

by dilution. (cf. 2-hydroxymethyltetrahydropyran, $v_{max}$ ($CCl_4$) 3579

($\varepsilon_a$ 55).

### p-Bromobenzoate of Alcohol (20)

Alcohol (20) (20 mg, 0.09 mM) was dissolved in pyridine (0.2ml)

and a solution of freshly crystallised p-bromobenzoyl chloride

(20 mg, 0.10 mM) in pyridine (0.2 ml) added. The solution was

left overnight at 0°C and partitioned between 1N hydrochloric

acid (20 ml) and diethyl ether (40 ml). The ethereal extract was

dried over anhydrous sodium sulphate, and yielded after evaporation

under vacuum, a crystalline solid (22 mg) mp.60.5-61.5° (following recrystallisation from light petroleum). Found, C, 61.48; H, 6.99% Calcd. for $C_{21}H_{29}BrO_3$, C, 61.61; H, 7.14%. Recrystallization from a wide range of solvents yielded crystals of a fibrous nature which were unsuitable for X-ray crystallography.

## Trimethylsilylation of Diastereoisomeric Diols (23) Derived from Caparrapi Oxide

The diastereoisomeric diol mixture (1 mg) was dissolved in hexamethyldisilazane (2 drops) and a catalytic quantity of trimethylchlorosilane added. The solution was heated in a sealed vial at 70° for 24 hours and evaporated with a stream of nitrogen. Extraction of the white residue with ether (1 ml) yielded bis-trimethylsilylation products, as shown by GC-MS. Diols: $I_{SE-30}^{140°}$ 1800 and 1820. Bis-trimethylsilylation products (molecular ion $m/e = 400$): $I_{SE-30}^{140°}$ 1890 and 1910.

## Methoxyamination of Aldehyde (19)

To aldehyde (19) (3 mg) in pyridine (0.5 ml) was added O-methylhydroxylamine hydrochloride (3 mg) in pyridine (0.1 ml). The solution was heated in a sealed vial for 3 hours, filtered, and an aliquot of the solution injected directly on to GLC. Complete derivatization was indicated: Aldehyde, $I_{SE-30}^{120°}$ 1450 O-methyloxime, $I_{SE-30}^{120°}$ 1620. (Molecular ion on GC-MS, $m/e$ 253). No evidence of syn and anti-isomers was seen.

## Bromination of Oxide (16)

Caparrapi oxide (1 mg) was dissolved in carbon tetrachloride
(1 ml) and bromine in carbon tetrachloride added dropwise till
the red colour persisted. Excess bromine was removed by addition
of aqueous sodium metabisulphite. An aliquot of the organic
layer was injected directly onto GLC, and the presence of two dia-
stereoisomers indicated ($I_{SE-30}^{120^{o}}$ approximately 2000 and 2010).
The expected molecular ion multiplets were observed by GC-MS
($^{m}/e$ 382, 384, 386 in the ratio 1: 2: 1).

## Attempted Cyclizations of (+)-S-Nerolidol
### to Caparrapi Oxide

(+)-S-Nerolidol was obtained by preparative TLC of Sample 1
of Caparrapi oil, and divided into several portions.

(i) 98% Formic Acid at 60$^{o}$.

(+)-S-Nerolidol (50 mg) was dissolved in 98% formic acid (1 ml)
yielding a white, opaque solution, which after 2 minutes warming at
60$^{o}$ became brown. Neutralisation with aqueous sodium bicarbonate
was followed by ether extraction. The extract was dried over
anhydrous sodium sulphate, yielding a yellow oil (35 mg). Comparative
TLC indicated complete reaction of nerolidol, and formation of
products with retention indices $R_f$ 0.95 and 0.89 ( cf. caparrapi oxide
0.89). GLC indicated a multiplicity of products; only one component
(approximately 1% of the total) had a retention index ($I_{SE-30}^{100^{o}}$ 1420)
corresponding to that of caparrapi oxide.

(ii) 98% Formic Acid at Room Temperature.

(+)-S-nerolidol (10 mg) was dissolved in 98% formic acid
(0.5 ml) and the solution shaken for 5 minutes at room temperature.
The products were isolated and examined as in (i), and shown to
be essentially similar to (i) by TLC and GLC, although some
starting material was unreacted.

(iii) Dilute Anhydrous Formic Acid.

(+)-S-nerolidol (10 mg) was dissolved in Analar tetrahydrofuran
(2 ml) and 98% formic acid (2 drops) added. After standing
overnight, no reaction had occurred, as shown by TLC and GLC.
Warming at 40°C for several minutes produced no reaction.

(iv) Concentrated Sulphuric Acid.

(+)-S-nerolidol (10 mg) was dissolved in 2-nitropropane
(1 ml, freshly distilled from phosphorus pentoxide), and
concentrated sulphuric acid (0.1 ml) in 2-nitropropane (1 ml)
added at -70°C. The solution was stirred magnetically for
5 minutes, becoming brown. Ice-cold water was added and the solution
extracted with light petroleum. TLC indicated complete reaction,
giving products with retention indices corresponding to
hydrocarbons. GLC indicated a complex mixture in which no oxide
could be detected.

(v) p-Toluenesulphonic Acid.

To (+)-S-nerolidol (1 mg) in benzene (0.5 ml) was added a
crystal of p-toluenesulphonic acid monohydrate. The solution was

warmed on a steam bath for 30 minutes. TLC indicated the presence of compounds corresponding in polarity to hydrocarbons and mono-alcohols. No oxide was evident.

(vi) Boron Trifluoride Etherate.

To (+)-S-nerolidol (1 mg) in benzene (1 ml) was added boron trifluoride etherate (1 drop). The solution was left at room temperature for 5 minutes, and the excess reagent neutralized with water. The benzene extract was dried over anhydrous sodium sulphate and was shown by TLC to contain several compounds of polarity consistent with that of olefins. No oxide could be detected.

## Separation of Samples of Caparrapi Oil into Neutral and Acid Components

The five samples of caparrapi oil (~0.5 g of each) were dissolved in ether (50 ml) and extracted twice with saturated aqueous sodium bicarbonate (50 ml). The bicarbonate extracts were back-washed with ether to remove traces of neutrals. Acidification of the bicarbonate extract with 2N hydrochloric acid was followed by three extractions with ether, the extracts being dried over anhydrous sodium sulphate. Analytical data are presented in Table 3.

## Preparative TLC on Caparrapi Oil Samples

Samples (~100 mg of the neutral fraction) were adsorbed on to Kieselgel preparative TLC plates and eluted with 50% benzene/diethyl ether. Analytical data are presented in Table 3.

### Saponification of Sample 5

The neutral fraction of sample 5 (1.6 g) was dissolved in methanol (5 ml). 30% sodium hydroxide (4 ml) and methanol (5 ml) were added, and the solutions refluxed at $100^{\circ}C$ for 15 minutes. Water was added and the neutral constituents extracted with methylene chloride. The basic aqueous solution was acidified and extracted with methylene chloride, which, after drying over anhydrous sodium sulphate, yielded acids (0.640 g).

### Saponification of Sample 4

The neutral fraction of sample 4 (10 mg) was saponified as above, and the acids isolated. Comparative TLC in benzene (90)/dioxan (25)/acetic acid (6) of the acids obtained by saponification of samples 4 and 5 with the free acids in sample 4 gave the following data:

| Free acids from 4 | Acids from saponification of sample 4. | Acids from saponification of sample 5 |
|---|---|---|
| $R_f$ 0.85 (brown)[*] <br><br> 0.70 (brown) | $R_f$ 0.70 (brown) <br><br> 0.40 (brown) | $R_f$ 0.90 (blue) |

[*] colours produced by ceric sulphate spray.

2.1 <u>INTRODUCTION TO THE STUDY OF THE ESSENTIAL OIL OF</u>
<u>BRACHYLAENA HUTCHINSII</u>

Botanical Description.

Brachylaena Hutchinsii is a hardwood, indigenous to East Africa. The tree is known variously among the natives as Muhuhu, Muhugu, Mububu, Ol-Magogo or Muhugwe, the vernacular term varying according to the dialect. A member of the compositae, the tree is found from the coast land up to a height of 6,500 feet, and reaches its full development in altitudes where its growing zone joins that of Juniperus procera, the Kenya cedar-tree. Its wood, dark brown in colour, is tough, easy to split and highly odorous.

Background Aspects

Literature references to Brachylaena Hutchinsii are sparse. The principal accessible references[36,37] describe the essential oil (product of steam distillation) as being reminiscent of cedarwood and vetiverwood oil in fragrance. Analytical descriptions of the oil indicated predominantly sesquiterpenoid constitution, comprising alcohols, and to a lesser extent, ketones. It is probable that the complex nature of the oil restricted further examination by classical methods.

The ready availability of this wood in Britain (as flooring blocks) together with the paucity of chemical information about the sesquiterpenoid constituents, prompted investigations, using modern techniques of separation and spectral analysis.

2.2      DISCUSSION

## Initial Problems of Isolation

Ethyl acetate extraction of the heartwood of Brachylaena hutchinsii afforded an oil, comprising 20% by weight of the wood. GLC examination of the oil indicated a complex mixture, many of the constituents of which had retention data characteristic of sesquiterpenoids.

Column chromatography resulted in the isolation of the principal constituents, two isomeric ketoaldehydes and the corresponding pair of ketoalcohols, as intractable mixtures. Following exhaustive chromatographic attempts at separation, which afforded only partial resolution of the constituents, chemical modification was examined in the hope of obtaining isolable products. It was subsequently found that treatment of the isomeric ketoaldehyde mixture with sodium borohydride resulted in reduction to a mixture of three epimeric diols which were easily separable and which could, by Sarett oxidation, be reconverted to two homogeneous ketoaldehyde isomers.

Fig. 23
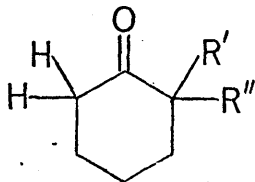
Functional groups                    Physical data (Ketoaldehyde 36a)

IR      $1720 \text{ cm}^{-1}$(S)

NMR     $\tau$ 7.49 (2H)
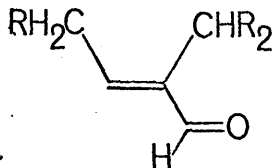
IR      1660(MS),1680(VS),2810 $\text{cm}^{-1}$(W)

NMR     $\tau$ 0.49(1H),3.20(1H), 7 12(1H)
        7.34 (2H)

UV      244 m$\mu$ ( $\varepsilon \sim$9000)
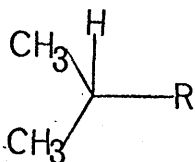
NMR     $\tau$ 9.06,9.19(2 doublets,
        J $\sim$ 7cps, 6H).
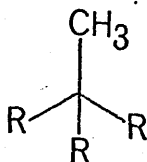
NMR     $\tau$ 9.25 (singlet, 3H).

## Preliminary Examination of Principal Constituents

Elemental analysis of each of the ketoaldehydes indicated the composition $C_{15}H_{20}O_2$. Uptake of one mole of hydrogen was indicated by mass spectrometry of the products. The ketoaldehydes were therefore tricyclic.

The spectral characteristics (infra-red, ultraviolet, nuclear magnetic resonance and mass spectra) of the two ketoaldehydes were very similar. The infra-red spectra (carbon tetrachloride solution and liquid film) indicated the possible presence of a cyclohexanone system and a conjugated aldehyde group (Fig. 23). In the ultraviolet spectrum, absorption at 244 mµ ($\varepsilon$ =9,000) was attributed to the conjugated aldehyde group[*]. The mass spectra of the two compounds were superimposable, but were highly complex in appearance. The NMR spectra were also similar. A detailed decoupling analysis is described in pages 52 to 60. Preliminary examinations revealed in each case an aldehydic proton ($\tau$ 0.49), a vinylic proton ($\tau$ 3.20), a tertiary allylic proton ($\tau$~7.15), two secondary allylic protons ($\tau$ 7.34) and two protons $\alpha$ to the ketone group ($\tau$ 7.49). In the methyl region a tertiary methyl group was evident at $\tau$ 9.25, and two doublets (J=7 cps) were ascribed to an isopropyl group.

---

[*] c.f. myrtenal (40); absorption predicted by Woodward's rules, 230 mµ; absorption observed,[38] 247 mµ ($\varepsilon$ = 8,500).

Fig. 24

35a

35b

36a

36b

37a,b,c

38 → 39

40
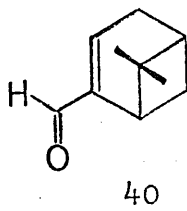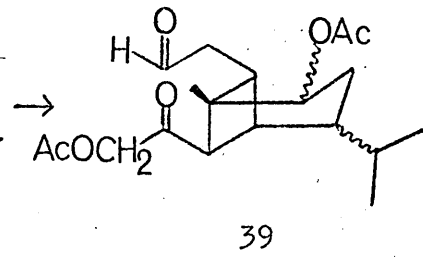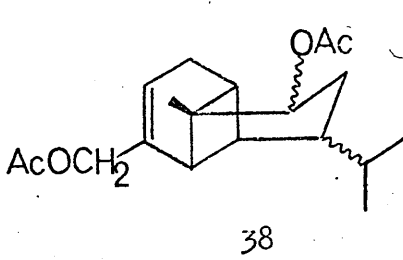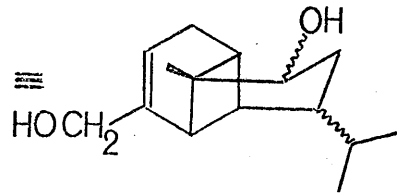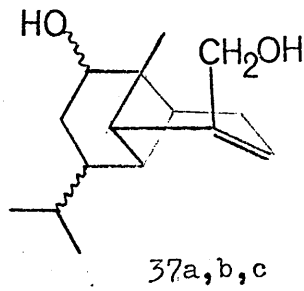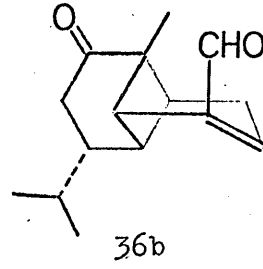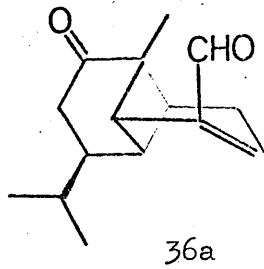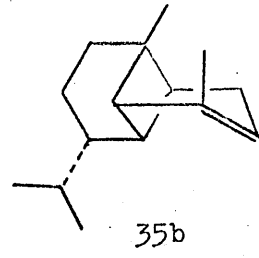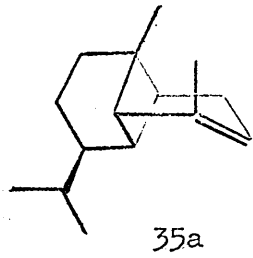
The presence of the functional groups depicted in Fig. 23 was therefore indicated. Consideration of the known tricyclic sesquiterpene skeletons revealed that the only one which could accommodate these functional groups was the ylangene/copaene type (35a,b). The problem thus resolved itself into assigning structures (36a) and (36b) to the ketoaldehydes, or postulating a novel tricyclic ring system.

A pair of ketoalcohols which occur together with the ketoaldehydes as major constituents of <u>Brachylaena hutchinsii</u> were separated, with difficulty, in very low yield. NMR examination revealed the presence of a primary allylic alcohol functionality ( $\tau$ 5.9, 2H) and an isopropyl group (two doublets centred on $\tau$ 9.0 and $\tau$ 9.13, J = 7 cps, 3H each) and a tertiary methyl group (singlet, $\tau$ 9.14, 3H). Oxidation of one ketoalcohol isomer to ketoaldehyde (36a) established a simple relationship.

Further investigations were centred on the ketoaldehyde isomers, which were more easily separated, and the diols (37a), (37b) and (37c) which were obtained by reduction of the ketoaldehydes.

<u>High Resolution NMR Analysis of the Isomeric Ketoaldehydes</u>

The potential structural affinity of the isomeric ketoaldehydes with the monoterpene aldehyde myrtenal (which has recently been the subject of detailed NMR studies[39,40]) prompted comparative NMR examination. Accordingly, the NMR spectrum 100 Mc/s.) of each isomer was subjected to spin decoupling of every

Fig. 25
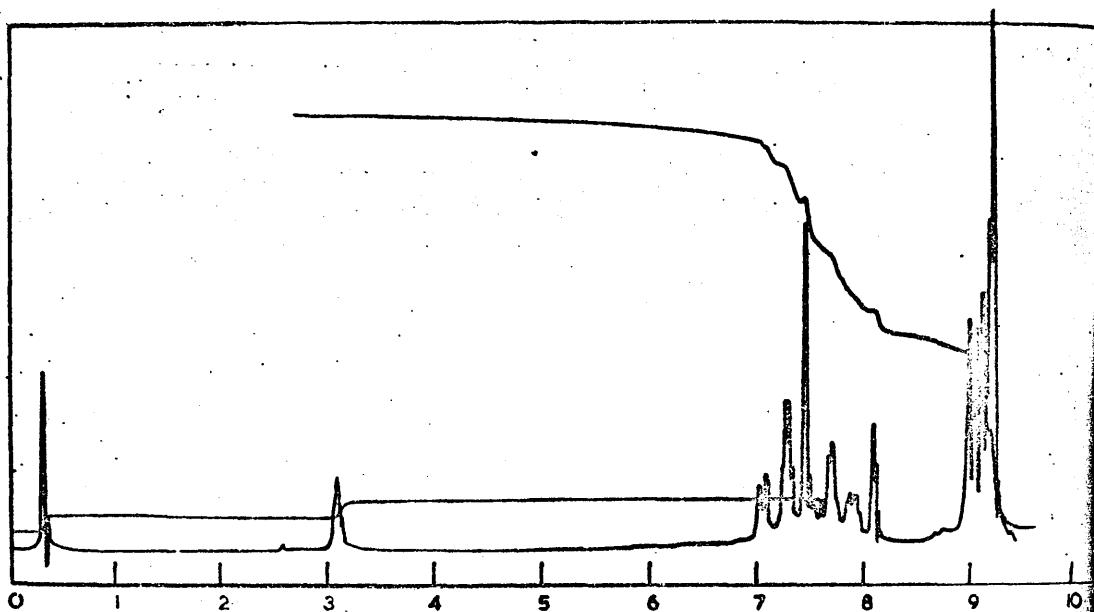


NMR spectrum (100Mc/s) of ketoaldehyde (isomer 36b) in $CDCl_3$

<div align="center">

## TABLE 6

### Observed Coupling Constants (cps)

</div>

| | Myrtenal | Ketoaldehyde (36b) | Ketoaldehyde (36a) |
|---|---|---|---|
| $J_{bc}$ | 1.4 | 1.5 | 1.5 |
| $J_{bd_1}$ | 3.0 | 3.0 | 3.0 |
| $J_{bd_2}$ | 3.0 | 3.0 | 3.0 |
| $J_{bh}$ | 1.4 | 1.5 | 1.5 |
| $J_{ch}$ | 5.8 | 6.5 | 6.5 |
| $J_{ci}$ | 0.04 | < 0.05 | < 0.05 |
| $J_{cj}$ | 5.4 | – | – |
| $J_{d_1 d_2}$ | * | * | * |
| $J_{d_1 h}$ | 2.8 | 3 | 3 |
| $J_{d_1 i}$ | 0 | 0 | 0 |
| $J_{d_2 h}$ | 3.0 | 3 | 3 |
| $J_{d_2 i}$ | 0 | 0 | 0 |
| $J_{e_1 e_2}$ | – | 0 | * |
| $J_{e_1 f}$ | – | 0 | 0 |
| $J_{e_2 f}$ | – | 0 | 0 |
| $J_{fg}$ | – | ∓ | ∓ |
| $J_{fi}$ | – | 2.0 | 2.0 |
| $J_{gl}$ | – | 6 | 6 |
| $J_{gm}$ | – | 6 | 6 |

\* The spectrum is insensitive to this constant, which cannot be evaluated because $\delta H_{gem} \cong \delta H'_{gem}$.

∓ $\delta H_f = \delta H_g$ therefore $J_{vic}$ cannot be determined.

Fig. 26
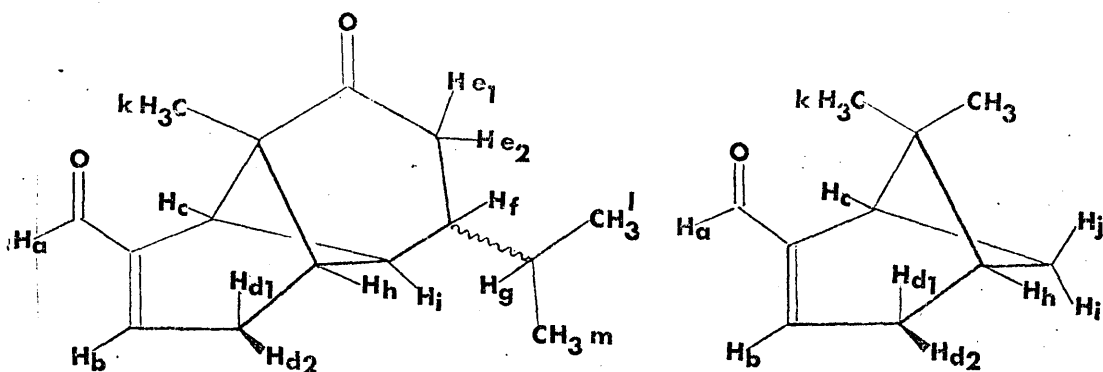


Ketoaldehyde (36)          Myrtenal (40)

TABLE 7

Comparative Chemical Shift Data (100 Mc/s)

|  | Myrtenal | Ketoaldehyde (36b) | Ketoaldehyde (36a) |
|---|---|---|---|
| a | $0.48\ \tau$ | $0.49\ \tau$ | $0.49\ \tau$ |
| b | 3.29 | 3.20 | 3.20 |
| c | 7.12 | 7.12 | 7.20 |
| $d_1$ | 7.43 | 7.34 | 7.34 |
| $d_2$ | 7.43 | 7.34 | 7.34 |
| $e_1$ | - | 7.49 | 7.49 |
| $e_2$ | - | 7.49 | 7.49 |
| f | - | 7.76 | 7.62 |
| g | - | 7.76 | 7.62 |
| h | 7.80 | 7.96 | 7.82 |
| i | 8.96 | 8.15 | 8.10 |
| j | 7.51 | - | - |
| Me(k) | 9.26 | 9.25 | 9.26 |
| Me(1) | - | 9.06 | 9.04 |
| Me(m) | - | 9.19 | 9.11 |

Fig. 27
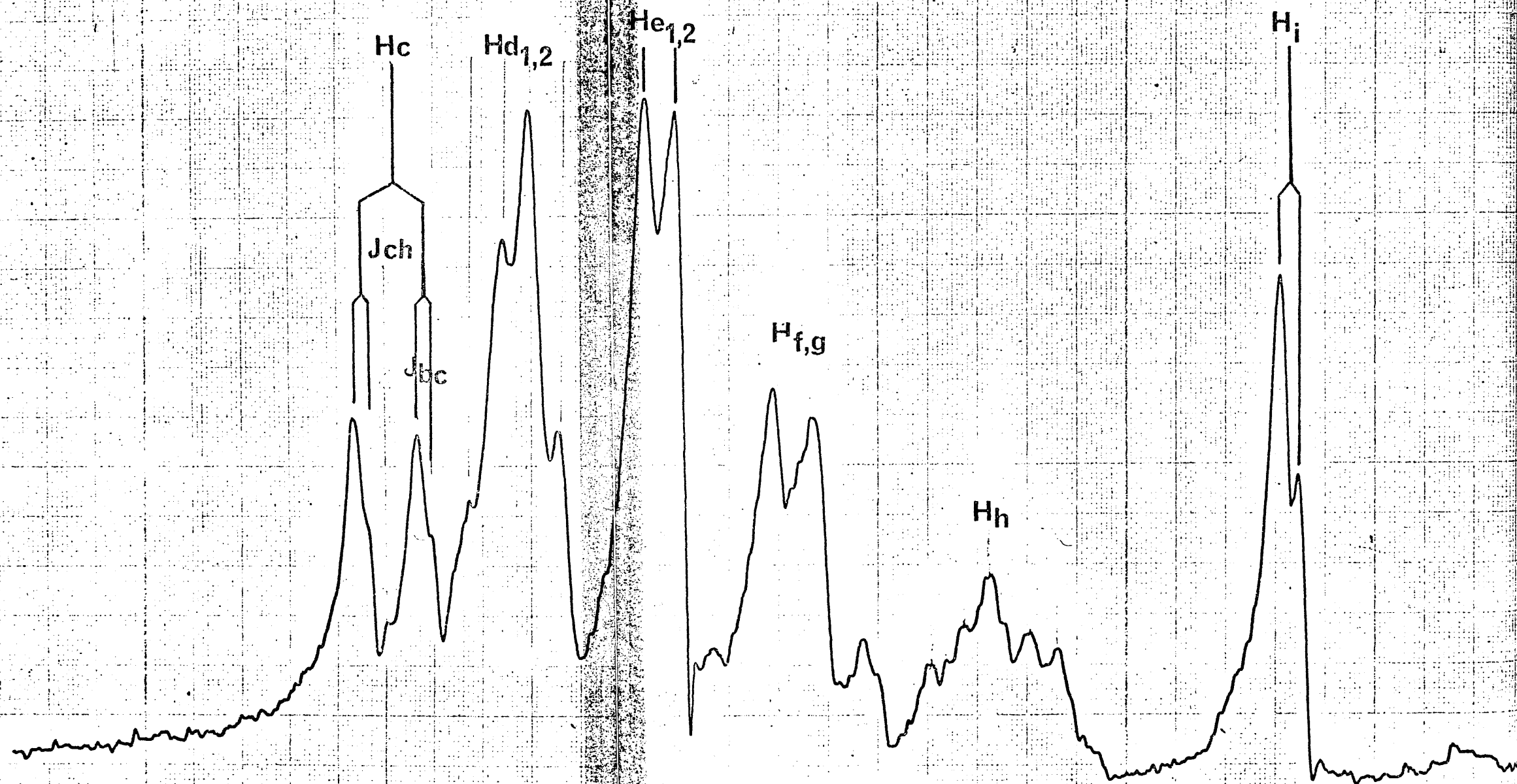
Ketoaldehyde isomer (36a)

Fig. 28

Irradiation on $H_b$
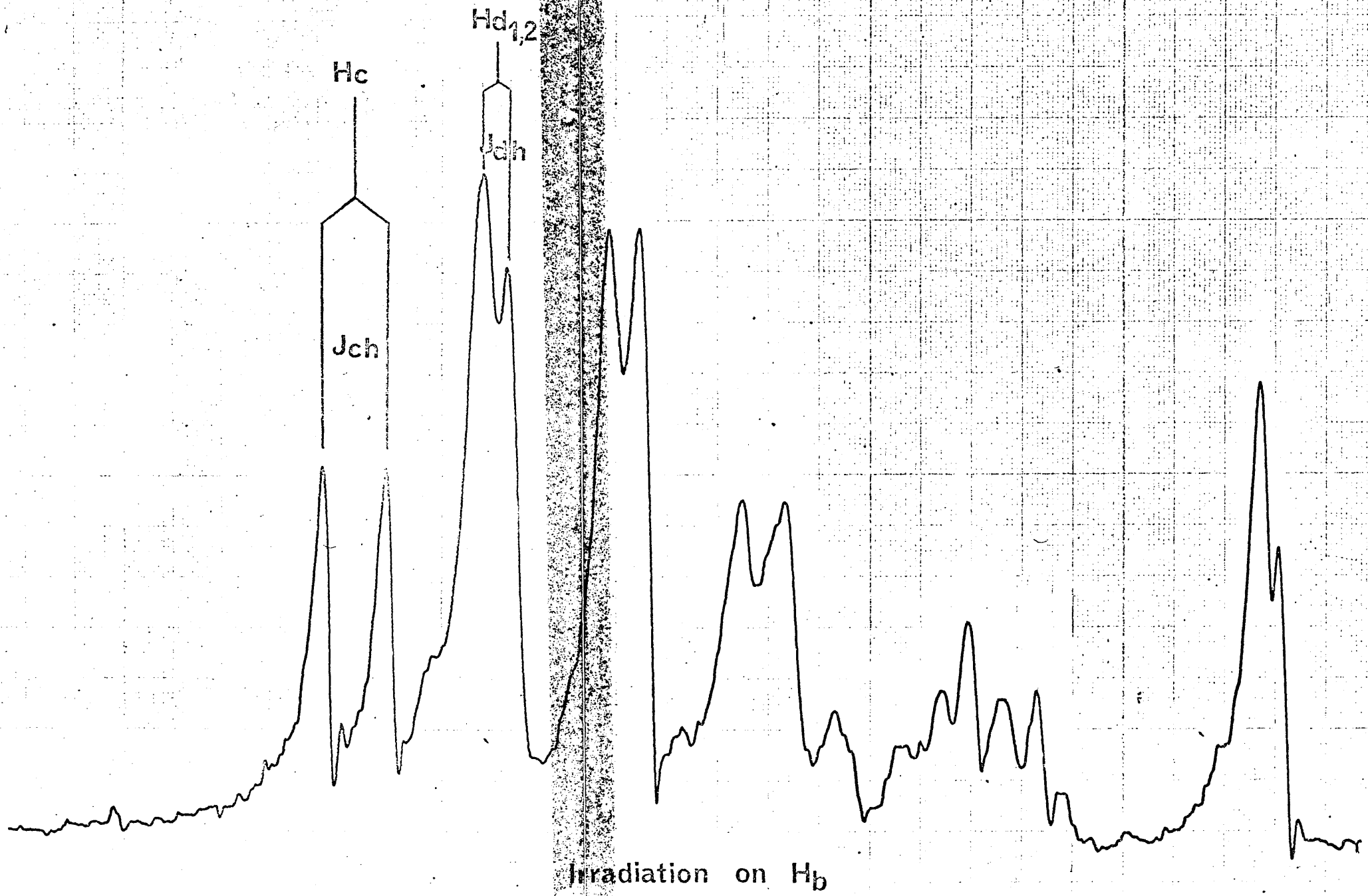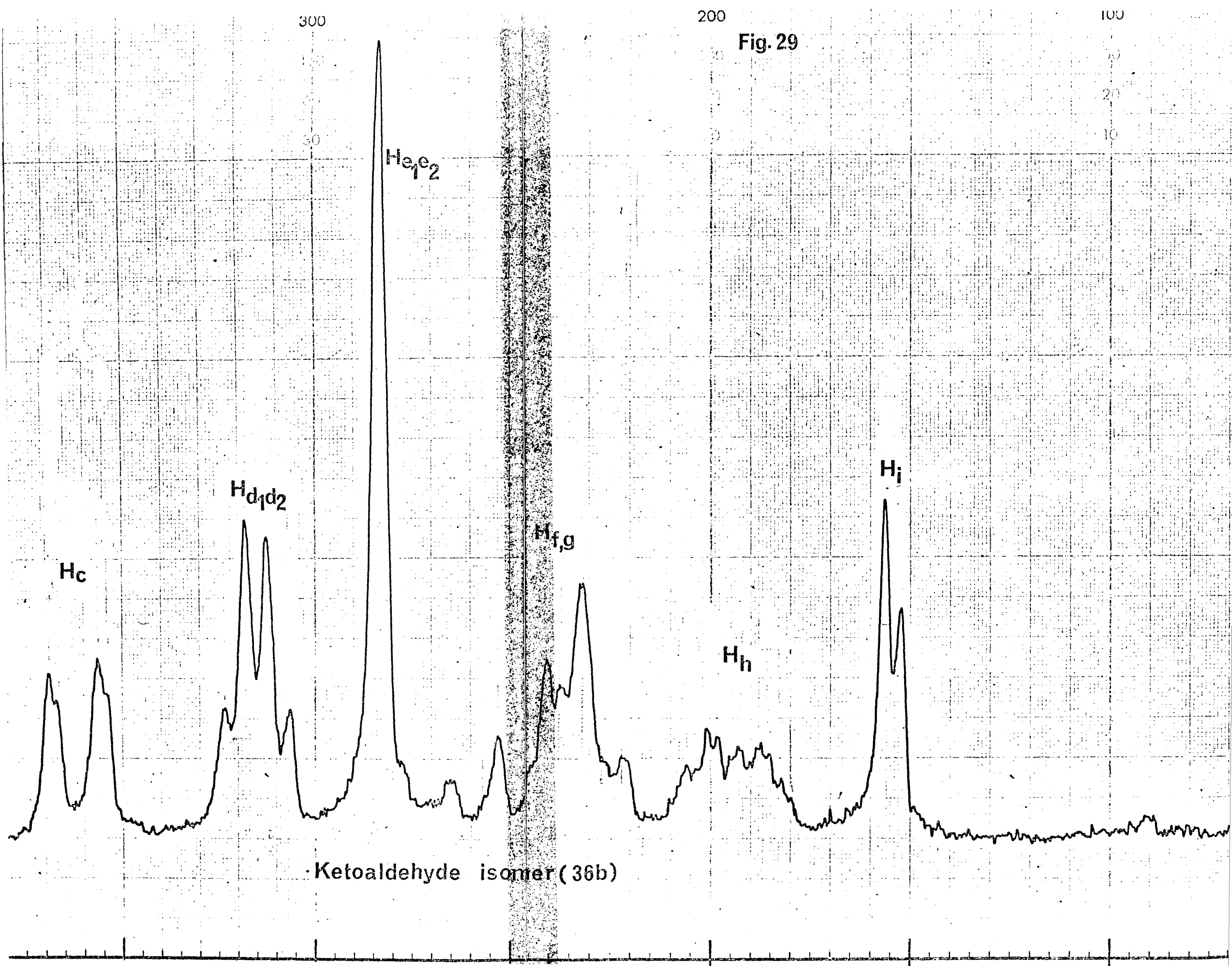
Fig. 29

Ketoaldehyde isomer (36b)

Fig. 30

Irradiation on $H_b$
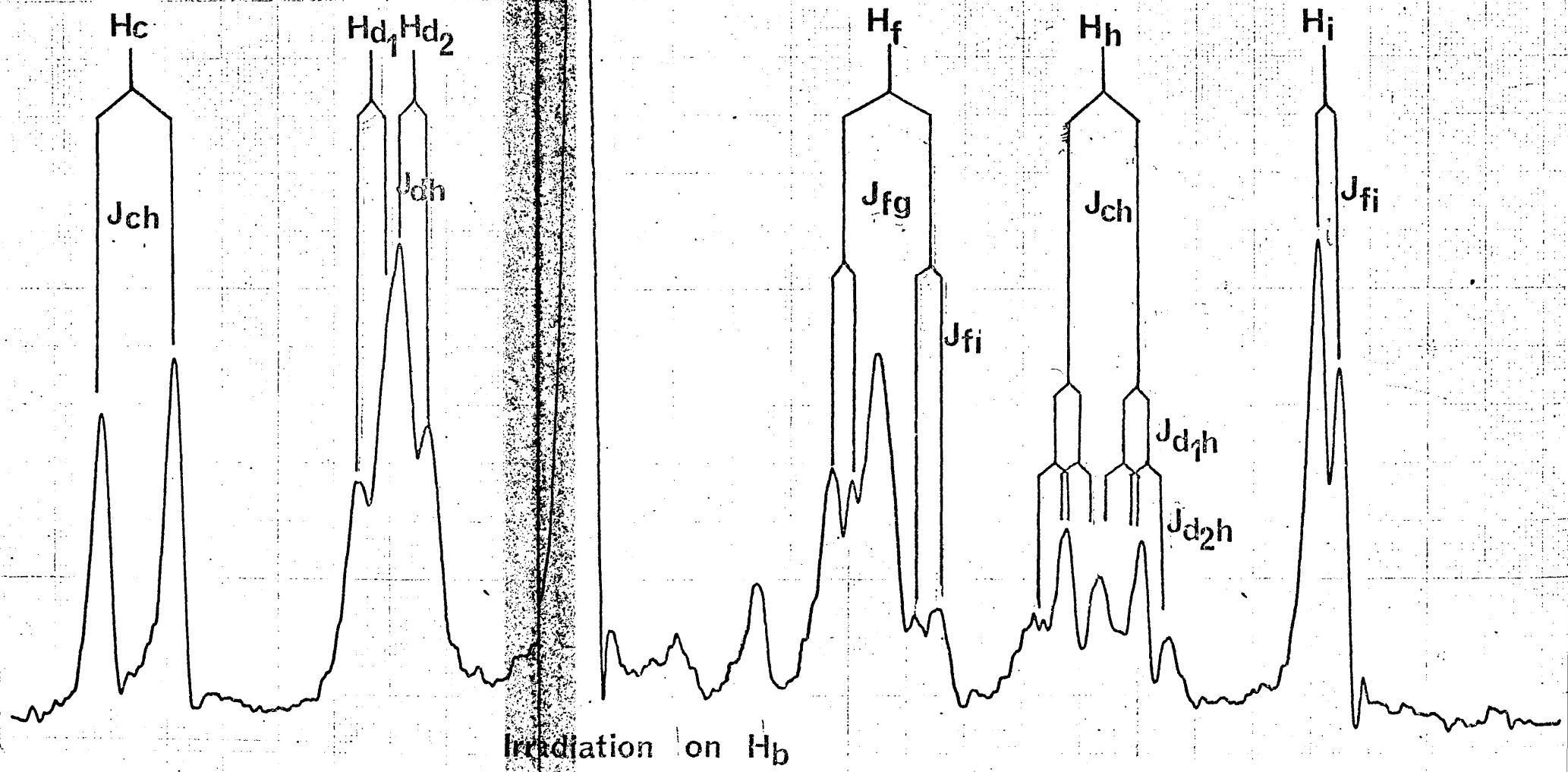
proton in turn, in order that a definite correlation with the monoterpene analogue, myrtenal, could be established. Comparative chemical shift and decoupling data are presented in Tables 6 and 7.

## Ketoaldehyde (36b)

Aldehydic proton Ha was unsplit under the conditions of resolution employed, and corresponded exactly in chemical shift to the aldehydic proton of myrtenal.

Vinylic proton Hb corresponded closely with Hb in myrtenal, in both chemical shift and multiplicity. Double irradiation on Hb resulted in several structurally significant changes in the rest of the spectrum (Figs. 29 and 30). Coupling of 1.5 cps. to allylic methine proton Hc was removed, sharpening the Hc doublet. This compares favourably with $J_{bc} = 1.4$ cps. in myrtenal. The quartet ascribed to the allylic methylenic protons $Hd_1$ and $Hd_2$ collapsed to a triplet. This is in accordance with the proposal that $Hd_1$ and $Hd_2$ comprise an 'AB' system in which the chemical shift of A is closely similar to that of B. The resultant AB quartet is manifested as two closely-separated peaks, the satellite peaks of the quartet being vanishingly small. These two peaks are further split equally by Hh (since $Hd_1$ and $Hd_2$ subtend equal angles with Hh) by 3 cps, affording the observed triplet (Fig. 30). In myrtenal, the observed values are $Jd_1h = 2.8$ cps. and $Jd_2h = 3$ cps. The complex multiplet ascribed to Hh was considerably simplified by removal of long-range coupling with Hb of approximately 1.5 cps., compared with $J_{bh} = 1.4$ cps in myrtenal. Irradiation on vinylic

proton Hb thus revealed significant parallelisms between the
ketoaldehyde and myrtenal, and corroborated several of the
proposed assignments.

Tertiary allylic methine proton Hc appeared as a doublet
(J = 6.5 cps.) further split by vinylic proton Hb by 1.5 cps, at
$\tau$ 7.12. This chemical shift, to which the double bond, the
aldehyde, the tertiary substitution and the anisotropic shielding
of the cyclobutane ring all contribute, corresponds exactly to that
of Hc in myrtenal. This coincidence in chemical shift strongly
supports a partial structure for the ketoaldehyde including a
myrtenal-type moiety. Double irradiation on Hc resulted in
coalescence of the Hh multiplet by removal of 6.5 cps. coupling.
Back-irradiation on Hh caused the Hc doublet to collapse to a
singlet, confirming the coupling. The magnitude of this coupling,
which occurs through 4 favourably-oriented $\sigma$-bonds is similar to
the corresponding coupling across the cyclobutane ring in myrtenal,
indicating the similarity between the molecules.

The signal at $\tau$ 7.34 ascribed to the allylic methylenic protons
occurs at similar chemical shift in myrtenal. Double irradiation
at this point resulted in a simplification of the Hh multiplet to a
diffuse doublet, confirming the vicinal relationship of the Hd protons
and Hh. A similar result was obtained in the myrtenal study.

The 2-proton singlet at $\tau$ 7.49 corresponds in chemical shift
to protons $\alpha$ to a carbonyl group. Examination of models of the
possible ketoaldehyde structure (36) reveals that protons $H_{e_1}$ and $H_{e_2}$

can, by virtue of facile interconversion of ring conformers, become magnetically equivalent on the NMR time scale, and subtend in this time-averaged configuration a dihedral angle of 90° with the vicinal $H_f$ proton. The observed unsplit signal can therefore be accommodated within structure (36) without invoking unprecedented assumptions*.

Double irradiation on the 2-proton multiplet ascribed to the tertiary protons $H_f$ and $H_g$ resulted in collapse of the two isopropyl doublets (J=6 cps.) centred on $\tau$ 9.06 and 9.19 to two singlets, confirming the assignment of the isopropyl methine proton $H_f$. Back irradiation confirmed this result. The doublet assigned to $H_i$ ($J_{fi}$ = 2 cps.) also collapsed, supporting the vicinal assignment of $H_f$ and $H_i$ and pointing to a time-averaged dihedral angle of 60°.

Double irradiation on the complex $H_h$ multiplet produced the expected changes in the spectrum. Coupling of 6.5 cps. to $H_c$ was removed, as already noted. Vicinal coupling to $H_{d1}$ and $H_{d2}$ was removed, causing the quartet at $\tau$ 7.35 to collapse to a diffuse doublet (residual coupling of 3 cps to $H_b$ as in myrtenal). The chemical shift of $H_h$ in this isomer is $\tau$ 7.96, compared to $\tau$ 7.82 in the other isomer and $\tau$ 7.80 in myrtenal. This additional shielding is possibly a consequence of the isopropyl group being in

---

*In bicyclic systems such as bicyclo-(3,3,1)-nonan-2-one and bicyclo -(3,2,2)-nonan-2-one the methylenic protons appear as a singlet in spite of apparent magnetic non-equivalence (private communication from Dr.W. Parker, of this department).

the <u>syn</u> relationship which would result in strong Van der Waals
interactions and consequent shielding of $H_h$. The stereochemistry
of this isomer (36b) may therefore be indicated provisionally as
that of ylangene (35b).

The $H_i$ proton appears at $\tau$ 8.15, compared with $\tau$ 8.95 in
myrtenal, but this is immediately explained by the fact that it
lies 2.5$\overset{o}{A}$ directly behind the carbonyl group in a line drawn through
the 2-fold C=O axis, and is therefore in a position of maximum
deshielding. Coupling with $H_f$ ($J_{if}$ = 2 cps.) was demonstrated by
double irradiation at $H_i$.

The shielded tertiary methyl group at $\tau$ 9.25 corresponds
exactly with Me(k) in myrtenal[*]. The upfield shift exhibited by
these methyl groups is indicative of their position above the
$\overline{\Pi}$-cloud of the olefinic double bond.

In conclusion, there is accordance between the spectral
parameters derived for myrtenal by the American workers, and the
parameters evaluated for the ketoaldehyde. The chemical shifts and
couplings of the additional protons ($He_1$, $He_2$, $H_f$, $H_g$, Me(l), Me(m) )
are also accommodated within structure (36) by invoking rapid
interconversion of the cyclohexanone ring conformers, as indicated
by examination of models. The methine proton resonance at $\tau$ 7.96
may indicate the ylangene stereochemistry (36b) for this particular
isomer.

------

[*]The $\alpha$-keto group in this particular environment would not be
expected to alter the chemical shift appreciably cf. bornane,
Me(10), $\tau$ 9.17; camphor, Me(10), $\tau$ 9.14.

## Ketoaldehyde (36a)

Systematic decoupling was also performed on this isomer, but the more congested nature of the spectrum (Fig.27) caused some of the results to be obscured by side-bands. Nevertheless, significant data were obtained, supporting the epimeric structure (36a).

Aldehydic proton $H_a$ was unsplit under the resolution conditions employed, and appeared at the same frequency as the aldehydic proton of myrtenal.

Vinylic proton $Hb$ again exhibited the same chemical shift and multiplicity as in myrtenal. Double irradiation at this point removed the 1.5 cps. coupling to $H_c$ which is also evident in myrtenal. The allylic methylenic quartet at $\tau$ 7.34 was reduced to a doublet, due to the magnetically equivalent protons $Hd_1$ and $Hd_2$ subtending equal angles with $Hh$ ($Jd_1h=Jd_2h=3$ cps, as in myrtenal). The complex spin system which causes $H_h$ to appear as a multiplet is simplified by removal of a small (perhaps virtual) coupling, again corresponding to myrtenal.

Tertiary allylic methine proton $H_c$ again differed only slightly in chemical shift from the corresponding proton in myrtenal, and was shown by double irradiation on $H_c$ and $H_h$ to be coupled with $H_h$ ($Jch = 6.5$ cps.)

The secondary allylic methylenic protons $Hd_1$ and $Hd_2$ appeared as virtually equivalent protons at $\tau$ 7.34 (myrtenal, $\tau$ 7.43). The effects of irradiation on the quartet were obscured by side bands.

The geminal protons $\alpha$ to the carbonyl group in the cyclohexanone ring appeared in this isomer at $\tau$ 7.49 as a doublet. Making the reasonable assumption that the ring is again conformationally mobile, it is probable that the two protons are almost magnetically equivalent (differences in this isomer being ascribed to different steric and anisotropic effects of the isopropyl group). In this instance, an extreme AB system, in which $\delta_A \rightarrow \delta_B$, occurs. The inner limbs of the theoretical AB quartet are enhanced in intensity relative to the outer limbs, which disappear.

Double irradiation on the multiplet ascribed to $H_f$ and $H_g$ resulted in collapse of the isopropyl doublets to two singlets, confirming the assignment of $H_g$. Coupling to $H_i$ ($J_{hi}$ = 2 cps.) was also removed.

Double irradiation on the $H_h$ multiplet at $\tau$ 7.82 ( $\tau$ 7.80 in myrtenal) again caused the $H_c$ doublet to coalesce to a singlet due to removal of the $4\alpha$ coupling ($J_{hc}$ = 6.5 cps. cf. $J_{hg}$ = 5.8 cps. in myrtenal). The effect on the vicinal protons $Hd_1$ and $Hd_2$ was obscured by a side band.

$H_i$ appears in this isomer at $\tau$ 8.10 compared with $\tau$ 8.95 in myrtenal. A similar explanation to that invoked for the epimeric ketoaldehyde probably holds. Decoupling effects on the relevant regions of the spectrum were lost because of side bands.

The comparative chemical shift data and decoupling results for the two ketoaldehydes and myrtenal thus stress the probable

similarity between the ring systems. The similar NMR parameters obtained for the isomeric ketoaldehydes and myrtenal appear to preclude the possibility of any ring system other than the copaene-ylangene skeleton.

Fig. 31
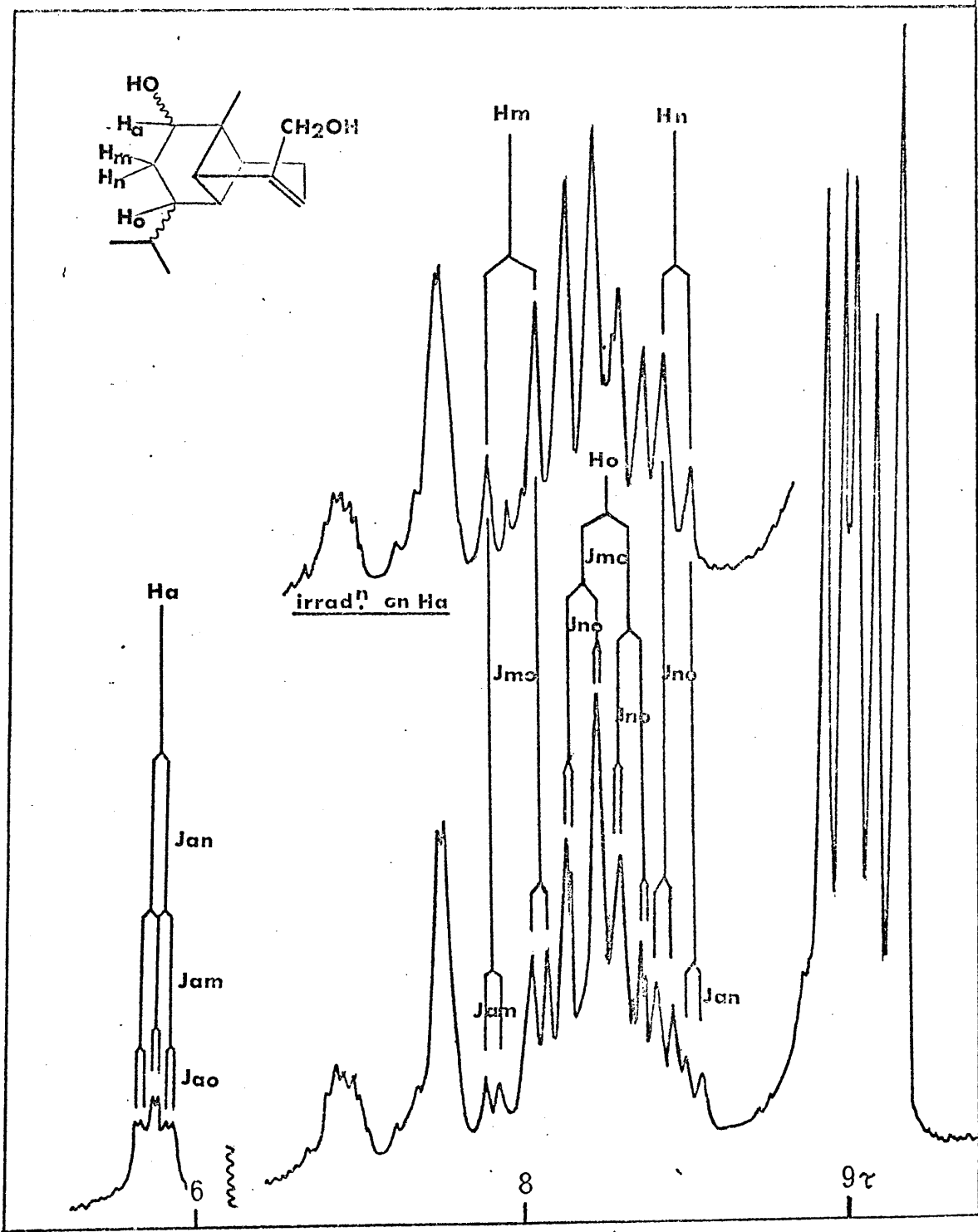
NMR spectrum (100Mc/s.) of diol (37a)
inCDCl$_3$

## Stereochemistry of Diol (37a) – Spin Decoupling Results

In an attempt to elucidate the relative stereochemistry of
the hydroxyl group and the isopropyl group, the three diol isomers
(obtained by reduction of the ketoaldehydes, p. 50) were subjected
to close scrutiny by NMR at 100 Mc/s. Diol (37a) was particularly
amenable to NMR spin-decoupling, since this was the only isomer
in which the secondary alcoholic _methine_ proton differed in
chemical shift from the primary allylic _methylenic_ protons
( $\tau$ 5.8 and 6.1 respectively).

The signal ascribed to $H_a$ (Fig. 31) is apparent as a triplet,
from which it can be deduced that $J_{am} = J_{an}$. The $H_a$ triplet is
further complicated by long-range $4\sigma$ coupling of magnitude
$J_{ao}$ = 1.5-2.0 cps. Irradiation on the secondary methine proton ($H_a$),
which comprises one part of a 4-spin AMNO system, resulted in an
illuminating simplification of the spectrum; a quartet of doublets
collapsed to two doublets (Fig. 31) of an MNO system in which
$J_{mn} = 0$ and $J_{mo} \neq J_{no}$.

The magnitudes of the observed couplings were: $J_{am} = 5$ cps,
corresponding to a time-averaged dihedral angle of 45 or 130° ;
$J_{an} = 5$ cps. (dihedral angle 45 or 130°) ; $J_{ao} \cong$ 1.5 cps. ;
$J_{mn} = 0$ (this is a consequence of the mobility of the ring
which results in magnetic equivalence of $H_m$ and $H_n$) ;
$J_{mo} = 14$ cps (dihedral angle 160°) ; $J_{no} = 8$ cps (dihedral angle
28 or 135°). The hydroxyl and isopropyl group in each of the
diols were conformationally mobile, however, so that assignment.

of stereochemistry on the basis of Karplus relationships
was deemed to be unsafe. It is possible that low-temperature
NMR analysis of this diol would result in stereochemically
significant results.

## Chemical Modification of the Molecule

The crystalline diols (37a,b,c) were more readily available
than the non-crystalline ketoaldehydes (36a,b). Accordingly,
several degradative approaches involving oxidative cleavage of the
unsaturated ring were examined. The instability of the ring
system was rapidly manifested, as conventional degradative
procedures such as ozonolysis (reductive and oxidative), lead
tetra-acetate cleavage and osmium tetroxide-sodium periodate
cleavage afforded heterogeneous product mixtures which precluded
further examination. The most profitable approach, however,
involved treatment of the bis-acetate (38) of one of the diols
with osmium tetroxide, followed by partial periodate cleavage[41],
affording a product whose spectral data were consistent with
structure (39):

NMR,    60 Mc/s ;

$\gamma$ 0.16  (1H, triplet, J=1 cps)   :   $- CH_2 - C\underline{H}O$

5.41  (2H, singlet)        :   $- CO-C\underline{H}_2-OCOCH_3$

6.42  (1H, 2 doublets, $J'_{vic}$=13 cps, $J^2_{vic}$=8 cps) : $-CH_2-C\underline{H}R-OCOCH_3$

7.86  (3H, singlet)  :   $-OCO\underline{CH}_3$.

7.98  (3H, singlet)  :   $-OCO\underline{CH}_3$.

9.01  (3H, singlet)  :   $CR_3- C\underline{H}_3$.

9.08  (3H, doublet, J=7 cps) )

) $(\underline{CH}_3)_2-CHR_2$

9.19  (3H, doublet, J=7 cps) )

The product from the corresponding diol decomposed rapidly on standing.

Fig. 32

36b, R=CHO

41, R= (1,3-dithiolane)
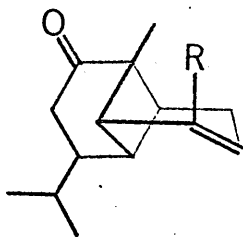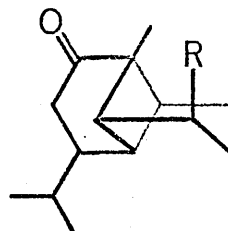
42, R=CH₃

43, R=CH₃
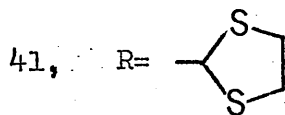
44, R=CHO
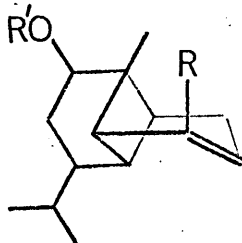
45, R=CHO
R'=H
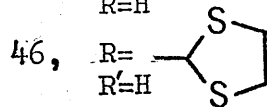
46, R= (1,3-dithiolane)
R'=H

47a, R=Me
R'=H

47b, R=Me
R'=SiMe₃
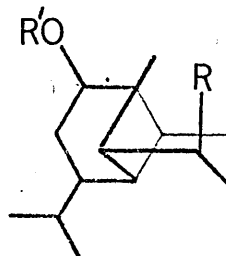
48a, R=Me
R'=H

48b, R=Me
R'=H

Fig. 33



49 (m/e 97)

50 (m/e 99) R=H

51 (m/e 171)R=SiMe$_3$
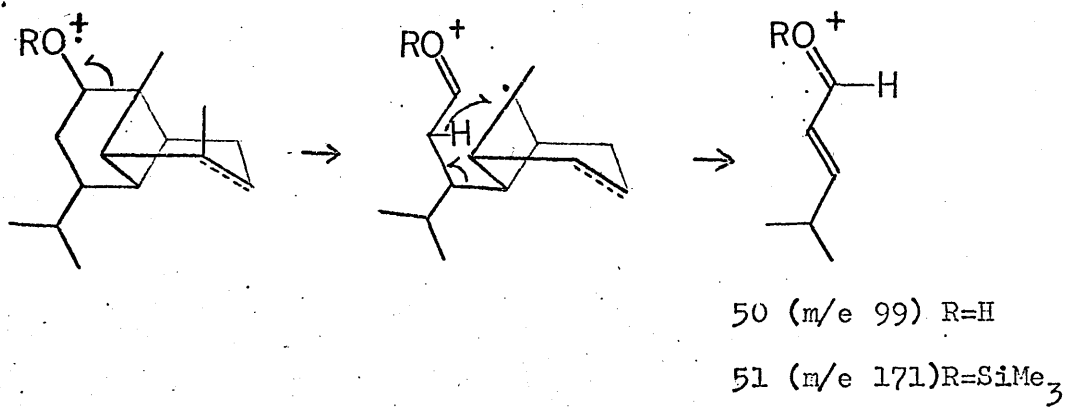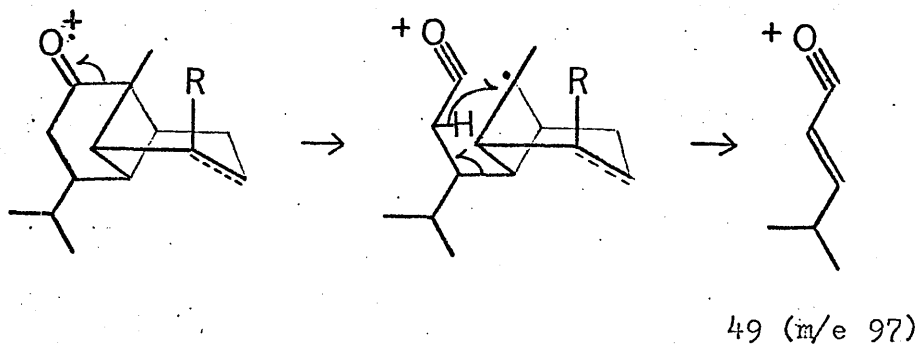
Mass spectral examination of the derived compounds [*] (41)-(48b) revealed in each case a highly complex fragmentation, especially when the double bond was present. Fragment ions such as (49), (50) and (51) were of significant abundance in compounds (41), (46) and (48b), but were absent, or present only as minor species, in the other compounds. Structures (41)-(48b) were therefore not readily reconciled with the complex mass spectra. This was not surprising in view of the strained tricyclic system, which probably complicated the fragmentation processes.

Conversion of the ketoaldehydes to the corresponding olefinic hydrocarbons was not satisfactorily achieved because of lack of time. Preliminary small-scale investigations revealed two potential routes, Wolff-Kishner treatment of ketoaldehyde (36a) ($I_{SE-30}^{125^{o}}= 1760$) afforded a very low yield of a mixture of hydrocarbons, one of the major constituents of which corresponded to copaene when examined on three different GLC phases (See Experimental Section). Similarly, the bis-thioethyleneketal when treated with Raney nickel afforded a low yield of a mixture of hydrocarbons.

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* These transformations were performed on a small scale, and the products evaluated by GLC (where possible) and spectral techniques.

## Conclusions

The essential oil from extraction of the heartwood of Brachylaena hutchinsii contains, as major constituents, two isomeric ketoaldehydes which can be separated as the corresponding diols formed by reduction and recovered by re-oxidation. Consideration of spectral data, and comparison with the apparent monoterpenoid analogue myrtenal (40), indicates that they are oxygenated ylangene and copaene types, i.e. they are isopropyl epimers. The spectral evidence presented strongly supports structures (36a) and (36b), but conclusive evidence will necessitate a direct correlation with the known copaene and ylangene skeletons. Modification of the Wolff-Kishner reduction, or the Raney nickel desulphurization of the bis-thioethylene glycol derivative should result in unambiguous stereochemical assignment of each epimer. Correlations between the two ketoaldehydes (36a) and (36b) and the three diols (37a,b,c) have been established, but the relative stereochemistry of the diols has not been investigated. It is difficult to envisage an alternative sesquiterpene ring system which could accommodate the data presented.

The ketoalcohol isomers which occur with the ketoaldehydes were isolated in very low yield. They were shown to correspond in ring structure to the ketoaldehydes.

Ylangene and copaene co-occur in the same plant.[42] The occurrence of the two ketoaldehydes together, is therefore not unusual.

2.3             <u>EXPERIMENTAL</u>

<u>Typical Extraction of Heartwood of</u>

<u>Brachylaena Hutchinsii</u>

The heartwood of <u>Brachylaena hutchinsii</u> (64g) was reduced
to sawdust and refluxed in a Soxhlet apparatus with ethyl
acetate (1 L.) for 24 hours. The light-brown solution thus
obtained was concentrated in a continuous-feed rotary evaporator
at $50^{\circ}$ to a brown oil. The last traces of ethyl acetate were
removed azeotropically with benzene, yielding 12 g. of a viscous
oil. Extraction with chloroform showed this oil to be almost
completely soluble, apart from a very small quantity of an
amorphous brown solid. The chloroform extract was washed 4 times
with water (which assumed a yellow colour), dried over
anhydrous magnesium sulphate and concentrated, yielding a
red-brown oil (11.5 g).

## TABLE 8.

| Fractions combined following TLC | Eluent | | Weight obtained | Content. |
|---|---|---|---|---|
| 1-2 | 25% ether-petrol | | 1.20 g | |
| 3-13 | " | " | 0.65 g | (37a) |
| 14-30 | " | " | 0.10 g | (37a)+(37b) |
| 31-39 | " | " | 0.10 g | (37a)+(37b) |
| 40-63 | " | " | 2.00 g | (37b) |
| 64-74 | 50% ether-petrol | | 0.20 g | (37b)+(37c) |
| 74-100 | 100% ether | | 1.00 g | (37c) |

## TABLE 9.

| Diols | (37a) | (37b) | (37c) |
|---|---|---|---|
| $R_f$ (ether) | 0.50 | 0.40 | 0.30 |
| $I_{SE-30}^{125}$ | 1790 | 1790 | 1790 |

| Ketoaldehydes | (36a) | (36b) | (36c) |
|---|---|---|---|
| $R_f$ (ether) | 0.70 | 0.70 | 0.70 |
| $I_{SE-30}^{125}$ | 1760 | 1725 | 1760 |

i.e. (36a) = (36c)

$$37b \xrightarrow{\text{O}} 36b$$

$$\left.\begin{array}{l}37a) \\ 37c)\end{array}\right\} \xrightarrow{\text{O}} 36a$$
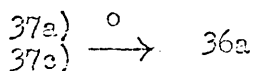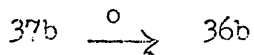
## Sodium Borohydride Reduction of Ketoaldehydes.

The isomeric ketoaldehyde mixture (6.3 g) was dissolved in
60% methanol-water (50 ml) and 1.5 g sodium borohydride added.
The solution was stirred overnight, and partitioned between ether
and water. The ethereal extract was dried over anhydrous
magnesium sulphate and evaporated in vacuo, yielding an oil
(6.2 g) which crystallised on standing. Comparative TLC (ether)
indicated the formation of three well-separated reduction products
($R_f$ 0.5, 0.4 and 0.3). GLC indicated complete reaction of
starting material: the following products were evident;
$I^{125}_{SE-30}$ 1700 and 1720, unreacted impurity in ketoaldehyde mixture;
$I^{125}_{SE-30}$ 1790, mixture of reduction products, not resolved by
1% SE-30.

## Chromatography of Reduction Products

The total reaction product obtained from the reduction of the
ketoaldehydes was dissolved in 25% diethyl ether-light petroleum
(15 ml) and adsorbed on to neutral alumina (250 g Woelm grade III).
Elution was continued with the same mixture. Aliquots of 80 ml.
were collected. Results of the chromatography are tabulated
opposite.

Fractions 3-13 on evaporation yielded isomer (37a) (0.650 g)
pure (mp. 111.5-112°). Analysis: C, 76.15; H, 10.03: required
for $C_{15}H_{24}O_2$ ; C, 76.23 ; H, 10.24% : $[\alpha]_D -10.3°$
(c, 1.0 in $CHCl_3$): $I^{125°}_{SE-30}$ 1790 : $R_f$ (ether) 0.50.

Fractions 40-63 yielded isomer (37b) (2.0 g) as a crystalline

solid (mp. 113.5 - 114$^{\circ}$). Analysis: C, 76.45; H, 10.39;

required for $C_{15}H_{24}O_2$ : C, 76.23 ; H, 10.24% : $[\alpha]_D$ - 85.6

( c, 1.0 in $CHCl_3$) : $I_{SE-30}^{125}$ 1790 : $R_f$ (ether) 0.40.

Fractions 74-100 yielded isomer (37c) (1.0 g) as a crystalline

solid (mp. 141-142$^{\circ}$). Analysis : C, 76.12; H, 10.21 :

required for $C_{15}H_{24}O_2$: C, 76.23; H, 10.24% : $[\alpha]_D$ + 18$^{\circ}$.

$R_f$ (ether) 0.30.

## Correlations Between Diols and Ketoaldehydes. (Sarett Oxidation)

1 mg of each diol was dissolved in dry pyridine (0.2 ml) and

0.5 ml of a stock suspension of chromium trioxide in pyridine

(1.0 g in 10 ml) added. The solutions were left standing overnight,

and partitioned between 2N hydrochloric acid (15 ml) and ether

(25 ml). The ether extracts were washed with bicarbonate and dried

over anhydrous magnesium sulphate. The resultant oxidation

products were examined by GLC and the correlation between the

epimeric alcohols and the epimeric ketoaldehydes established

(Table 9).

## Correlation between Ketoalcohols and Ketoaldehydes.

Preparative TLC of the mixture of ketoalcohol isomers

($I_{SE-30}^{125}$ 1780 and 1790) which co-occur with the ketoaldehydes in

the essential oil of Brachylaena hutchinsii afforded one isomer

($I_{SE-30}^{125}$ 1790) pure, in low yield. To 8.3 mg of this isomer in

pyridine (0.1 ml) was added 1.5 ml of a stock suspension of

chromium trioxide (50 mg) in pyridine (1 ml). After 5 minutes the product was isolated as above. Comparison of the retention data on GLC and TLC, and of the spectral data (infra-red and ultraviolet) indicated that the product corresponded to ketoaldehyde (36a) ($I_{SE-30}^{125}$ 1760).

## Reductive Ozonolysis of Diol. (37b)

Diol (8 mg) in ethyl acetate (10 ml) was cooled to $-70°$ and ozone bubbled through for 8 minutes, till the solution acquired a purple colour. Oxygen was bubbled through for 3 minutes, and the solution allowed to attain room temperature. Glacial acetic acid (1 ml) and zinc dust ($\sim$30 mg) were added, the solution stirred for 12 hours and filtered through Celite. Evaporation in vacuo, using benzene for azeotropic removal of the last trace of acetic acid, afforded a white residue, from which the product (6 mg) was extracted with methylene chloride. TLC (1% ethyl acetate-ether) indicated two products ($R_f$ 0.70, 0.65) which could only be observed with an iodine spray. GLC (1% SE-30, $125°$) also indicated two products, but peak resolution was very poor.

## Oxidative Ozonolysis of Diol. (37b)

Diol (41 mg) in ethyl acetate (20 ml) was treated as above, but the ozonide was decomposed by stirring overnight with 30% hydrogen peroxide (30 ml). Evaporation in vacuo afforded an oil (30 mg) which was soluble with effervescence in bicarbonate

solution. TLC (benzene/dioxane/acetic acid; 90/25/6) indicated three principal products. Following methylation by diazomethane, the products could not be detected by GLC.

## Acetylation of Diol (37b)

Diol (100 mg) was dissolved in pyridine (0.5 ml) and acetic anhydride (0.5 ml) added. The solution was warmed at $60^{\circ}$ for four hours and partitioned between 2N hydrochloric acid (50 ml) and ether (100 ml). The ether extract was washed with aqueous sodium bicarbonate water, and then dried over anhydrous sodium sulphate. Evaporation in vacuo afforded a colourless oil (105 mg). Preparative TLC yielded the bis-acetate (38) as an oil (85 mg). Analysis: C, 71.26 ; H, 8.78 : required for $C_{19}H_{28}O_4$ : C, 71.22 ; H, 8.81%.

## Reaction of Bis-Acetate (38) with Osmium Tetroxide

Bis-acetate (80 mg) was dissolved in ether (50 ml) and excess osmium tetroxide in ether (approx. 100 mg in 10 ml) added. 5 drops of pyridine were added as catalyst, and the solution, which darkened immediately, was kept standing overnight. Saturated aqueous sodium metabisulphite (20 ml) was added, and the heterogeneous mixture stirred vigorously for 30 minutes, till the aqueous layer became red. The aqueous solution was extracted 4 times with ethyl acetate, which, after drying over anhydrous

sodium sulphate and evaporation in vacuo yielded 85 mg of
a white solid. TLC indicated the presence of one principal product,
together with a minor by-product (presumably the epimeric
hydroxylation product).

The diol mixture thus obtained was dissolved in methanol
(3 ml). Sodium metaperiodate (150 mg) in water (3 ml) was,
added and the solution stirred for 10 minutes at ambient
temperature. The solution was diluted with water (50 ml) and
extracted 4 times with methylene chloride (50 ml). The extract
was dried over anhydrous sodium sulphate and evaporated in vacuo
yielding an oil (55 mg). Purification by preparative TLC (ether)
afforded an oil (50 mg). Infra-red (liquid film) : 1740-1705 (VS),
1240 (VS), 1070(S), 1030cm$^{-1}$(S). NMR: see p. 62.

<u>Modification of Functional Groups for Mass Spectroscopy</u>

Ketoaldehyde (36a) (28 mg) was dissolved in ether (2 ml) and
boron trifluoride etherate (3 drops) added. Ethane dithiol
(10 drops) was added and the solution kept an ambient temperature
for 5 minutes. TLC (ether) showed complete reaction of starting
material, with one main product, although several by-products
were observed. 2N sodium hydroxide (5 ml) was added and the solution
shaken till it became orange. The ethereal extract was dried over
anhydrous sodium sulphate and evaporated in vacuo, affording an
oil (35 mg). Preparative TLC (30% ether-light petroleum) yielded
the principal constituent (41) (12 mg) as a white solid

(Mp. (decomp.)~100-120°) Infra-red (CCl$_4$ solution): 1738, 1235, 1045cm$^{-1}$ Mass spectrum (most abundant ions): $m/e = 308$ (19%) (M$^+$), $m/e = 158$ (30%), $m/e = 131$ (45%), $m/e = 105$ (75%), $m/e = 91$ (35%), $m/e = 41$ (100%). GLC was precluded by the thermal lability of the derivative.

To the thioacetal (41) (6.8 mg) in absolute ethanol (5 ml) was added approximately 50 mg Raney nickel (W4 grade) in ethanol. The solution was refluxed for one hour, filtered through Celite and evaporated _in vacuo_, yielding (42) as an oil (5 mg) : Infra-red (liquid film), 1710cm$^{-1}$(S): Molecular ion (GC-MS), $m/e = 218$ ; $I^{125}_{SE-30}$1560). (15% impurity, molecular ion m/e 220 $I^{125}_{SE-30}$1620.

Ketone (42) was dissolved in ethyl acetate (5 ml) and hydrogenated over a period of 5 minutes using 10% palladium-charcoal (5 mg) as catalyst. The product (43) (3 mg) was homogeneous on GLC ($I^{125}_{SE-30}$1575): Molecular ion (GC-MS), $m/e = 218$: infra-red (liquid film), 1710cm$^{-1}$(S).

A similar sequence of small-scale reactions on hydroxyaldehyde (45), obtained by partial Sarett oxidation of diol (37b), afforded the derivatives (46-48b). Product purity was evaluated by TLC and GC-MS. The following physical data were obtained:

(45) : infra-red (liquid film); 3500(S), 1680(S), 1660cm$^{-1}$(S) GC-MS; molecular ion at $m/e = 384$; $I^{125}_{SE-30}$ 1790;

(46) : infra-red (liquid film); 3500(S), 1050cm$^{-1}$(MS); GC-MS; examination precluded by thermal lability of derivative on GLC. Mass spectral molecular ion (probe) at $m/e = 310$ (NB; significant ion at $m/e = 99$ (25%) );

(47a) : infra-red (liquid film); 3500cm$^{-1}$ (S);

GC-MS; molecular ion at $^m/e$ = 220; $I_{SE-30}^{125}$1535;

(47b) : infra-red (liquid film); 1250(M), 850(M), 760cm$^{-1}$(W);

GC-MS; molecular ion at $^m/e$ = 308; $I_{SE-30}^{125}$1680;

(48a) : infra-red (liquid film). 3500cm$^{-1}$(S);

GC-MS; molecular ion at $^m/e$ = 222; $I_{SE-30}^{125}$1580;

(48b) : infra-red (liquid film); 1250(M), 850(M), 760cm$^{-1}$(W);

GC-MS; molecular ion at $^m/e$ = 310 (NB : significant ion

at $^m/e$ = 187 (15%) ).

The bis-thioethylene ketal of (36a) was also prepared on a
small scale by prolonged heating at 70$^o$ of (36a) in ethane dithiol
and boron trifluoride etherate. TLC indicated the formation of a
homogeneous derivative. The derivative was thermally labile and
could not be examined by GLC. Mass spectral data (probe):
Molecular ion at $^m/e$ = 418 (100%). Abundant ions at $^m/e$ = 105
(65%), 124 (30%), 145 (65%), 325 (35%).

## Wolff-Kishner Reduction of Ketoaldehyde (36a)

Ketoaldehyde (36a) (42 mg) was dissolved in dry diethylene
glycol (2 ml) and sodium hydroxide (0.3 g) added: 100% hydrazine
hydrate (0.44 ml) was added and the solution stirred under nitrogen
as the temperature was raised to 120$^o$. The water was distilled off,
and the red/brown solution left overnight at 160-170$^o$. The products
were partitioned between ether and water. The ether extract (5 mg)

was examined by GLC on three phases:

a. Cyano-P (20%) at 75°.

| Product | % (GLC) | $I^{75}_{cyano-P}$ |
|---------|---------|--------------------|
| 1 | 5 | 1510 |
| 2 | 20 | 1545 |
| 3 | 15 | 1625 (ketone ? ) |
| 4 | 30 | 1650 ( " ) |
| 5 | 30 | 1670 ( " ) |

cf. copaene; $I^{75}_{cyano-P}$ 1545

b. APL (10%) at 150°

| Product | % (GLC) | $I^{150}_{APL}$ |
|---------|---------|-----------------|
| 1 | 20 | 1395 |
| 2 | 15 | 1410 |
| 3 | 35 | 1440 |
| 4 | 30 | 1460 |

cf. copaene; $I^{150}_{APL}$ 1410

c. SE-30 (1%) at 80$^{\circ}$.

| Product | % (GLC) | $I^{80}_{SE-30}$ |
|---------|---------|---------|
| 1 | 20 | 1375 |
| 2 | 35 | 1390 |
| 3 | 45 | 1400 |

cf. copaene ; $I^{80}_{SE-30}$ 1375

APPENDIX

## Spectroscopic Examination of the Drimenol Epoxides

The structure elucidation of drimenol and the chemical reactivity of the molecule due to the homo-allylic primary, alcohol function were the subject of papers by Brooks and Overton[43,44] in 1957 and 1959. In the absence of more modern techniques of separation and spectral characterization only the principal constituents of some of the reactions of the molecule were accessible. Epoxidation, for example, afforded a mixture of products, from which the principal constituent, drimenol $\alpha$-epoxide, was separated by fractional crystallization. The configuration was assigned primarily on the basis of certain chemical reactions which could be accommodated only by assuming $\alpha$-stereochemistry.

It was thought to be of interest to compare the chemical reactivities of both oxides as a function of relative stereochemistry. Accordingly, the crude reaction mixture from epoxidation was re-investigated. Careful chromatography afforded as the principal constituents two isomeric epoxides. One of these was the $\alpha$-oxide previously described: the other displayed closely similar infra-red absorption and mass spectrometric behaviour consistent with the isomeric $\beta$-oxide. NMR spectral examination was undertaken in order that the relative epoxide configurations could be more confidently assigned. Although the $\beta$-oxide structure has not been rigorously

proved, the spectroscopic evidence, in conjunction with the mode of preparation strongly supports this formulation and is difficult to reconcile with other formal representations.

The similarity of the carbon tetrachloride solution infra-red spectra confirmed the affinity of the two compounds. Dilution studies showed that in neither isomer was there any evidence of hydroxyl to epoxide intramolecular hydrogen bonding. The inference was that rotational freedom about the $C_{(9)}$ and $C_{(11)}$ bond was hindered in such a manner that the hydroxyl group was precluded from approaching the epoxide function. It had been expected, from an examination of Fieser and Dreiding models, that the $\beta$-oxide would show intramolecular bonding. The apparent absence of such bonding suggests that the necessary conformation of the hydroxymethyl group is sterically disfavoured as further mentioned below.
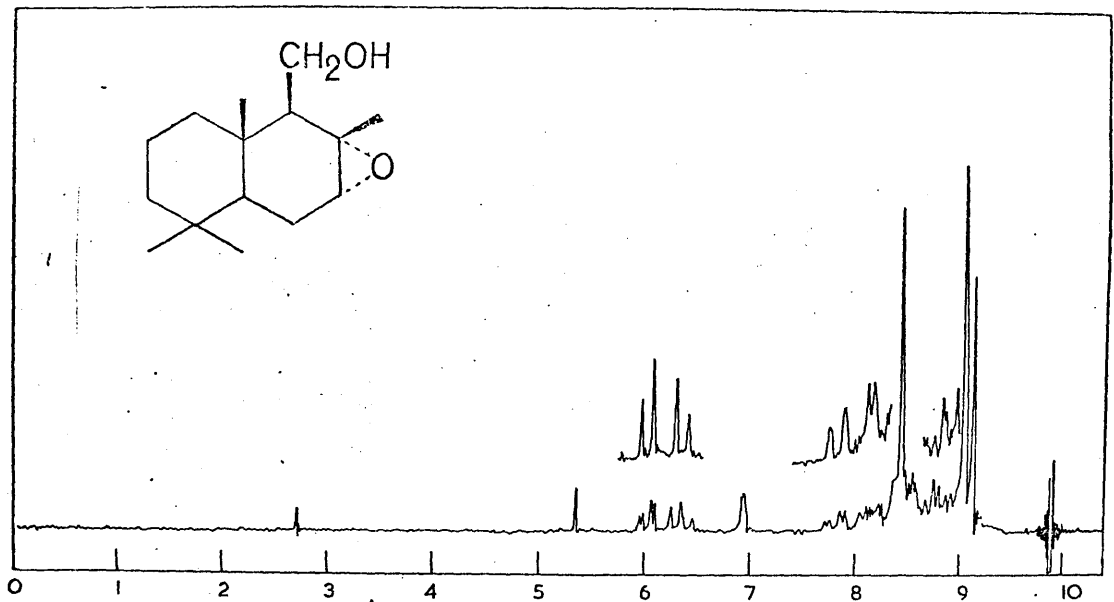
Fig. 34
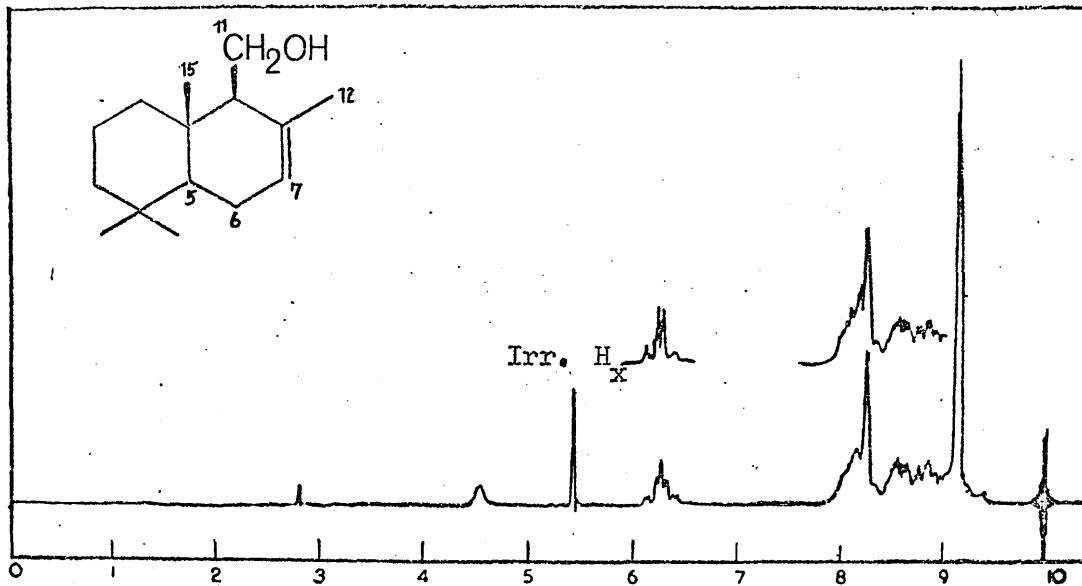
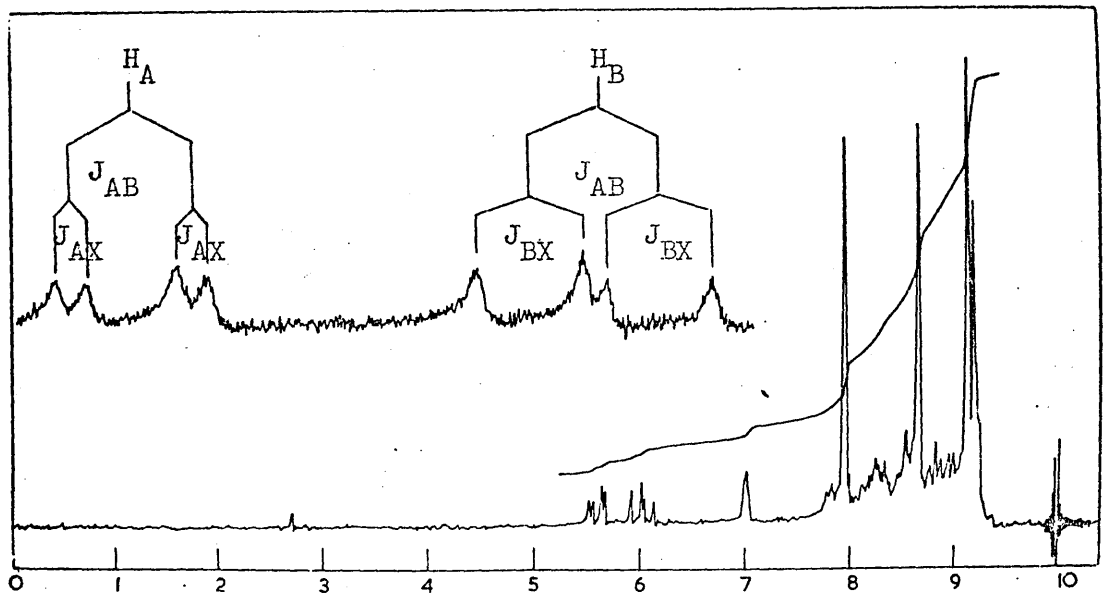NMR spectrum (100Mc/s.) of drimenol in CDCl$_3$



Fig. 35
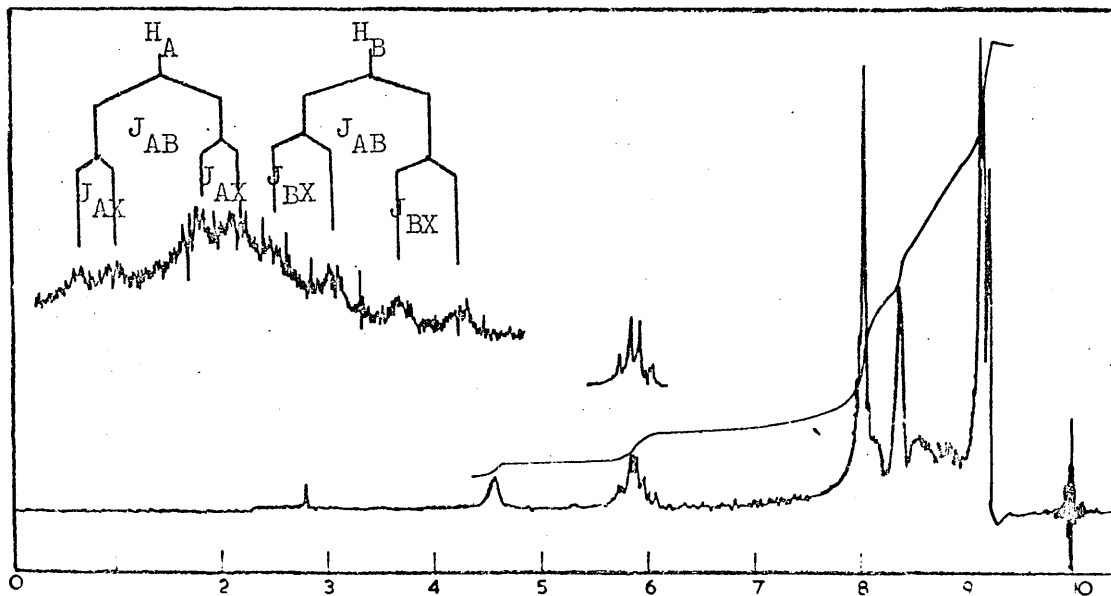
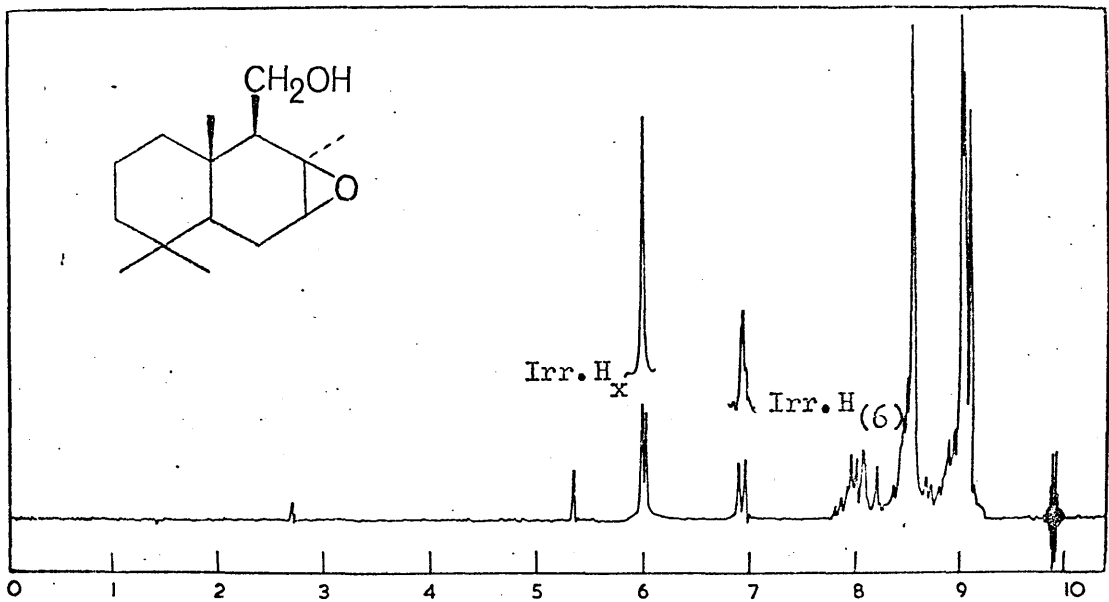NMR spectrum (100Mc/s.) of drimenyl acetate in CDCl$_3$

Fig. 36

NMR spectrum (100Mc/s.) of drimenol α-epoxide in CDCl₃



Fig. 37

NMR spectrum (100Mc/s.) of drimenyl acetate α-epoxide in CDCl₃

Fig. 38

NMR spectrum (100Mc/s.) of drimenol $\beta$-epoxide in CDCl$_3$



Fig. 39
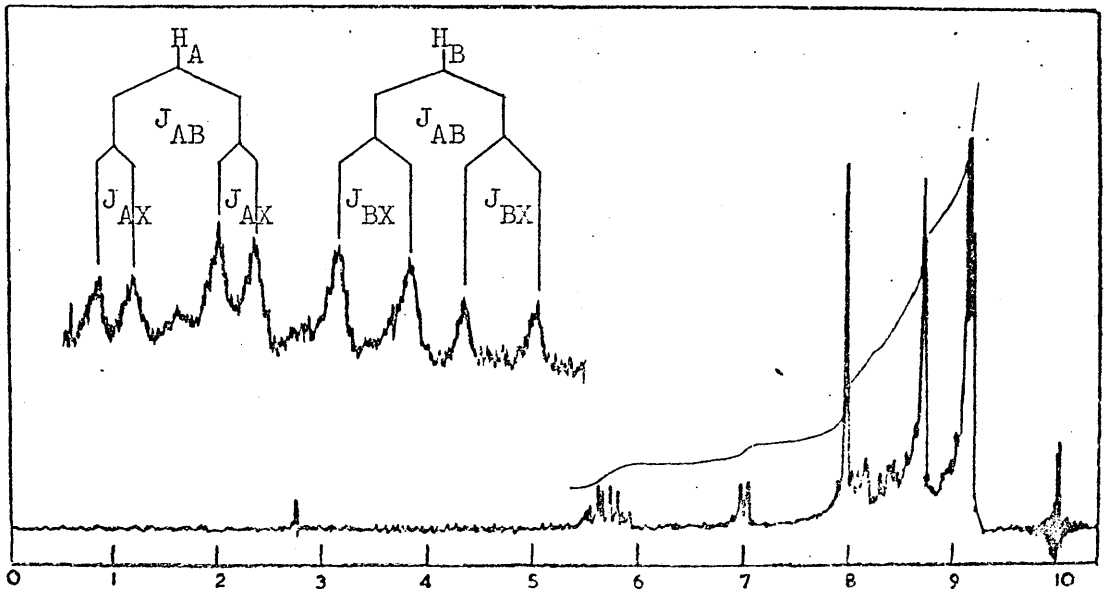
NMR spectrum (100Mc/s.) of drimenyl acetate $\beta$-epoxide in CDCl$_3$

TABLE 10

|  | Drimenol | Drimenyl acetate | α-oxide | α-oxide acetate | β-oxide | β-oxide acetate |
|---|---|---|---|---|---|---|
| Me(12) | 8.28 broad | 8.34 | 8.56 | 8.65 | 8.64 | 8.70 |
| Me | 9.06 | 9.18 | 9.25 | 9.18 | 9.18 | 9.18 |
| Me | 9.05 | 9.12 | 9.08 | 9.12 | 9.13 | 9.15 |
| Me | 9.05 | 9.10 | 9.08 | 9.12 | 9.10 | 9.12 |
| H(9) | 8.9? | 8.6,9.9? | ~8.55 | ~8.5 | ~8.6 | ~8.6 |

TABLE 10 (Cont'd).

| | Drimenol | Drimenyl acetate | α-oxide | α-oxide acetate | β-oxide | β-oxide acetate |
|---|---|---|---|---|---|---|
| 2H(11) | 6.3 8 lines AB X | 5.85 8 lines AB X | 6.3 8 lines AB X | 6.3 8 lines AB X | 6.1 2 lines J=2 cps | 5.7 8 lines AB X |
| H(7) | 4.55d broad double mult. | 4.55 broad mult. | 7.08 diffuse triplet small coupling possibly overlap doublets | 7.02 two overlapping doublets | 7.0 doublet J=3 cps | 7.0 doublet J=3 cps |
| 2H(6) | 8.20 broad mult. | 8.02 hidden under CH$_3$CO singlet | 8.0 multiplet | 7.9 multiplet hidden partially | 8.0 multiplet | 8.0 multiplet |
| H(5) | 8.9? | 8.6-8.9? | 9.0 two doublets | 8.9 multiplet | 8.6-8.9? multiplet | 8.6-8.9? multiplet |
| O ‖ Me-C- | - | 8.00 | - | 7.95 | - | 7.98 |

NMR studies on steroidal epoxides[45,46] have indicated
that the angles subtended by the epoxidic C-H bond with the
vicinal protons permit a differentiation between the $\alpha$- and
$\beta$ -isomers through a study of J - values. Examination of models
showed that in the $\alpha$ -oxide, $H_{(7)}$ (which should be the lowest
field signal of an AMNX multiplet) subtended angles of between
$40\text{-}60^{\circ}$ and $50\text{-}70^{\circ}$ with each of the vicinal $H_{(6)}$ protons.
Using the modified[46] Karplus relationship for epoxidic protons,
coupling constants of $J_{H(7)} - J_{H(6)} \sim 2$ cps were predicted. The
expected pattern for $H_{(7)}$ ( $\tau$ 7.0), a diffuse triplet of band
width $\sim 5\text{-}6$ cps was in fact observed (Fig. 36).

Similarly, in the $\beta$ -oxide, for dihedral angles $\angle H_{(7)}H_{(6a)}$
$= 0\text{-}20^{\circ}$ and $\angle H_{(7)}\text{-}H_{(6b)} = 100\text{-}120^{\circ}$ coupling constants
$J_{H(7)H(6a)} = 7$ cps and $J_{H(7)H(6b)} \sim 0$ were predicted. The signal
for $H_{(7)}$ was in fact observed at $\tau$ 7.0 as a doublet, $J = 7$ cps
(Fig. 38). Structure (56) was therefore indicated for drimenol
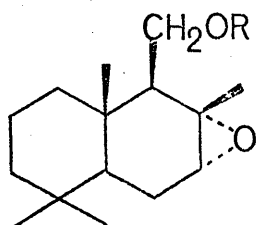$\beta$ -oxide.

Support for these assignments was provided by comparing
the chemical shift of Me (12) in each epoxide. The contribution
to the shielding of this group due to the proximity of the
hydroxyl should be greater in the $\beta$ -oxide. In accord with this,
chemical shifts of $\tau$ 8.56 and $\tau$ 8.64 were observed for the $\alpha$ -oxide
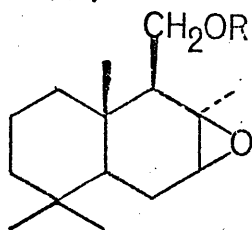and $\beta$ -oxide respectively.

Fig. 40
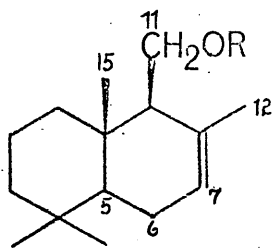


52, R=H
53, R=Ac


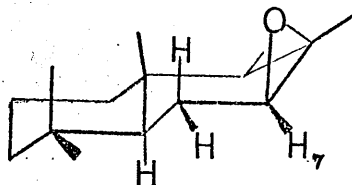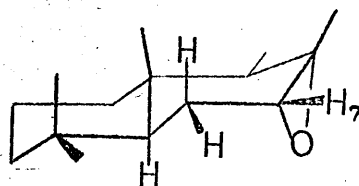
54, R=H
55, R=Ac



56, R=H
57, R=Ac

Table 10 summarizes the chemical shift data for the series of related compounds, drimenol (52), drimenyl acetate (53), drimenol $\alpha$-epoxide (54), drimenyl acetate $\alpha$-epoxide (55), drimenol $\beta$-epoxide (56) and drimenyl acetate $\beta$-epoxide (57). The considerable differences in the AB part of the ABX multiplets (Figs. 34-39) due to coupling of $H_{(11a)}$ and $H_{(11b)}$ with $H_{(9)}$ (henceforth referred to as HA, HB and HX) indicated time-averaged preferred conformations. Coupling constants were readily established by double irradiation and are listed in Table II.

TABLE II.

| | Chemical Shift ($\tau$) | | | Coupling Constants (cps) | | | Dihedral Angle (calculated) | |
|---|---|---|---|---|---|---|---|---|
| | HA | HB | HX | $J_{AB}$ | $J_{AX}$ | $J_{BX}$ | $\angle_{AX}$ | $\angle_{BX}$ |
| drimenol | 6.2 | 6.3 | 8.2 | -12 | 4 | 4 | 50 or 120° | 50 or 120° |
| drimenyl acetate | 5.8 | 5.9 | 8.0 | -12 | 3.6 | 6 | 53 or 117° | 38 or 128° |
| $\alpha$-epoxide | 6.1 | 6.3 | 8.5 | -11 | 3 | 10 | 55 or 113° | 0 or 140° |
| $\alpha$-epoxide acetate | 5.6 | 6.0 | 8.0 | -12 | 3 | 10 | 55 or 115° | 0 or 140° |
| $\beta$-epoxide | 6.1 | 6.1 | 8.6 | 0 | 0 | 4 | 90° | 50 or 120° |
| $\beta$-epoxide acetate | 5.6 | 5.8 | 8.0 | -12 | 4 | 7 | 50 or 120° | 30 or 130° |

In each case, apart from (56) (which exhibits coincidental magnetic isochrony) Jgem was approximately - 12 cps. (negative by convention). The chemical shifts of HA and HB differ in each case (apart from (56) ) due to the prochiral nature of the protons.

More important, however, from the point of view of the preferred conformation of the hydroxyl group, was the fact that $J_{AX} \neq J_{BX}$ except in (52).

From the data for drimenol (52), application of the Karplus equation leads to two possible dihedral angles, $50^\circ$ and $120^\circ$ for the time-averaged $\angle AX$. If the former value holds, then a Newman projection shows that $\angle BX = 170^\circ$; but $\angle BX = 50^\circ$ or $120^\circ$ (observed), therefore $\angle AX = 120^\circ$ and $\angle BX = 120^\circ$ are the only possible values. Thus, the hydroxyl group points away from the double bond and the $C_{(11)}-O$ bond eclipses the $C_{(9)}-H_{(9)}$ bond. A similar result is obtained from a consideration of drimenyl acetate.

In drimenol $\alpha$-oxide $J_{BX} = 10$ cps. therefore $\angle BX$ could be $0^\circ$ or $140^\circ$. The former value is precluded because of severe steric inter-action between the hydroxyl and Me(12), therefore $\angle BX = 140^\circ$, and the time-averaged conformation is one in which the hydroxyl group points away from the epoxide, and the $C_{(11)}-O$ bond eclipses the $C_{(9)}-H_{(9)}$ bond. Drimenyl acetate $\alpha$-epoxide has a similar conformation.

Four possible time-averaged conformations are possible in drimenol $\beta$-epoxide, which are compatible with $\angle AX = 90^\circ$. Two involve the hydroxyl group pointing towards the epoxide group and two result in the hydroxyl group pointing away. Of the latter two, one is precluded by severe hydroxyl-Me(15) inter-actions. The other should result in intra-molecular hydrogen bonding (O -O distance 2.3Å ). No such bonding was observed in the infra-red spectrum, however,

and the hydroxyl group must therefore point away from the epoxide. The $\beta$ -oxide acetate must, for steric reasons, point away from the epoxide, so that dihedral angles subtended by HA HX and HB HX are $120^{\circ}$, and $C_{(11)}$-O eclipses $C_{(9)}$-$H_{(9)}$.

## EXPERIMENTAL

### Chromatographic Separation of Drimenol Epoxides

The crude epoxidation product (4.67 g) from drimenol and
perphthalic acid, from which several crops of drimenol $\alpha$-epoxide
has been obtained in 1959, was adsorbed on neutral alumina
(225 g Woelm, grade III). A gradient elution using 2.5 l. petrol
(bp. 60-80°) and 2.5 l diethyl ether was performed, collecting
50 ml aliquots. Drimenol $\beta$-epoxide (1.45 g) was obtained from
fractions 40-69. Recrystallization from light petroleum-diethyl
ether afforded a white crystalline solid mp. 78-79.5°. Found,
C, 75.73; H, 10.91 : required for $C_{15}H_{26}O_2$, C, 75.58; H, 10.99%.
Infra-red (carbon tetrachloride solution, 5 mg/ml): $\nu_{max}$ $(cm^{-1})$,
3620 (free OH), 3497 (intermolecularly-bonded OH, disappeared on
1:10 dilution), 1385(M), 1376(M), 1362(M), 1230 diffuse (M),
1120(S), 1077(MS), 1053(MS), 1037(MS), 1002(M), 986(M), 966(M),
951(M), 919(M). No absorption was noted in the carbonyl region.
$R_f$ (ether), 0.75. Unstable under GLC conditions.

Further elution afforded, from fractions 83-95, crude
drimenol $\alpha$-epoxide (2.00 g). Recrystallization from light
petroleum-diethyl ether afforded a white crystalline solid, mp. 96-97°
Found, C, 75.78; H, 11.15: required for $C_{15}H_{26}O_2$, C, 75.58;
H, 10.99%. The infra-red spectrum was similar to that of the
$\beta$-oxide: $\nu_{max}$ $(cm^{-1})$ (carbon tetrachloride solution, 5 mg/ml),
3620(S), 3497 (disappeared on dilution), 1384(M), 1376(M), 1362(M),

1235 diffuse (M), 1178(M), 1147(M), 1111(M), 1072(M), 1060(M),

1031(M), 981(M), 964(M), 946(M), 920(M). $R_f$ (ether) 0.60.

Unstable under GLC conditions.

## Acetylation of Drimenol Epoxides

Small-scale acetylation of each oxide (20 mg) was accomplished

by dissolution in acetic anhydride (0.1 ml) and pyridine (0.1 ml),

warming the solution for 5 minutes. The derivative was

partitioned between ether and dilute hydrochloric acid, sublimed

and checked by TLC for complete conversion.

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P A R T   II

PART II

4.1      <u>Introduction to the Use of Derivatives in</u>

           <u>Combined Gas Chromatography - Mass Spectrometry</u>.

        Structure elucidation is becoming progressively more dependent on spectroscopy as more subtle techniques are developed and increasingly complex systems examined. In previous years the chemist, having reached an impasse in his consideration of spectral data, would have resorted to classical procedures for chemical modification of the structure in question. This approach, in the light of recent developments in spectroscopy, is no longer obligatory; many structures yield to modern spectrometric investigations, coupled with chemical transformations designed to enhance the diagnostic value of spectrometric data.

        A major development in mass spectrometry, for example, has been the use of specific functional group derivatives. Groupings such as olefins, ketones and hydroxyls have been transformed in such a manner that predictable fragmentations should occur, affording structural data complementary to that obtained from the parent molecule. Since the work to be described in this section of the thesis involves a brief examination of certain derivatives in relation to combined gas chromatography - mass spectrometry(GC-MS) it is proposed to review critically some of the derivatives which may be suited to the technique. The utility of the derivatives in terms of the following criteria will be appraised:

(a) they should be amenable to simple small-scale preparation, preferably in good yield;

(b) they should have good gas chromatographic properties;

(c) desirable mass spectral characteristics are the formation of an abundant molecular ion and informative skeletal fragmentation – directing properties.

Deuterium labelling, which has proved to be an indispensable aid to the elucidation of mass spectral fragmentation mechanisms will not be included under this heading of derivatization.

Sources of information are the major text-books[1], mass spectroscopy bibliographies and the recent literature.
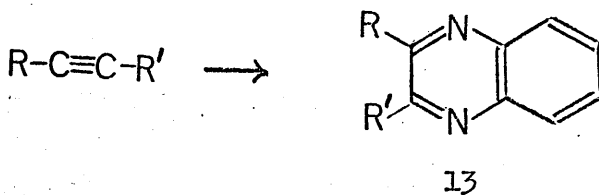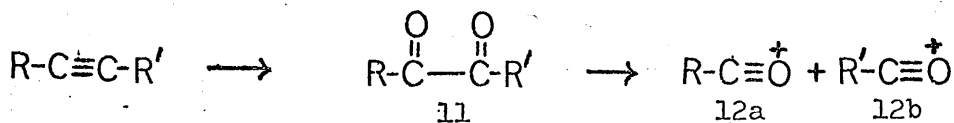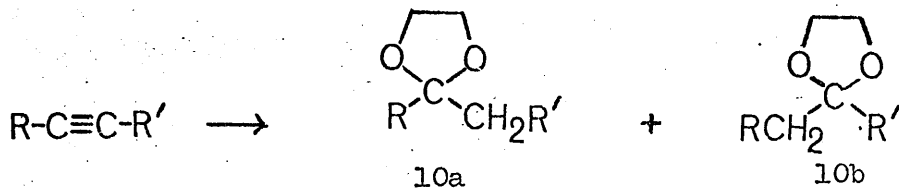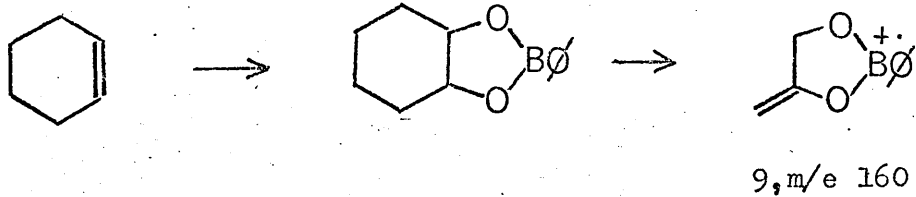
# Fig. 1
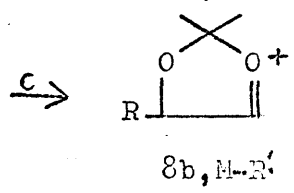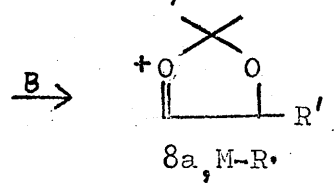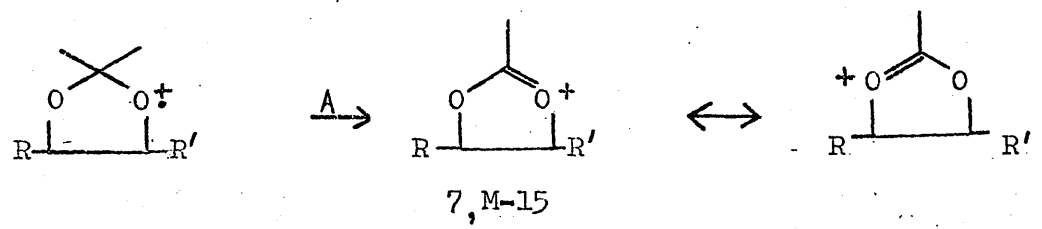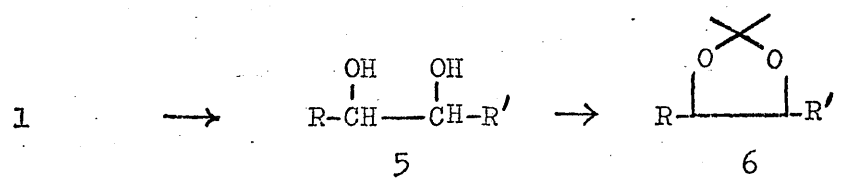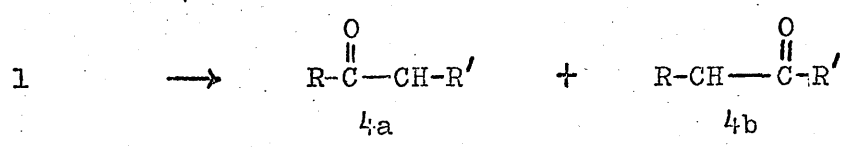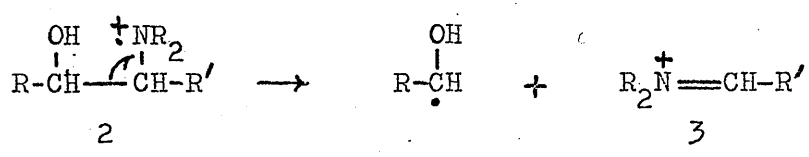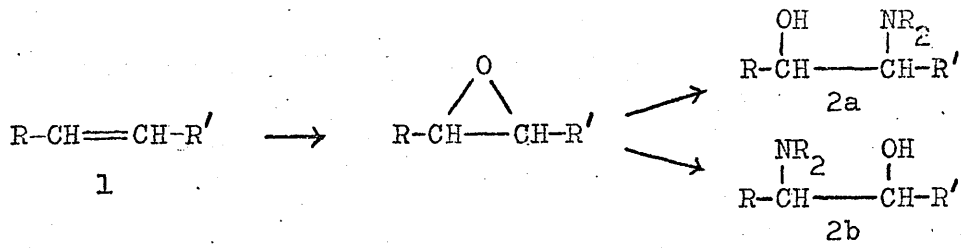
## Unsaturated Hydrocarbons

The mass spectral fragmentation of olefins is a complex process, especially in cyclic systems. Even in acyclic mono-olefins, hydrocarbon rearrangements occur in the molecular ion because of migration of the radical site along the chain. Hence, the mass spectra of isomeric acyclic mono-olefins are generally very similar[2]. The practical value of derivatization of the functional group, in such a manner that well-defined fragmentations may be observed, is therefore evident.

Several approaches have been successfully examined. Epoxidation, followed by treatment with dimethylamine, yields, in the case of the acyclic asymmetrically substituted olefin (1) the two isomeric vicinal dimethylamino alcohols (2a, b). Their principal fragmentation involves rupture of the bond connecting the hetero-functions, with formation of a stabilized immonium cation (3), defining the location of the double bond. This procedure could be of applicability in GC-MS depending on the polarity of the derivative. A reservation, not noted in the literature, is that in the case of a chiral olefin, diastereoisomerism may complicate the gas chromatographic, though not, perhaps, the mass spectrometric characteristics of the mixture. Epoxidation can also be followed by treatment with sodium iodide[3], affording the two possible ketones (4a, b) which undergo characteristic ketonic fragmentation. A third approach involves osmium tetroxide hydroxylation of the

Fig. 2



$9, m/e$ 160

$R-C\equiv C-R'$ ⟶ $\underset{10a}{}$ + $\underset{10b}{}$

$R-C\equiv C-R'$ ⟶ $\underset{11}{}$ ⟶ $R-C\equiv \overset{+}{O}$ + $R'-C\equiv \overset{+}{O}$

$\phantom{xxxxxxxxxxxxxxxxxxxxxxxxxxx}$ 12a $\phantom{xxx}$ 12b
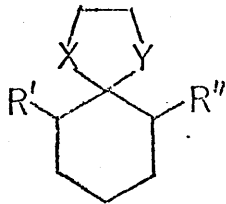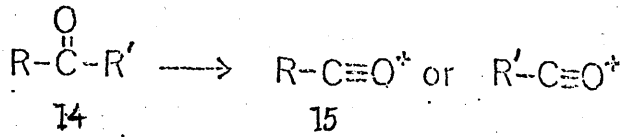
$R-C\equiv C-R'$ ⟶ $\underset{13}{}$

olefin to the vicinal diol (5) and formation of acetonide (6).

The characteristic fragmentation of acetonides involves [4,5]

$\alpha$ -cleavage, resulting in an abundant M-15 peak (path A) or,

more significantly, (but much less abundantly), loss of the

alkyl moiety (paths B and C). Several other complicating processes

occur, limiting the applicability of this procedure to simple

compounds such as unsaturated fatty acid esters[4]. Investigation

of the cyclic boronate esters of vicinal diols derived from

olefins, has revealed their ease of formation and useful properties

for GC-MS. Phenylboronate esters have been observed to give rise to

abundant fragments incorporating the boronate ring and characteristic

of its environment. The corresponding derivative of cyclohexene,

for example, affords a base-peak at $^m/e$ 160, corresponding to a

species $(C_6H_5BO_2C_3H_4)^+$ which may have structure (9)[7]. Studies on

substituted and deuterated species are necessary, however, to

elucidate the fragmentation process. The use of carbonates or

thiocarbonates as derivatives for GC-MS would appear to be precluded

by the complexity of their fragmentation[8].

Triple bonds in a simple molecule may be located[9] through

hydration of the acetylene in the presence of ethylene glycol, followed

by GC-MS examination of the resultant isomers (10a, b). Otherwise,

there is a paucity of procedures for the mass spectral derivatization

of acetylenes. Procedures such as $\alpha$ -diketone formation using

permanganate presumably yielding only one product (11) (cf. (10a)

and (10b) ) appear not to have been examined. If characteristic

fragmentation data were not obtained from the acylium ions
(12a, b), then derivatization with ortho-phenylenediamine should
afford the quinoxaline (13) which should have a characteristic
fragmentation and should be amenable to gas chromatographic
examination.

In conclusion, most of the derivatization procedures discussed
for unsaturated hydrocarbons can be applied successfully to
acyclic structures. There is no obvious derivative which could be
utilized in a complex unsaturated cyclic system, affording
definitive fragmentations. Designing such a derivative for GC-MS
would presumably necessitate the incorporation of the olefinic
moiety into an aromatic heterocyclic system of low polarity and
high mass spectroscopic stability.

Fig. 3

$$R-\overset{\overset{\displaystyle O}{\|}}{C}-R' \longrightarrow R-C\equiv O^{+} \text{ or } R'-C\equiv O^{+}$$

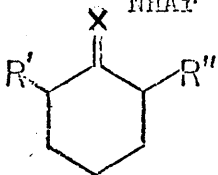$$\underset{14}{} \qquad\qquad \underset{15}{}$$

only one isomer possible
except when $X=O$ and $Y=S$,
with potential diastereo-
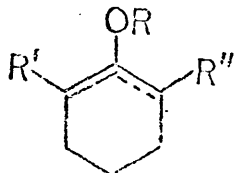isomerism

14a, X and/or Y
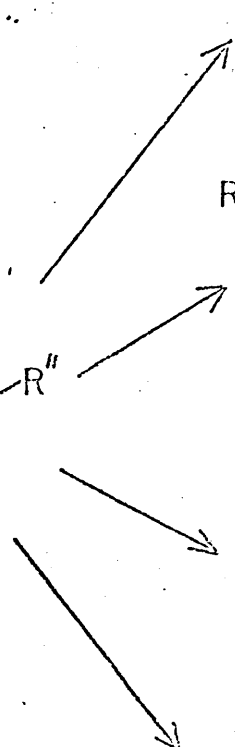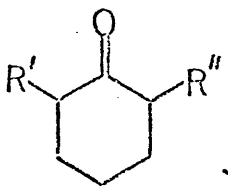= S and/or O
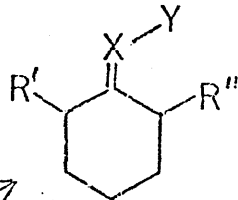
geometrical isomers
possible

14b , X=H
Y=NMe$_2$
NHAlk
NHAr

no isomers possible

14c , X=O$^{18}$, S

double bond isomers
possible

14d

## Carbonyl Groups

Fragmentation processes involving the carbonyl group can be understood most readily in terms of the decomposition of radical ion (14), generally by an $\propto$-cleavage process, the charge tending to remain with the stabilized oxonium ion (15). Without a double-focusing instrument the assignment of elemental constitution to the ions in the spectrum can often be ambiguous, and the elucidation of the fragmentation may be rendered more difficult. Indeterminate data concerning the environment of the carbonyl in an unknown structure may therefore be obtained. It is obvious why derivatization of the carbonyl group has concerned many mass spectroscopists in recent years.

Carbonyl derivatives of potential use in GC-MS can be divided into four classes (Fig. 3):

(a) Those in which the carbonyl $sp^2$ carbon is replaced by an $sp^3$ hybridized carbon of the type (14a) e.g. an acetal or thioacetal which are symmetrical derivatives, or a thioxolane derivative which is asymmetric and is potentially diastereoisomeric.

(b) Those in which the carbocyclic ring has one $sp^2$ centre, i.e. carbonyl analogues of the type (14b) in which R = $N-NR_2^1$ or $NR^1$. Derivatives of this type are also potentially capable of geometrical isomerism.

# Fig. 4



16, R=O, S

17

18

19

20, m/e 99

21, m/e 125
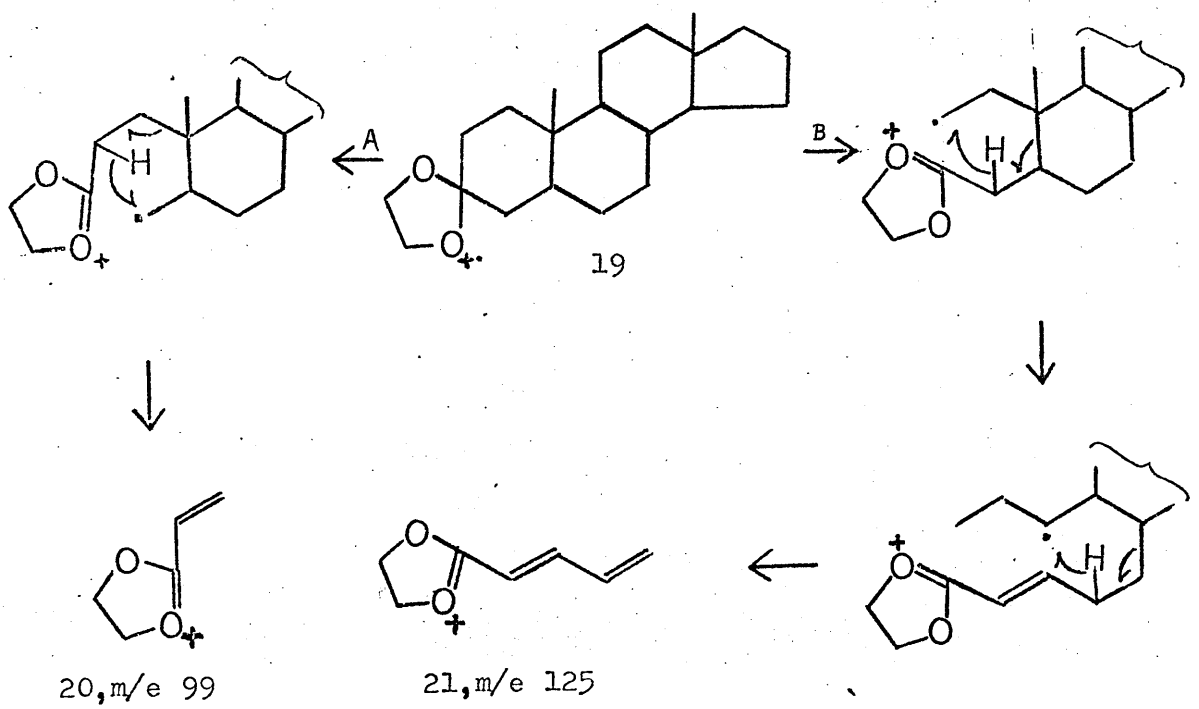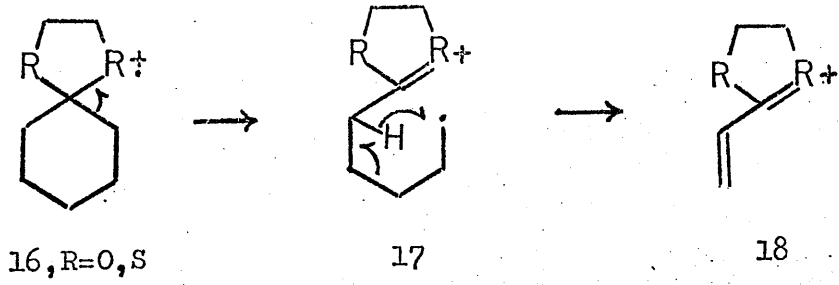
(c) Those symmetrical derivatives (14c) in which the carbocyclic ring has one $sp^2$ centre i.e. iso-electronic analogues of the carbonyl group such as $C = O^{18}$ and $C = S$

(d) Those systems in which two vicinal $sp^2$ centres are introduced e.g. enol acetates and enol methyl ethers of type (14d). Double bond isomerism is again possible.

## Class (a)

Among the most widely-applied derivatives are the ethylene ketals and thioketals (Fig. 4) which largely satisfy the criteria for general utility; thus, they fragment in a coherent manner and are potentially amenable to gas chromatography. The fragmentation processes are governed by the preferential homolysis of the bond attached to the 2-position of the dioxolane/dithioxolane ring (16) producing a resonance-stabilized oxonium/sulphonium ion (17). The species then undergoes 1,5-hydrogen transfer (substantiated in many instances by deuterium labelling) from the allylic to the primary site, followed by scission of the C-C bond yielding the resonance-stabilized fragment (18). An illustration of the utility of these derivatives is provided by the fragmentation of $5\alpha$-androstan-3-one ethylene ketal[10,11] (19). The two pathways A and B corresponding to the alternative initial $\alpha$-fissions are both evident, leading by a synchronous process to the predominant spectral fragments (20) and (21). The fragmentations have been

Fig. 5
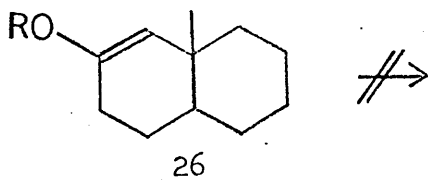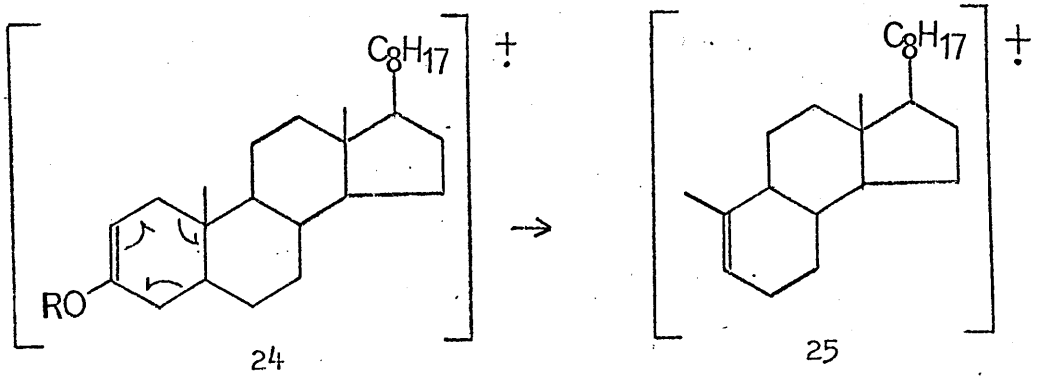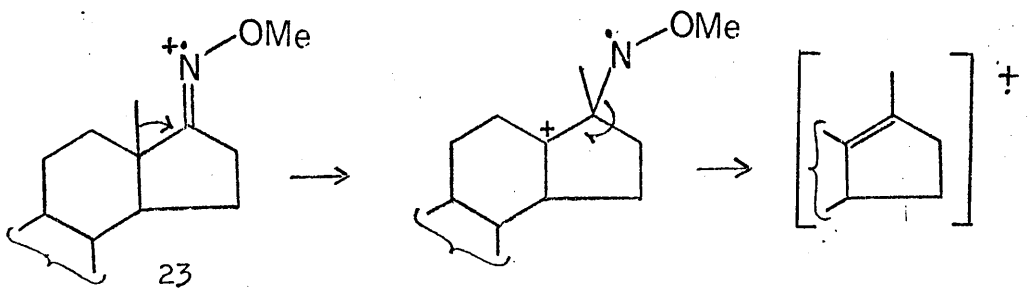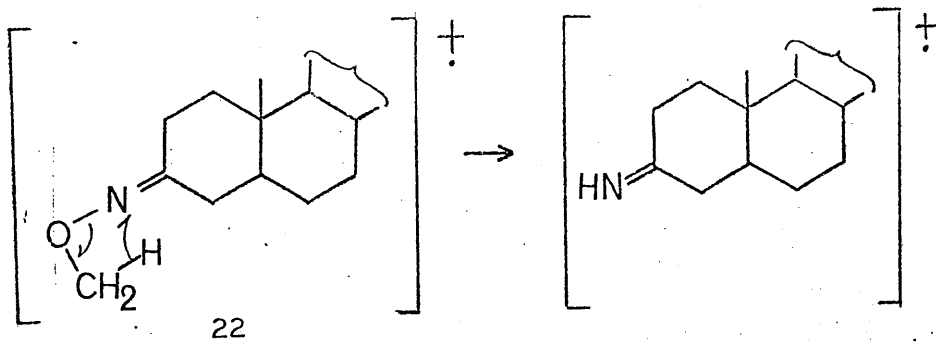
corroborated by thorough deuteration studies[10,11]. The
fragmentation patterns of the ketals and thioketals are practically
identical, but the intensity of the diagnostically significant
fragment ions of the thioketals is much lower than in the
corresponding ketal derivatives, thus making the sulphur derivatives
less desirable for structural determinations. This is, to a small
extent, offset by the enhanced molecular ion of thioketals. A
reservation must be made, however, about the applicability of these
derivatives in GC-MS, since they may be thermally labile.
Oxazolidines, formed by reaction of the carbonyl group with vicinal
hydroxyamines, exhibit adverse gas chromatographic properties[12].

Class (b).

This class of 'asymmetric' derivatives of carbonyls has
been the subject of detailed mass spectrometric study, e.g. oximes,
O-methyloximes, O-trimethylsilyloximes, hydrazones, alkylhydrazones,
arylhydrazones and semicarbazones. Of these derivatives, however,
only the substituted oxime type and dimethylhydrazones are of
relevance in this review of derivatives for GC-MS, the others being
of limited gas chromatographic applicability. The base peak of
cholestan-3-one at $m/e$ 231 [13] (loss of side chain and $C_{(15)}$, $C_{(16)}$
and $C_{(17)}$) is shifted to $m/e$ 260 (231 + NCH$_3$) in the O-methyl
oxime (22) as expected. Similarly, peaks in androstan-17-one at
$m/e$ 230, 218 and 217 due to cleavage of ring D are still found in

the O-methyl oxime (23) in lower abundance. Abundant loss of CH$_2$O probably occurs in the manner depicted in Fig. 5. A second abundant process in the derivative of androstan-17-one (23) involving loss of NOCH$_3$ perhaps[13] occurs as shown in Fig. 5. It is evident that several fragmentation-directing pathways are possible in O-methyl oximes, but valuable complementary data can be obtained by the 'shift' technique, for example, by comparing the O-methyloxime with the O-trimethylsilyl oxime. The extreme ease (and potential reversibility) of preparation of O-methyloximes and O-trimethylsilyl oximes, and the ready recognition of N-containing fragments, makes them useful derivatives despite complications due to syn and anti isomers.

Of the various substituted hydrazones, only the dimethyl derivatives have been shown to exhibit good gas chromatographic[14] and mass spectrometric[15] characteristics. The fragmentation-directing ability of the group is illustrated in Fig. 6.

The azomethine ketone derivative, which formally falls within category (b) is the simplest nitrogen analogue. Were it not for the instability[*] of the azomethines, requiring prompt measurement of the mass spectrum on freshly-prepared material, it would represent one

---

[*]The difficulty is to go from $>C=O$ to $>C=NR$: the excess RNH$_2$ being strongly basic usually destroys the ketone. The use of the reverse process $RNH_2 \xrightarrow[R_2CO]{excess} RN=CR_2$ is well known[16].

of the best nitrogen-containing derivatives for mass spectroscopy since the functional group directs the fragmentation in a highly specific manner[16].
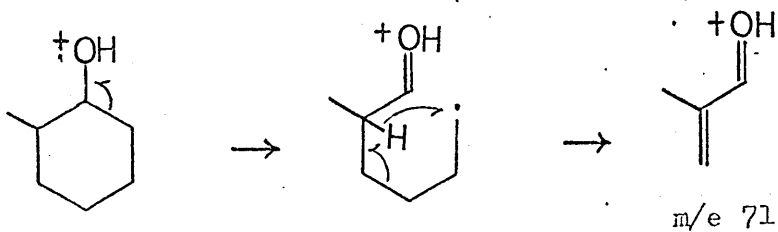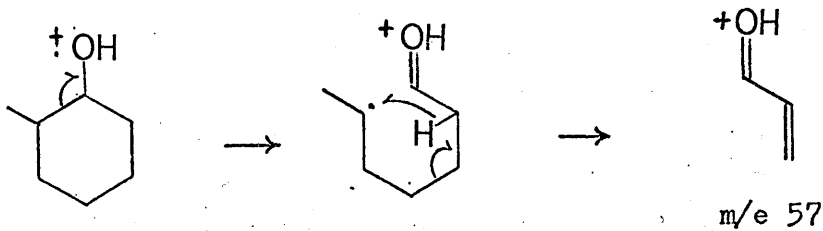
## Class (c)

The use of isotopically-labelled carbonyl groups ( $C=O^{18}$ ) in the elucidation of carbonyl-directed fragmentations has been reported[17]. However, the use of iso-electronic carbonyl analogues such as thiones has not been reported. This derivative, which is readily prepared in many instances, is the subject of an investigation in section 4.2 of this thesis.

## Class (d)

Enol acetates and enol methyl ethers, which are often capable of existing in isomeric forms, are the best-known examples of class (d). Audier et al[18] have examined the mass spectral properties of these derivatives (24, R=Me, Ac). In cases where Retro-Diels Alder processes were possible, the appropriate fragments (25, R= Me, Ac) were observed as abundant species. Information about ring A substitution in 3-oxo-steroids can thereby be deduced, and the further fragmentation of species such as (25) considered. The authors did not, however, explain the fragmentation of (26) in which the Retro-Diels Alder process is precluded by virtue of substitution. The work of Waight et al[19] on tetralone-type enol acetates has indicated that they may, in the absence of other processes, eliminate ketene by a McLafferty-type process giving rise to an energised

Fig. 7

$$RCH \overset{H}{\cdots} + \overset{O}{\cdots}{\overset{H}{\phantom{|}}} \quad \xrightarrow{-H_2O} \quad RCH\!-\!CH_2$$

$$\xrightarrow{-H_2O} \quad RCH = CH_2 \overset{+}{}$$

$$\xrightarrow[-C_2H_4]{-H_2O}$$

$$\xleftarrow[\text{dehyd}^n]{1,4-} \qquad \xrightarrow[\text{dehyd}^n]{1,3-}$$

m/e 57

m/e 71
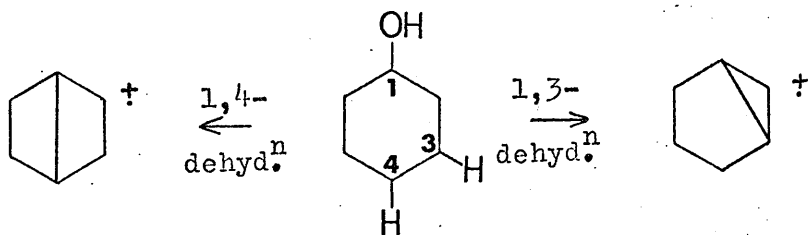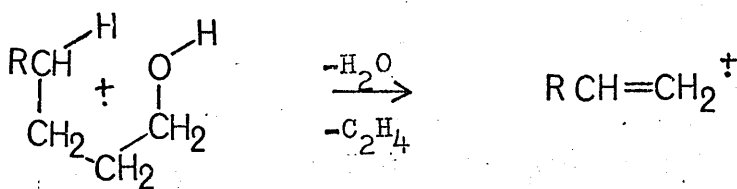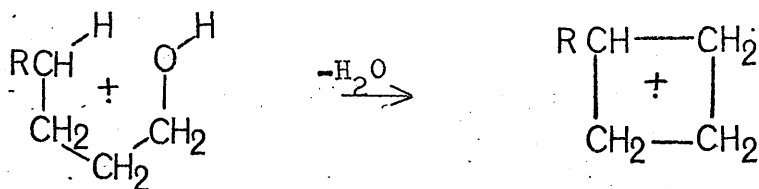
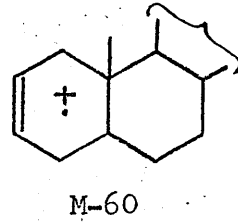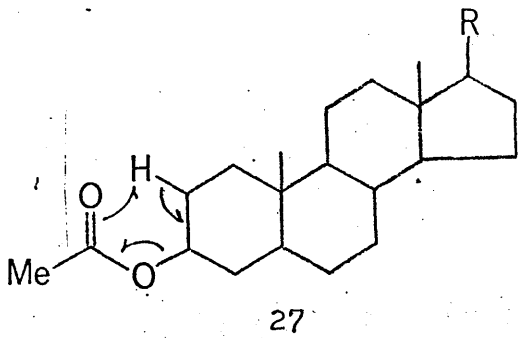species which is formally similar to the parent ketone, but which has a different mode of fragmentation. This type of derivative has obvious potential in GC-MS. Consequently, an examination of enol trimethylsilyl ethers was undertaken in order to evaluate their general utility (see p.103).

## Hydroxyl Groups

The fragmentation of alcohols is beset by a complexity of pathways. Dehydration frequently occurs, either by thermal 1, 2-elimination when a heated inlet system is used, or from the molecular ion through 1,3 or 1,4 elimination as depicted in Fig. 7. $\alpha$-Fission leading to the stabilized oxonium ion which then may have several alternative fragmentation routes, is also a general process. Higher aliphatic alcohols give rise to oxygen-containing as well as hydrocarbon ions with the latter generally predominating and obscuring characteristic hydroxyl-directed fragmentations. The abundance of molecular ions is often low. In cyclic alcohols, well-recognised processes occur, as shown in Fig. 7. In general, however, the spectra of cyclic alcohols such as the monoterpene alcohols, are sufficiently complex that useful generalisations applicable to other cyclic alcohols cannot safely be made, since many of the fragmentations are not directed by the hydroxyl group.

Acetates are perhaps the most common derivatives in organic chemistry. Their ease of formation and excellent gas chromatographic characteristics are well known, but unfortunately, in the mass

Fig. 8



27 → M-60

Retro- | Diels Alder

M-(60+54)

$Me_3Si-O-(CH_2)_{10}-\overset{+\bullet}{O}-SiMe_3$

28

$Me_3Si=\overset{+}{O}-CH_2-O-SiMe_3$

29, m/e 177

$Me_3Si-\overset{+}{\underset{H}{O}}-\underset{OH}{Si}Me_2$

30, m/e 165

31 →

32, m/e 85
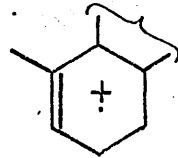
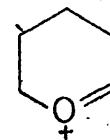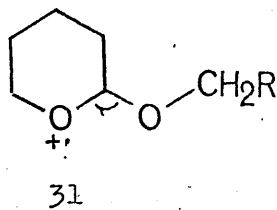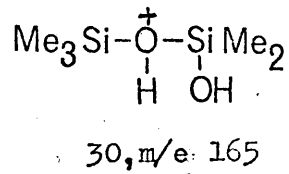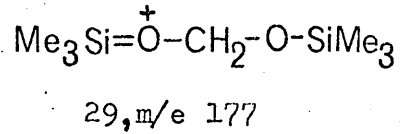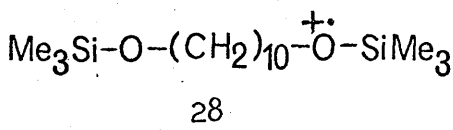spectrometer they eliminate acetic acid by both 1,2 and 1,3 - processes as readily as alcohols eliminate water, and consequently the molecular ion is often undetectable. The charged residue which remains often suffers complex olefinic fragmentations, although in the case of cholesteryl acetate[18] (27, Fig. 8) a straightforward fragmentation process occurs. Trifluoroacetates have been reported[20] to show abundant molecular ions and have less complex fragmentation pathways.

The analytical utility of trimethylsilyl ethers in GC-MS is widely appreciated, especially in biochemical investigations; the separation and identification of steroids in faeces[21], urine[22], and blood[23], through their trimethylsilyl derivatives constitute telling examples of the power of the technique. These derivatives have already been considered briefly in section 1.2. Characteristic steroid trimethylsilyl ether fragmentations were first recorded by Eneroth et al.[24] in 1964 and by Sjövall[25] in 1965. Useful correlations were noted for the ubiquitous $3\beta$-hydroxy-$\Delta^5$-steroids. The scope of these correlations was explored for many examples by Brooks et al[26] in 1966. The formation of characteristic ions at $^m/e=129$ and M-129 was shown also to occur for both $4\beta$-methyl- and 4,4-dimethyl-$\Delta^5$-sterols, and it was concluded that the fragmentations involved allylic fissions at $C_{(3)}$-$C_{(4)}$ and $C_{(1)}$-$C_{(10)}$. The proposed breakdowns (Fig. 9) were confirmed by Djerassi et al[27] in 1967 by deuterium labelling.

A reservation concerning the use of the derivative must be

Fig. 9
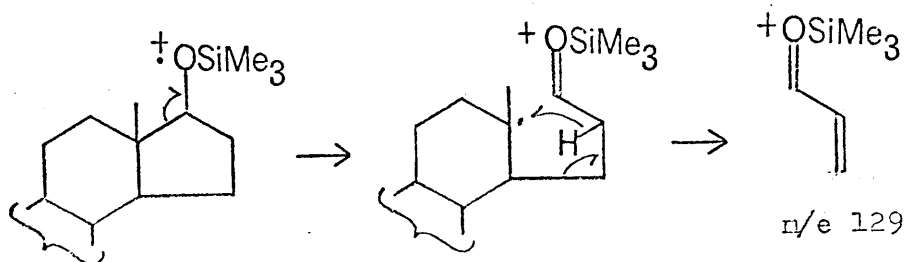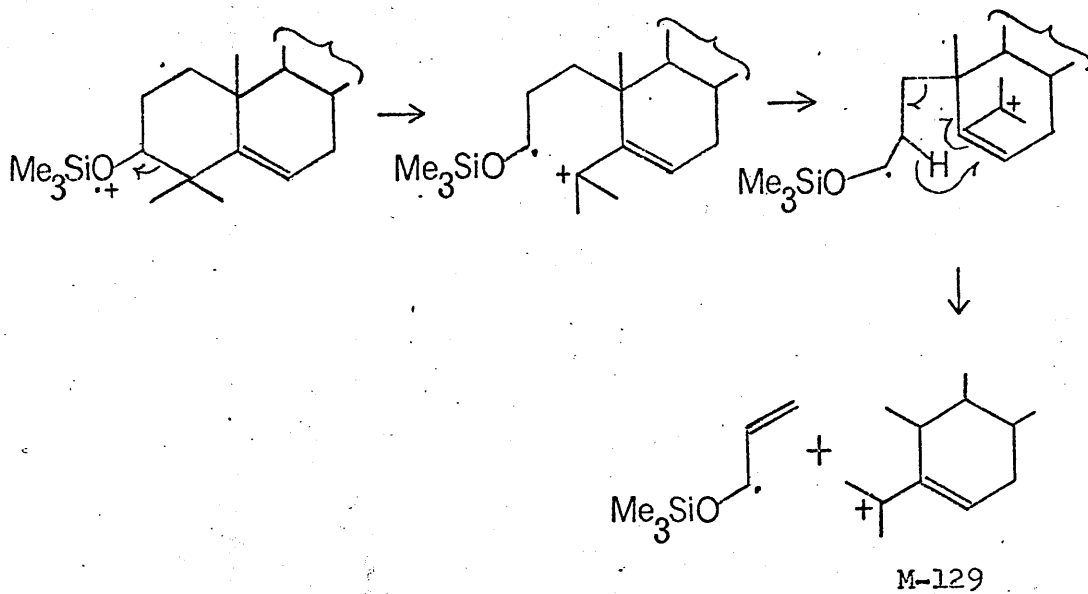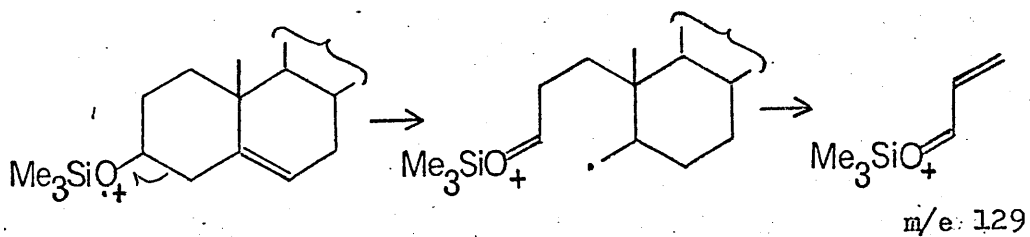
made, however. It has been observed that certain trimethylsilyl ethers undergo skeletal rearrangements upon electron impact, which can cause misinterpretation. An example of this is the formation of the ions (29) and (30) from the bis-trimethylsilyl ether (28)[28]. The use of perdeuterated trimethylsilylating reagents[28] is of potential value in the 'shift' technique. The versatility of other derivatives such as dimethylsilyl ethers and chloromethyl-dimethylsilyl ethers[29] (useful in mass spectrometry and electron-capture gas chromatography) should also be noted.

Tetrahydropyranyl ethers[30] are probably of limited applicability, since their molecular ions are weak, and the base peak in the spectrum is generally caused by $\alpha$-fission between the ether oxygens ( (31) → (32) ) with retention of charge by the tetrahydropyranyl moiety. The alternative $\alpha$-fission in the side chain is relatively unimportant.

Polyols are by virtue of their polarity unsuitable as such for GC-MS. Vicinal diols have already been considered in the section on olefinic derivatives. 1,3-Diols should be amenable to examination by GC-MS, using either the acetonide or boronate[6] derivative. No comprehensive data for their mass spectral fragmentations are as yet available. Poly-trimethylsilyl ethers have been demonstrated to be suitable for GC-MS e.g. mannitol hexa-trimethylsilyl ether[31] (mw. 614, cf. parent compound, mw. 182). Studies on saccharides[32], steroid pentols[33], and nucleosides [34] via the polytrimethylsilyl derivatives illustrate the capacity of

Fig. 10



$$CH_3CH(CH_3)\overset{+}{N}(CH_3)-CH_2CH_2CH_2CH_3 \longrightarrow CH_3CH=\overset{CH_3}{\underset{+}{N}}-CH_2CH_2CH_2CH_3$$

$$CH_2(CH_3)CH\overset{CH_3}{\underset{+}{N}}=CH_2 \longrightarrow H-\overset{CH_3}{\underset{+}{N}}=CH_2 \quad CH_3CH=\overset{CH_3}{\underset{+}{N}}-H$$

m/e 44

m/e 58

$$\overset{\cdot+}{N}R_2 \longrightarrow \overset{\cdot}{\underset{H}{N}}R_2 \longrightarrow \overset{+}{N}R_2 \longrightarrow \overset{+}{N}R_2$$

34

m/e 82

the derivative to render non-polar materials sufficiently volatile for GC-MS.

## Amines

The amine group has a greater electron-releasing capacity than the hydroxyl group. Types of cleavage analogous to those of the hydroxyl group occur in amines to a more pronounced degree. The fragmentation of methyl ethyl butylamine (33) illustrates the simpler processes to be expected (Fig. 10). The amine function thus has a greater ability to direct fragmentation and stabilize the resultant fragment as an immonium cation (e.g. bornylamine (34), Fig. 10), affording information about the environment of the nitrogen. Unfortunately, the amino function, because of its polarity, confers adverse gas chromatographic properties.

Functionalization of primary and secondary amines can improve gas chromatographic properties: acetylation and trimethylsilylation are recognised procedures. An example of the power of the GC-MS method is given by the study of derivatized biological amines, by Horning et al.[16]. $\beta$-Phenylethylamine readily affords the N-trimethylsilylated derivative which exhibits abundant ions at $m/e = 193$ (molecular ion) and at $m/e = 102$ (cleavage between the carbon atoms of the side chain with charge retention by the N-containing moiety). Catecholamine mixtures are specifically derivatized by treatment with hexamethyldisilazene, followed by addition of an excess of a simple ketone, resulting in an O-trimethylsilyl enamine, or O-trimethylsilyl Schiff's base which

Fig. 11

R-C≡O⁺     ←     R CÖ OR'     →     R'O-C≡O⁺

35                                          36



38, m/e 102          m/e 87

37

M<sup>c</sup>Lafferty process



$(CH_2)_5CO_2Me$

39                              40

m/e 130(M-76)                    m/e 174(M-32)
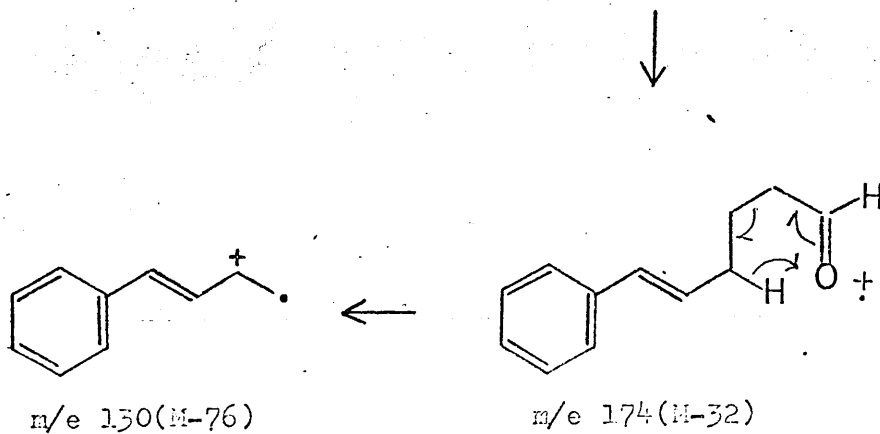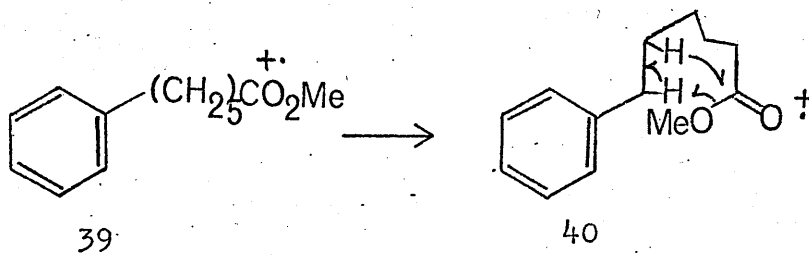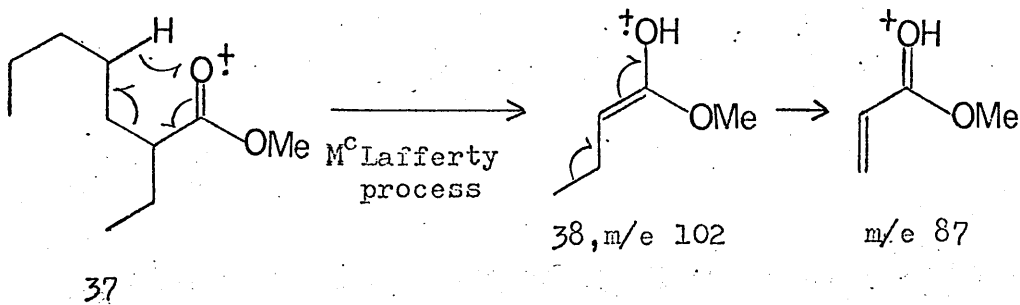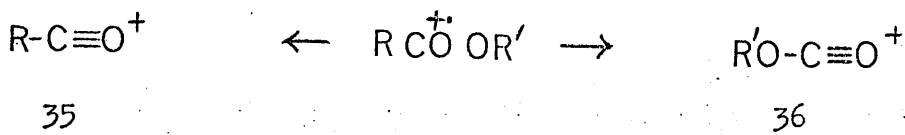
fragment in a characteristic manner. Pailer et al[35] have described

the analysis by GC-MS of the primary and secondary amines from

cigarette smoke, as their N-trifluoroacetyl derivatives.

## Acids

Carboxylic acids are, apart from the lowest molecular weight

species, unsuitable for GLC. Their involatility and potential

thermal lability may limit the value of mass spectroscopic

examination.

Routine investigations normally utilize the methyl esters,

which are more suitable for GC-MS; the derivatives are volatile

relative to the acids, the increase in molecular weight is small,

molecular ions are usually observed, and most important, the

fragmentation of the molecule is directed in a well documented[36]

manner. Characteristic fragmentations (Fig. 11) involve $\alpha$-cleavage,

often affording the two energetically favoured ions (35) and (36),

which undergo further breakdown. The well known McLafferty

rearrangement ( (37) → (38) ) involves $\beta$-cleavage with concomitant

hydrogen transfer, revealing information about neighbouring carbon

substitution. A complicating process in many branched chain and

long chain methyl esters is the ubiquitous loss of methanol which

often follows initial $\alpha$-cleavage ( (39) → (40) ).

Trimethylsilyl esters of acids have been reported[31] and are

worthy of mention because of the possibility of their formation

during trimethylsilylation of natural extracts containing hydroxylic

and carboxylic compounds. They are thermally stable and exhibit good gas chromatographic behaviour, but are very susceptible to hydrolysis. Citric acid,[31] for example, is suitable for study by GC-MS as its tris-trimethylsilyl ester, but the scarcity of derivatives reported does not permit an assessment of the fragmentation-directing properties of the group.

Amino acids, by virtue of their zwitterionic nature and resultant low volatility must also be derivatized in order to perform an examination by GC-MS. Many satisfactory derivatives for GLC have been described, e.g. Richards and Mason[37] have reported the use of N,O-bistrifluoroacetyl methyl esters of tyrosine and thyronine; Darbré[38] has described the derivatization of protein amino acids by conversion of the carboxylic acid to an ester followed by trifluoroacetylation of the amino, hydroxyl and thiol groups; Jaakonmaki[39] has demonstrated the utility of N,O-dipivalyl methyl esters of the thyroid hormones. Of the large volume of work done on peptide derivatives for GC-MS perhaps the work of Andersson[40] can be mentioned. An examination of trifluoroacetyl and hexafluorobutyryl methyl esters of peptides revealed that both derivatives rendered the peptides sufficiently volatile for gas chromatography. On Carbowax 20 M columns the retention times for the former were 50% less. Striking similarities in mass spectral fragmentations were observed, and a sequential analysis of derivatives of DL-Ala-DL-Phe-OMe and Gly-Gly-Gly-OMe was obtained.

The increasing importance of the GC-MS technique is becoming more obvious. The foregoing discussion indicates that a particularly important aspect of the technique, the use of derivatives designed to enhance the significance of GC-MS evidence, is under active development. It remains largely dependent on the types of derivative used classically for organic characterisation, and it is clear that gas-phase procedures both demand and allow a quite different approach to the recognition of structure through functionality.

4.2                    DISCUSSION OF RESULTS

Enol Trimethylsilyl Ethers as Derivatives for GC-MS

The efficacy of enol trimethylsilyl ethers of carbonyl

groups, which belong to derivative category (d), was deemed worthy

of examination.  It was expected by analogy with alcohol

trimethylsilyl ethers, that the ability of the trimethylsilyl

moiety to stabilize the high-energy radical cation in the mass

spectrometer would result in an abundant molecular ion.  The gas

chromatographic properties were potentially favourable since enol

trimethylsilyl ethers had been briefly reported by Grundy et al[41]

as by products during gas chromatographic examination of

trimethylsilylated steroid alcohol mixtures which contained ketonic

material.  They had also been observed by Horning et al[42] during

similar GLC group analyses.  The fundamental change in the carbon

skeleton due to the incorporation of two $sp^2$ centres was expected

to alter markedly the mass spectral characteristic.

Enol formation is a reversible process in which only small

quantities of the enol tautomer are present in the normal unperturbed

system.  The equilibrium can be altered, however, in the presence of

an acid and the enolic form abstracted irreversibly by acetylation.

By analogy, several ketones were treated at different temperatures

in hexamethyldisilazane with a catalytic trace of p-toluenesulphonic

acid.  Only in the simplest case, cyclohexanone, was enol

trimethylsilylation achieved.  Further investigations revealed that
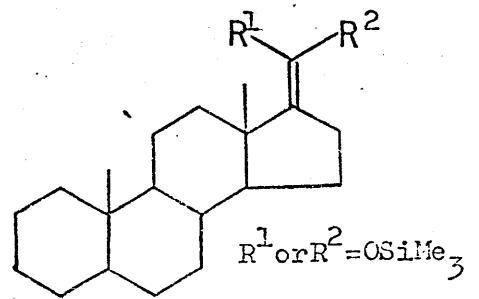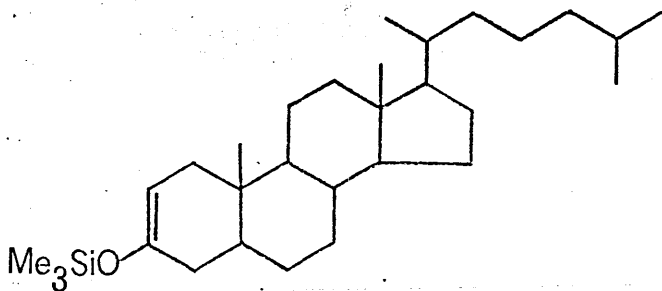
Fig. 12

Fig. 13

Fig. 14

m/e 169 → m/e 127

m/e 169 → m/e 113

m/e 173 → m/e 141

m/e 211 → m/e 155
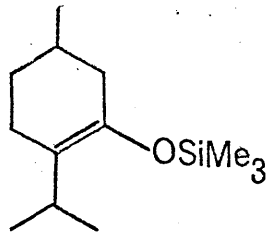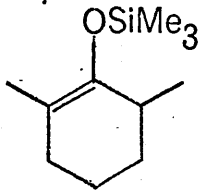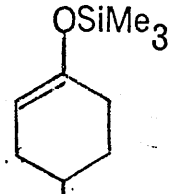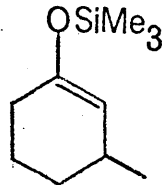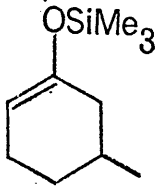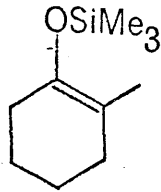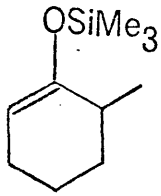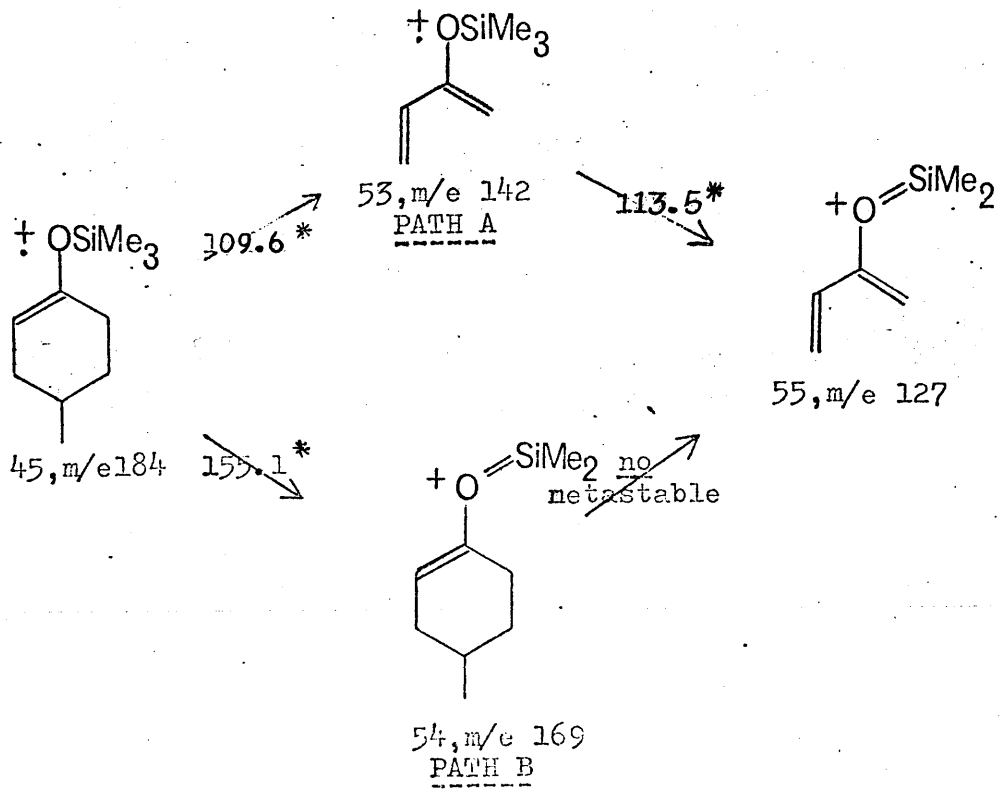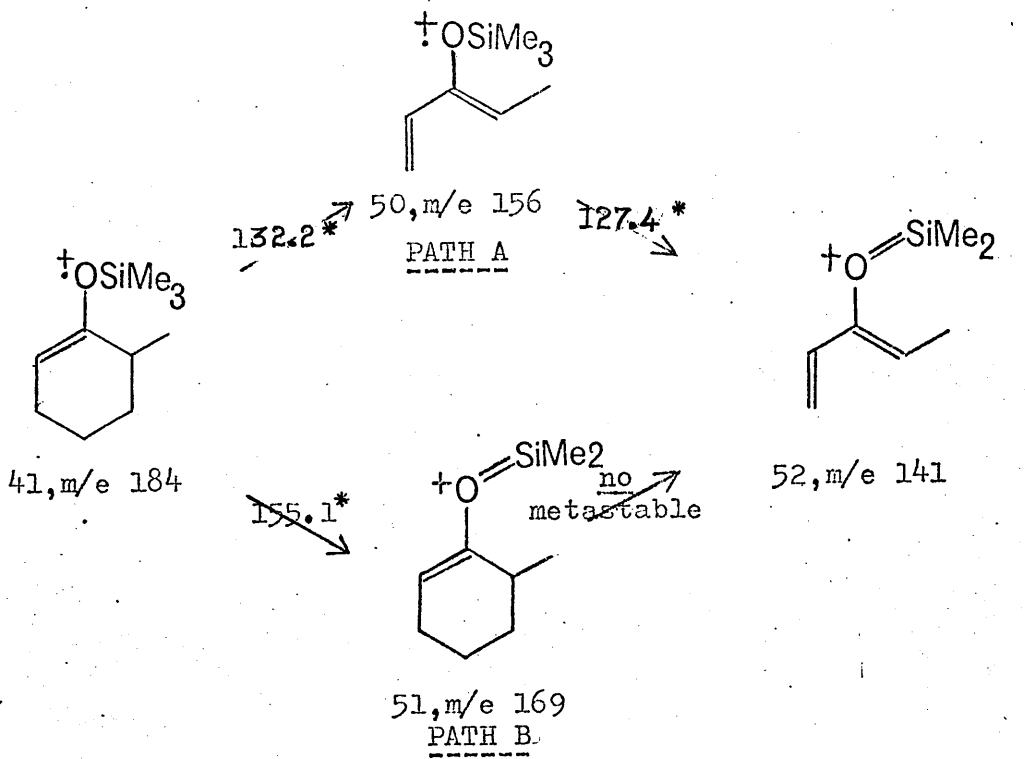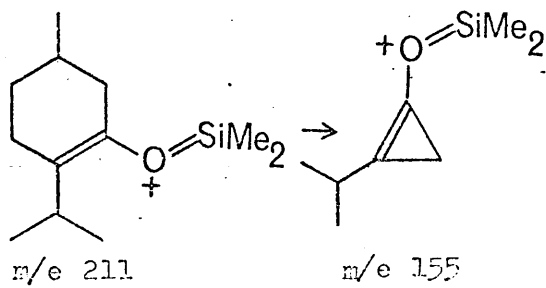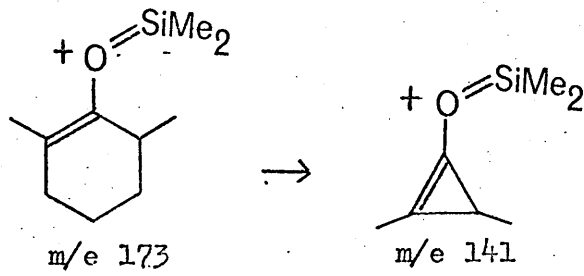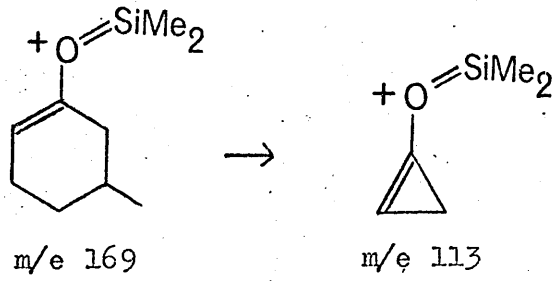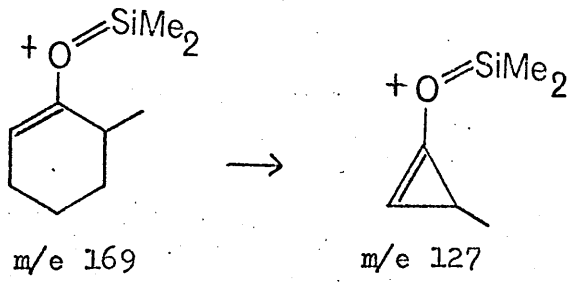
# TABLE 1.

| Ion m/e | % of base peak | | | | |
|---|---|---|---|---|---|
| | 41 | 42 | 43 | 44 | 45 |
| 184 | 48 | 48 | 35 | 15 | 28 |
| 183 | 11 | 13 | 15 | 4 | 4 |
| 169 | 70 | 55 | 67 | 100 | 43 |
| 156 | 10 | 15 | 8 | 3 | 2 |
| 155 | 15 | 10 | 2 | 1 | 5 |
| 142 | 6 | 30 | 15 | 23 | 59 |
| 141 | 37 | 17 | 10 | 12 | 10 |
| 127 | 3 | 25 | 35 | 1 | 90 |
| 75 | 85 | 92 | 100 | 45 | 100 |
| 73 | 100 | 100 | 85 | 95 | 60 |

# TABLE 2.

| transition | Calculated Metastable | Observed metastable | | | | |
|---|---|---|---|---|---|---|
| | | 41 | 42 | 43 | 44 | 45 |
| 184– 169 | 155.1 | 155.1 | 155.1 | 155.1? | 155.1 | 155.1 |
| 184– 156 | 132.2 | 132.2? | 132.2 | – | – | – |
| 184– 142 | 109.6 | – | 109.6 | –. | – | 109.6 |
| 184– 141 | 107.0 | – | – | – | – | – |
| 184– 127 | 87.75 | – | – | – | – | – |
| 169– 142 | 119.2 | – | – | – | – | – |
| 169– 141 | 117.6 | – | – | – | – | – |
| 169– 127 | 95.5 | – | – | – | – | – |
| 156– 142 | 129.1 | – | – | – | – | – |
| 156– 141 | 127.4 | 127.4 | 127.4? | – | – | – |
| 156– 127 | 103.4 | 103.4? | 103.4 | – | – | – |

the optimum conversion conditions necessitated the recently
introduced reagent bis-trimethylsilyl acetamide (BSA) with a
trace of p-toluenesulphonic acid (PTSA) monohydrate as catalyst.
The derivatives could be separated by preparative thin layer
chromatography, but were hydrolysed back to the ketone form on
exposure to air within two hours.

Dissolution of 2-methyl-cyclohexanone, 3-methyl-cyclohexanone,
4-methyl-cyclohexanone, 2,6-dimethyl-cyclohexanone and menthone in
BSA and PTSA resulted in total conversion, according to GC-MS,
to derivatives of molecular weight consistent with enol
trimethylsilylation. 2-methyl- and 3-methyl-cyclohexanone afforded
the isomeric derivatives (41 and 42) and (43 and 44) respectively.
Menthone, on the other hand afforded <u>one</u> derivative, shown by NMR
to correspond to structure (47), and displaying in the infra-red
spectrum characteristic trimethylsilyl absorptions at 850 $cm^{-1}$,
1180 $cm^{-1}$ and 1250 $cm^{-1}$

The most characteristic fragmentations of the isomeric
derivatives (41 to 46) (Table 1) involved Retro-Diels Alder processes
with charge retention by the trimethylsilylated moiety. Tentative
assignment of the isomeric structures (43) and (44) could be made
from the relative abundances of the Retro-Diels Alder peaks at
$^m/e$ = 142 and $^m/e$ = 156 respectively. No such assignment can be
made for isomers (40) and (42) since both afford Retro-Diels Alder
species of the same mass. This process is also evident in (45),

affording an abundant ion at $^m/e = 142$. Derivative (46) also exhibits a significant Retro-Diels Alder peak at $^m/e = 170$, but a fragment at $^m/e = 169$ is twice as abundant. Derivative (47), formed from menthone, exhibits an insignificant Retro-Diels Alder product ($^m/e = 184$), but shows an abundant ion at $^m/e = 183$.

Abundant fragment ions at $^m/e = 127$ in (43) and (45), $^m/e = 141$ in (41), (42) and (44), $^m/e = 155$ in (46) and $^m/e = 169$ in (47) are probably related species formed by a combination of Retro-Diels Alder reaction and loss of methyl group from the trimethylsilyl moiety (Fig. 13), resulting in stabilized species such as (52) and (55). Two pathways, A and B (Fig. 13) are possible. Path A is supported by the appearance of metastable ions (Table 2) corresponding to the transitions (41) → (50) → (52) and (45) → (53) → (55). The alternative path B is not corroborated by metastable ions. Ions (51) and (54) may undergo fragmentations other than Retro-Diels Alder processes.

A third group of abundant fragment ions at $^m/e = 113$ in (43), (44) and (45), $^m/e = 127$ in (41) and (42), $^m/e = 141$ in (46) and at $^m/e = 155$ in (47) is more difficult to account for. A suggested explanation is outlined in Fig. 14.

The corresponding derivative of 5α-cholestan-3-one, (48), was formed under similar conditions. A homogeneous derivative was indicated by GLC. The effect on gas chromatographic retention time is illustrated in Fig. 15. The NMR spectrum of the derivative

Fig. 15



ENOL TMS ETHER (B) OF
5α-CHOLESTAN-3-ONE (A).

1% SE30, 10′ COLUMN,
240°
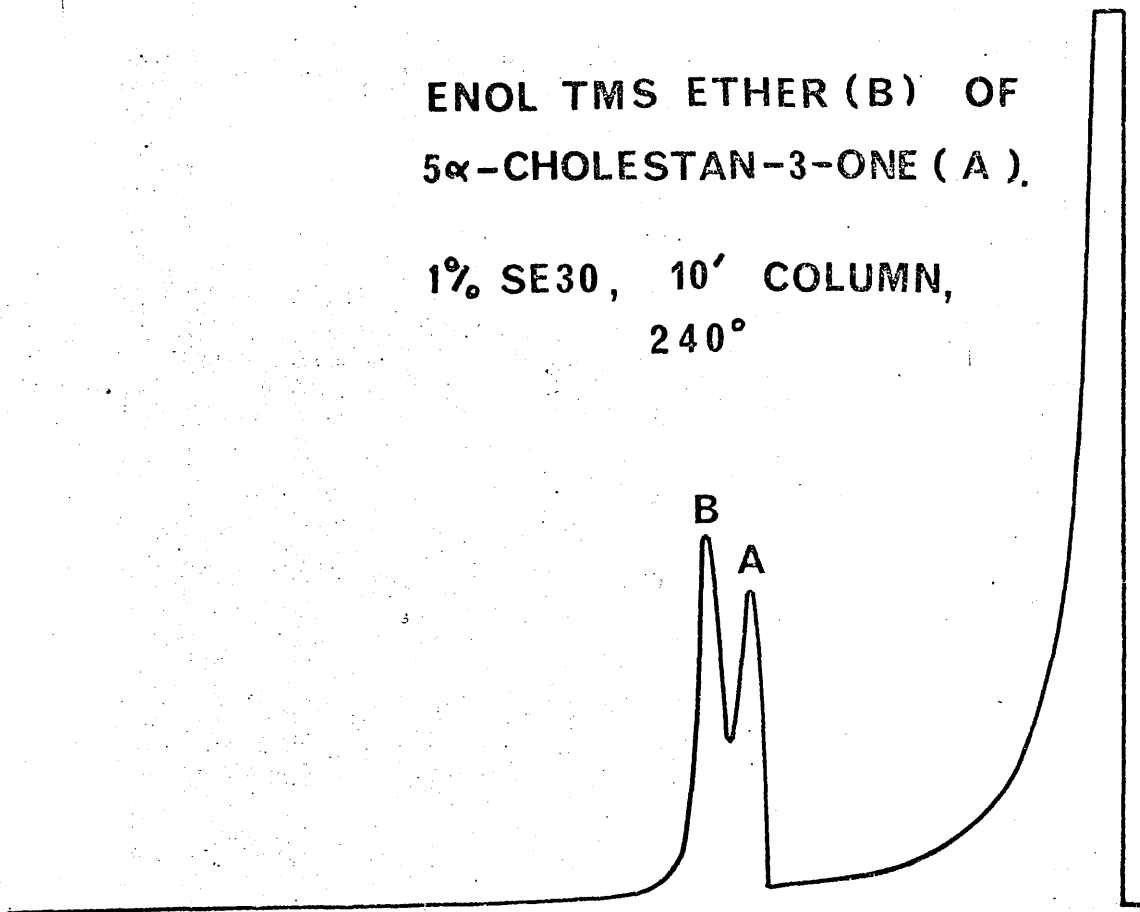
# Fig.16

## 5α–PREGNANE–20–ONE



## ENOL TMS ETHER OF 5α–PREGNANE –20– ONE

# Fig 17

5α–CHOLESTAN–3–ONE

386

231

163

81

55

300

200

100

ENOL TMS ETHER

458

C₈H₁₅

(CH₃)₃SiO

H
143

317

203

143

73

400

300

200

100

Fig. 18



56

57, m/e 143

56

RDA

58, m/e 142

59, m/e 127

60

61
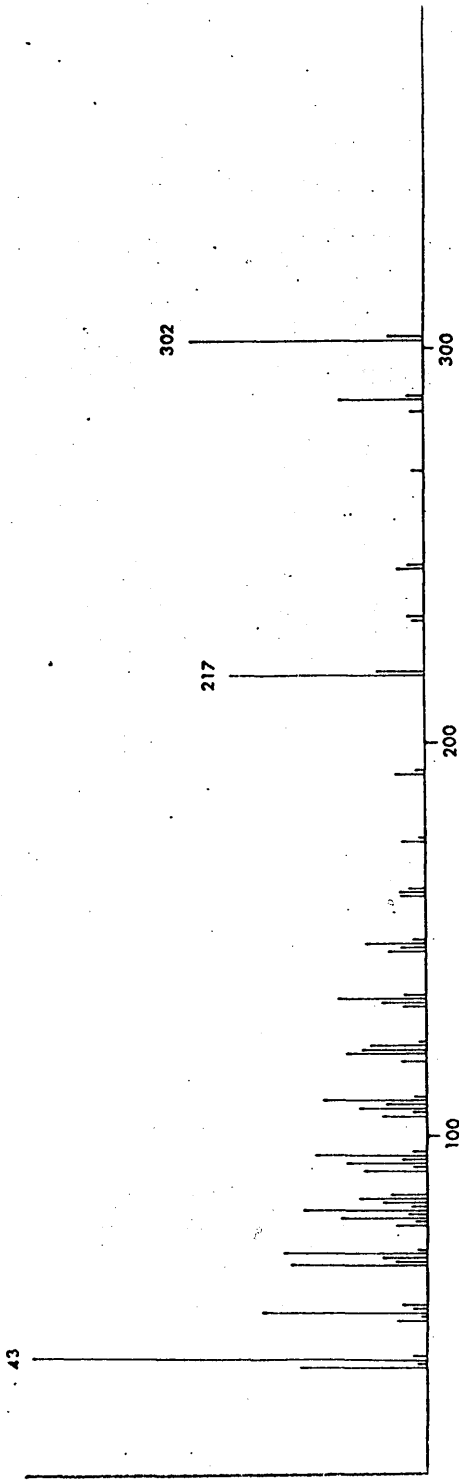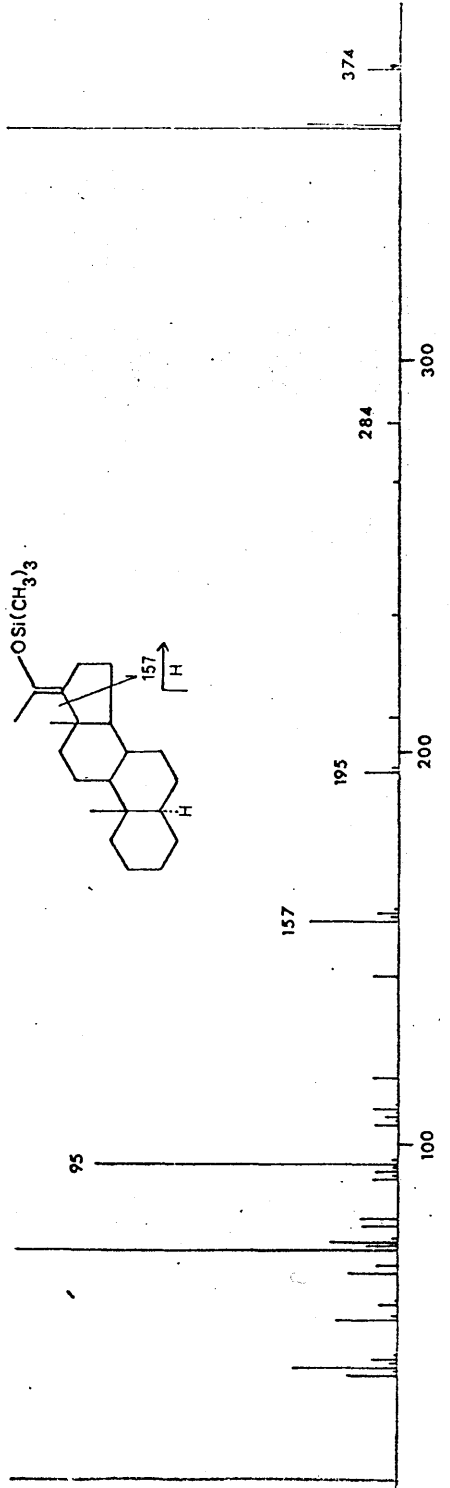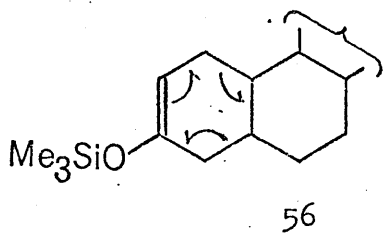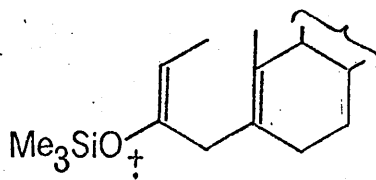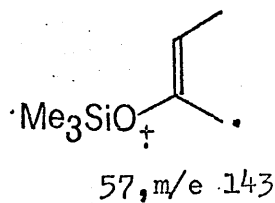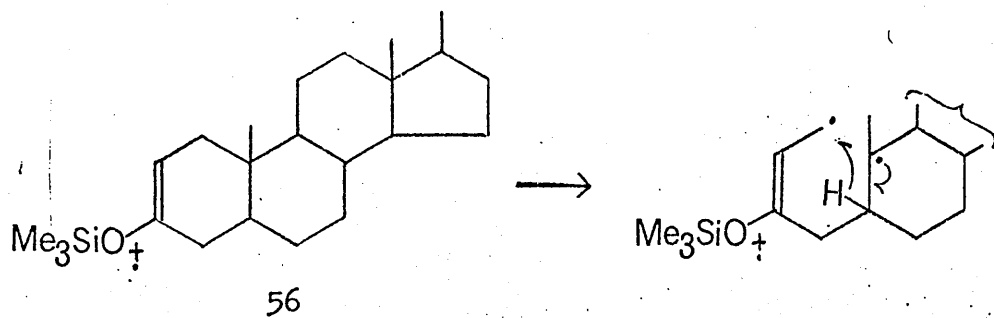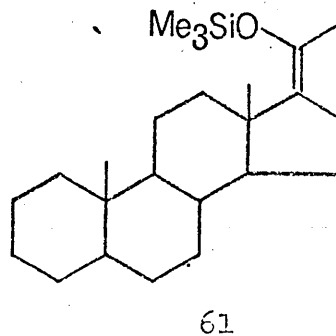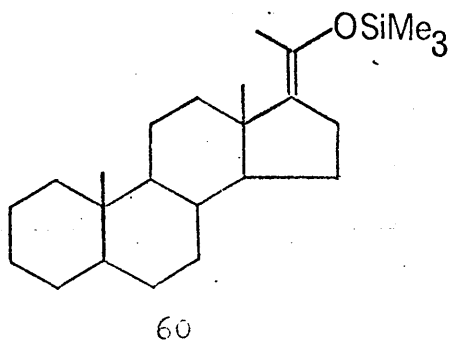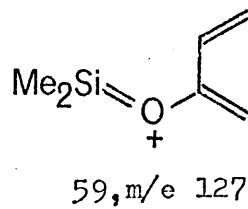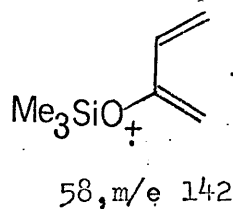
indicated 9 trimethylsilyl protons at $\tau$ 9.7, 4 allylic protons and one vinylic proton. Structure (48) was therefore suggested, analogous to that formed by enol acetylation[13]. Formation of the other possible isomer ($\Delta^3$) is apparently precluded on steric grounds[18].

The molecular ion ($m/e = 458$) was an abundant species (75% of the base peak). The spectrum as a whole was much simpler than that of $5\alpha$-cholestan-3-one, indicating that the enol trimethylsilyl ether group has a greater fragmentation-directing capacity than the 3-ketone group. The fragmentation was dominated by scission of ring A to give ions at $m/e$ 143 and 142. The former ion may arise through allylic cleavage and hydrogen transfer through a 6-membered transition state followed by vinylic cleavage as shown in Fig. 18. The expected retro-Diels Alder fragment (58) $m/e$ 142, (72% of base peak) was observed. The appearance of these two ions contrasts with the reported[18] mass spectrum of cholestan-3-one enol acetate, in which apparently only the Retro-Diels Alder process was observed, and the charge resided with the ring B-C-D moiety. In the case of (56) the charge is totally retained on the trimethylsilylated moiety. The ion at $m/e = 127$ (59) probably arises primarily from the Retro-Diels Alder product (58). The loss of trimethylsilanol (90 mass units) which normally occurs as a major process in alcohol TMS derivatives, does not occur, since eliminations at olefinic centres are difficult and the other fragmentations predominate.

Fig. 19

60/61

62

64, m/e 157

63, m/e 195
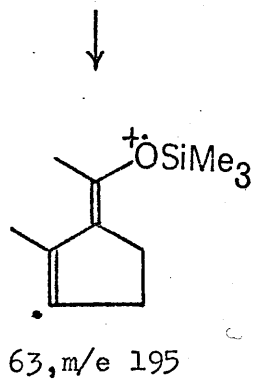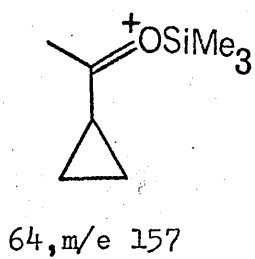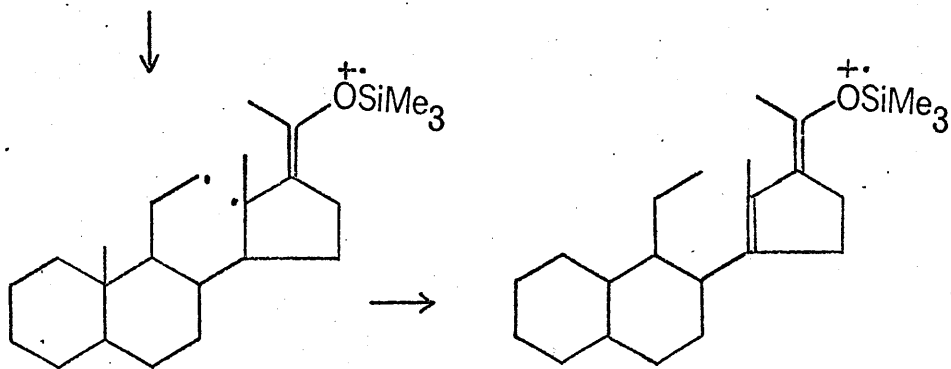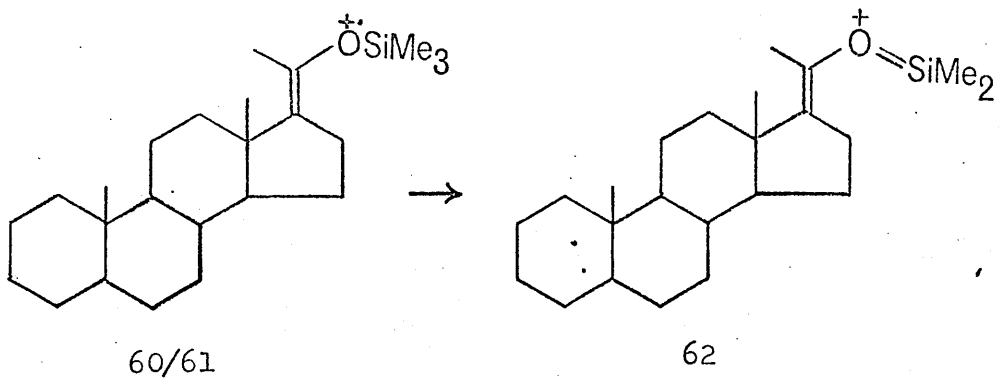
Pregnan-20-one, which represents a different type of carbonyl environment, was observed by GLC to form two derivatives when treated under enol trimethylsilylation conditions. NMR of the mixture, which was inseparable by preparative thin layer chromatography, indicated complete absence of vinylic protons. Structures (60) and (61) are therefore probable. The derivatives were again observed to be labile. GC-MS was performed, resulting in similar mass spectra for each of the geometrical isomers. The molecular ion in this case was less abundant, and the peak at $^m/e$ 73 ( $(CH_3)_3Si^+$ ) observed as the base peak: however, the significance of this ion is limited as it is very commonly encountered from trimethylsilyl ethers and can arise from reagents. The abundant loss of 15 is presumably due in part to formation of the conjugated oxonium ion (62). Loss of trimethylsilanol (P-90) is evident in this derivative, unlike the corresponding derivative of 5α-cholestan-3-one, as a minor peak at $^m/e = 284$. The ion at $^m/e$ 195 (63) (P-$C_8H_{10}$TMS) possibly arises through allylic cleavage between $C_{(12)}$ - $C_{(13)}$ as shown in Fig. 19. The process leading to the ion of $^m/e$ 157, which probably contains silicon, may involve scission as shown in Fig. 19 to give the stabilized cation (64).

In summary, enol trimethylsilylation results in direction of fragmentation, the resultant ions being stabilized by either the trimethylsilyl (radical cation) or dimethylsilyl (cation) moieties. The carbocyclic derivatives examined exhibited characteristic

fragmentations. Pregnan-20-one enol trimethylsilyl ether, the only

system examined which cannot undergo a Retro-Diels Alder process,

displayed a more complicated mode of fragmentation.

## Thiones as GC-MS Derivatives

Thiones, formally analogous to ketones, are in many instances readily prepared in high yield[43] by simple methods such as refluxing the ketone with phosphorus pentasulphide in xylene, or by bubbling hydrogen sulphide and hydrochloric acid gas through an ethanolic solution of the ketone. We have found thiocamphor(66), thiofenchone (68) and thiocamphenilone (70) to have excellent gas chromatographic properties, the slight increase in molecular weight being offset by increased volatility (thiones are less polar than the corresponding ketones); they are also thermally stable under gas chromatographic conditions. The retention factor (ratio of retention of derivative to ketone) is, on non-polar phases such as SE-30 or APL, approximately 1.5. Micro-scale reactions on mixtures using the conversion method described above should therefore be amenable to the 'Peak-Shift' technique in which changes in gas chromatographic retention may be correlated with the retention factor of the thione group.

Certain preliminary observations may be made concerning the mass spectral properties of thiones. The molecular ions of the thiones are substantially stronger than in the corresponding ketones. This would appear to indicate that the thione function has a greater capacity for stabilization of the high-energy radical cation. More importantly, the mass spectra of camphor(65), fenchone (67) and camphenilone (69) are totally dissimilar (Figs. 20-22) whereas those of thiocamphor and thiofenchone are

Fig. 20



Mass spectra of camphor and thiocamphor

Fig. 21
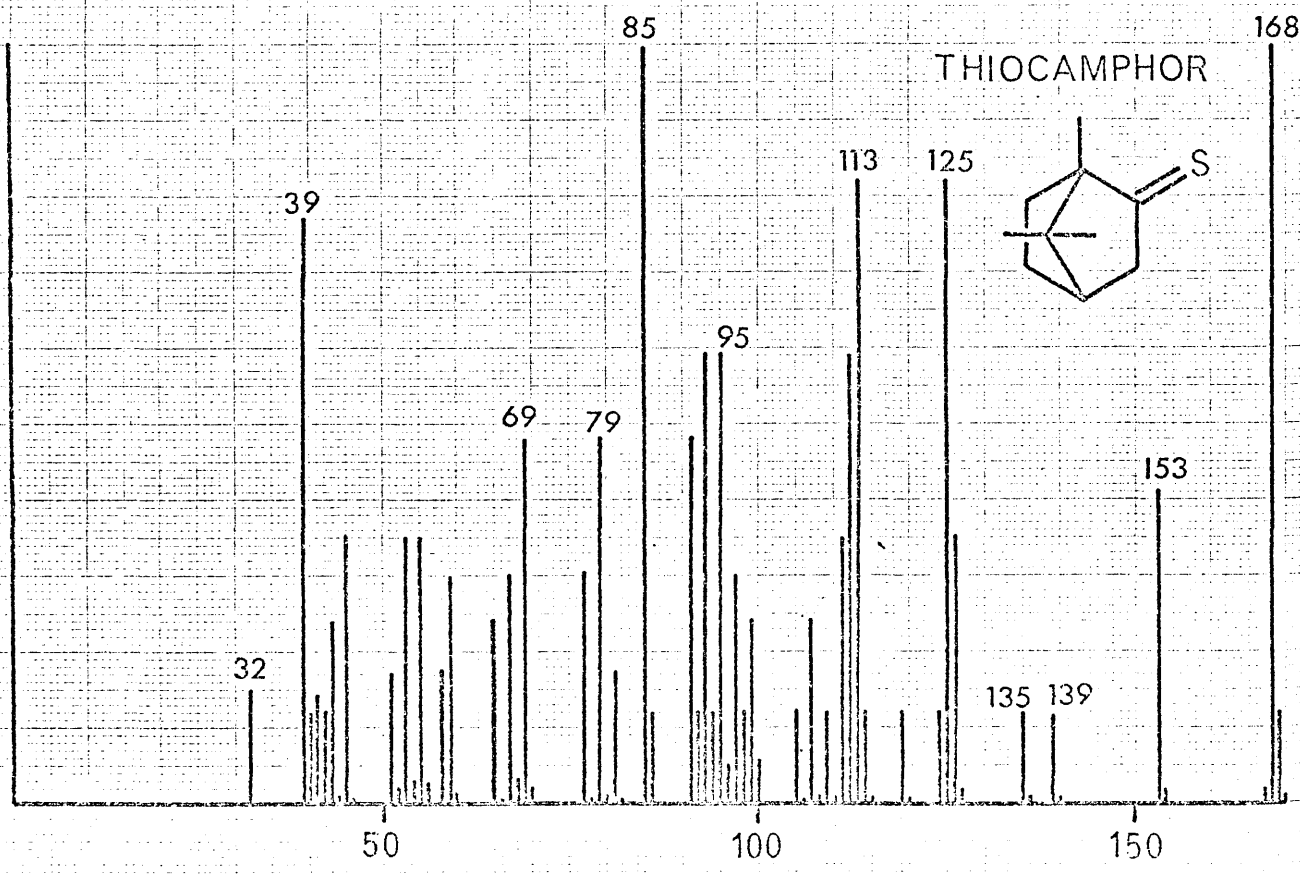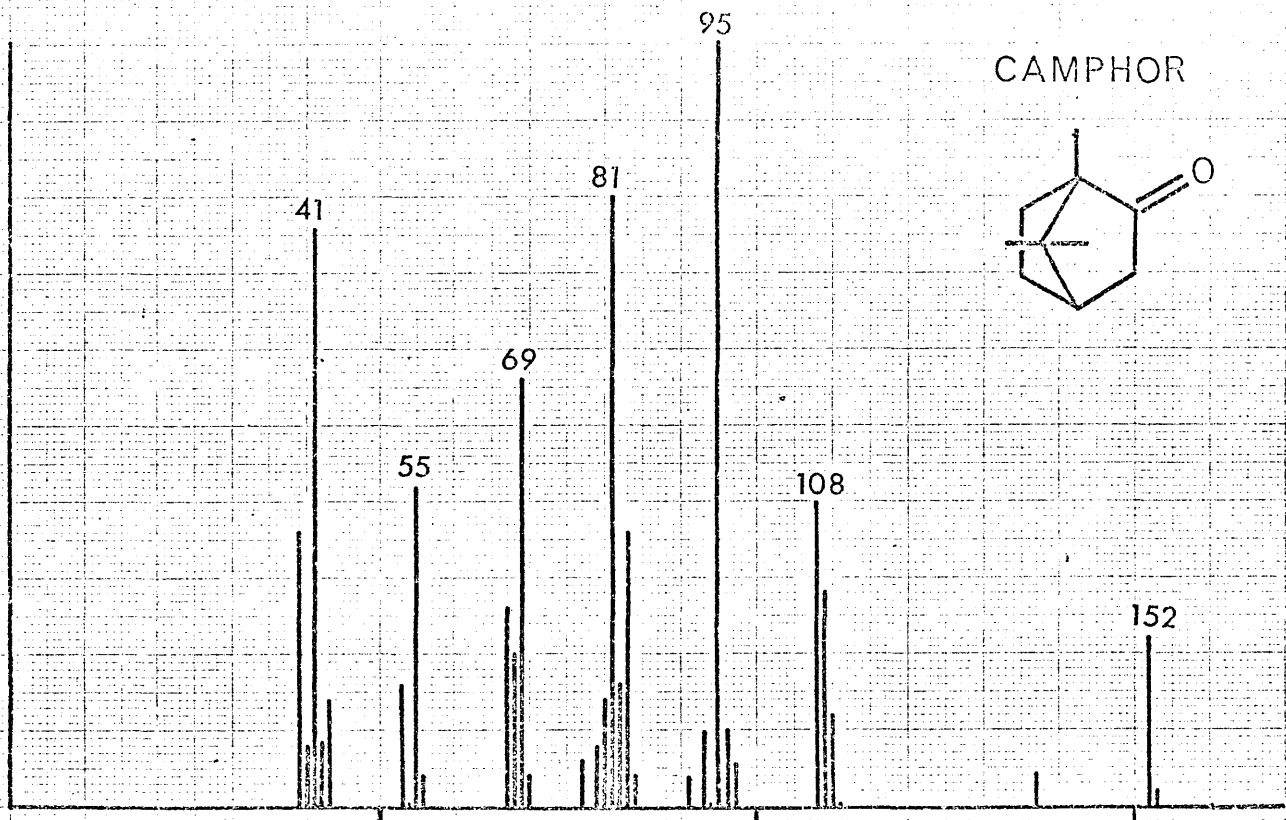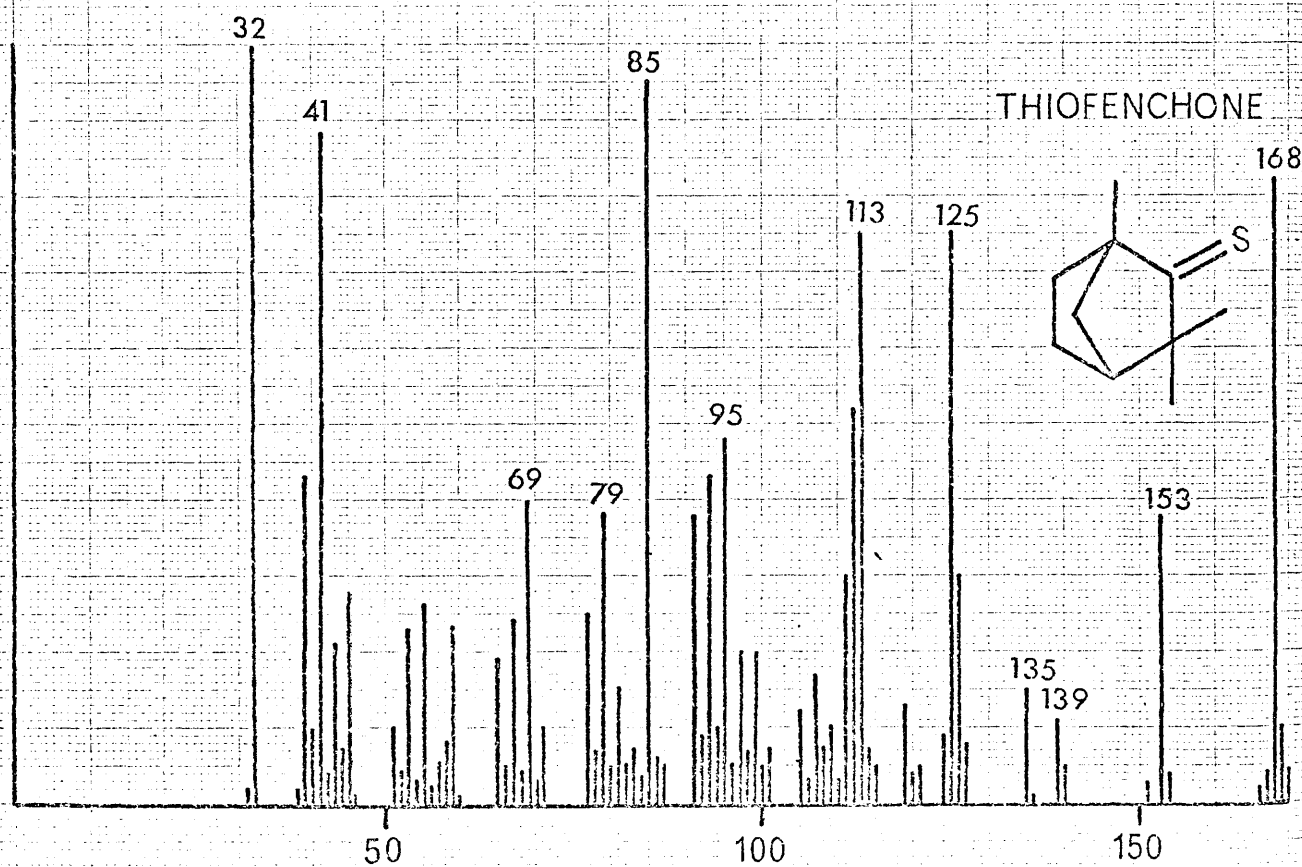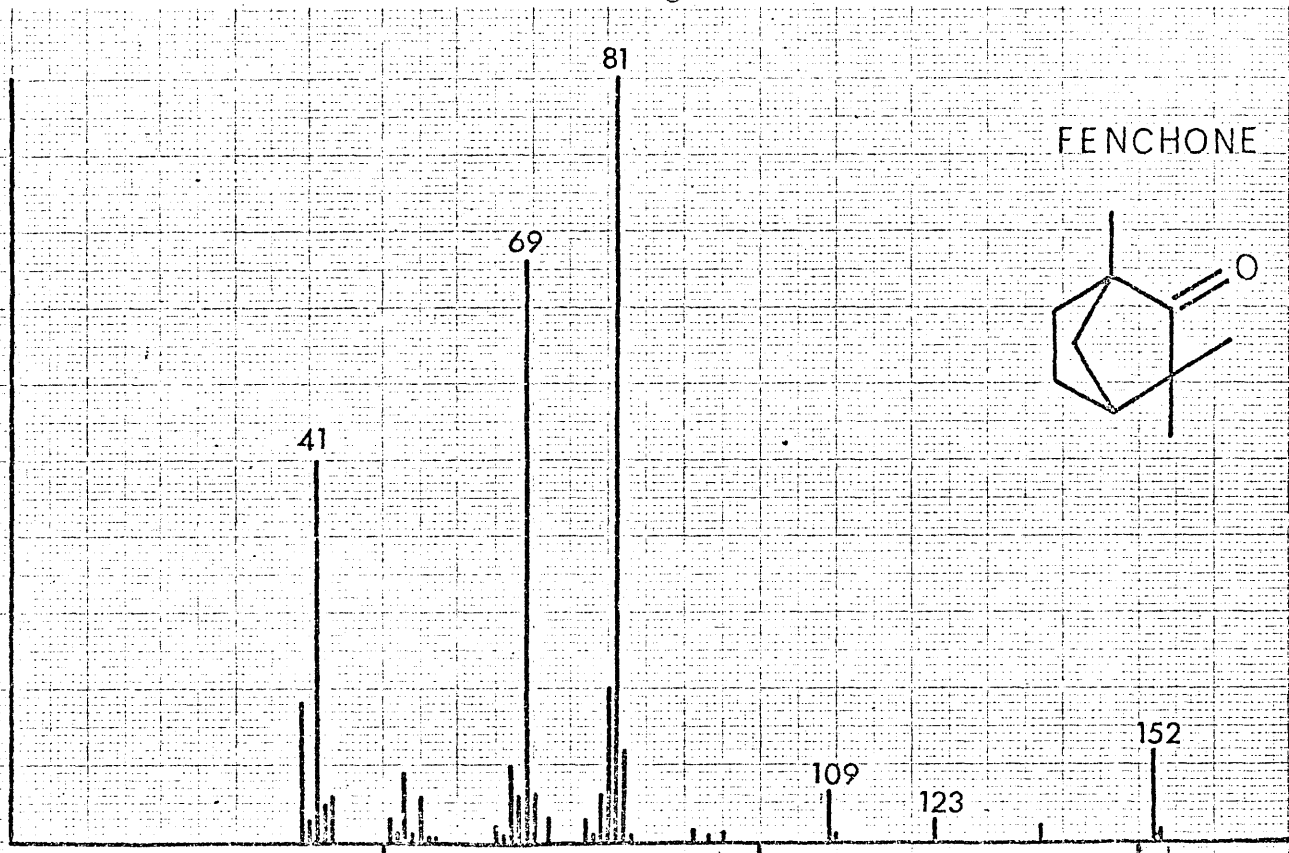


Mass spectra of fenchone and thiofenchone

Fig. 22



CAMPHENILONE
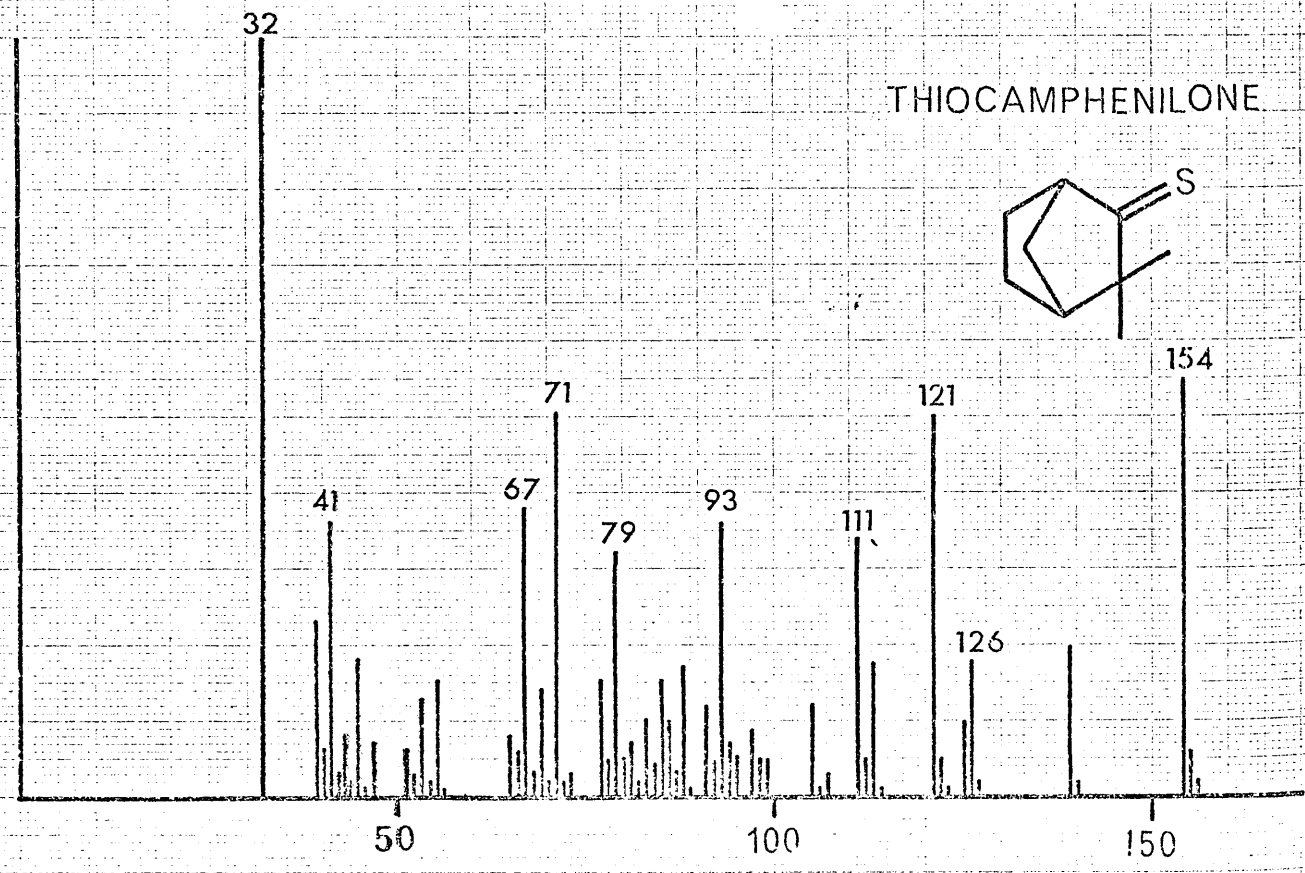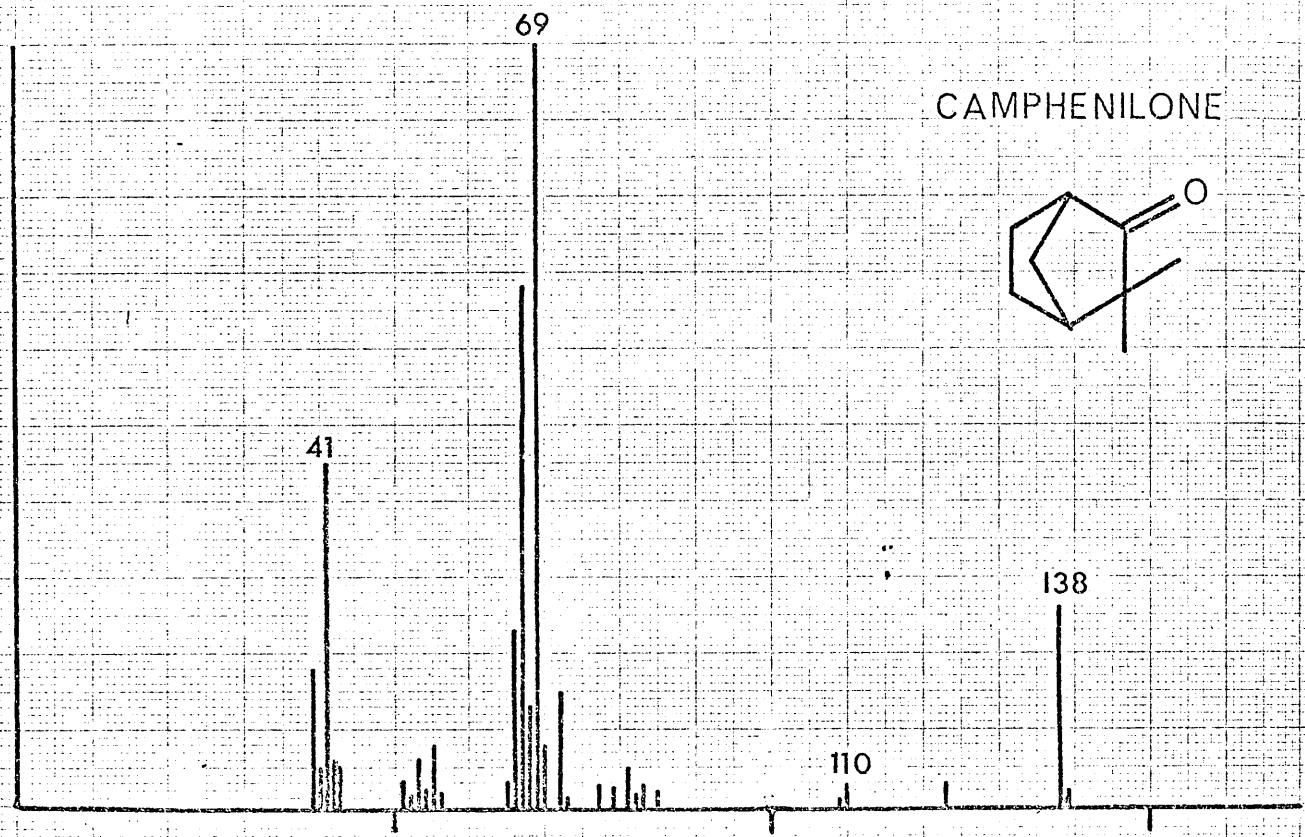
THIOCAMPHENILONE

mass spectra of camphenilone and thiocamphenilone

Djerassi et al,
J. Org. Chem., 31, 115, 1966

m/e 108

m/e 95

m/e 109

m/e 109

m/e 81

m/e 110

m/e 109

m/e 95

m/e 69

m/e 137

m/e 110

$-C_2H_2O$
Me shift
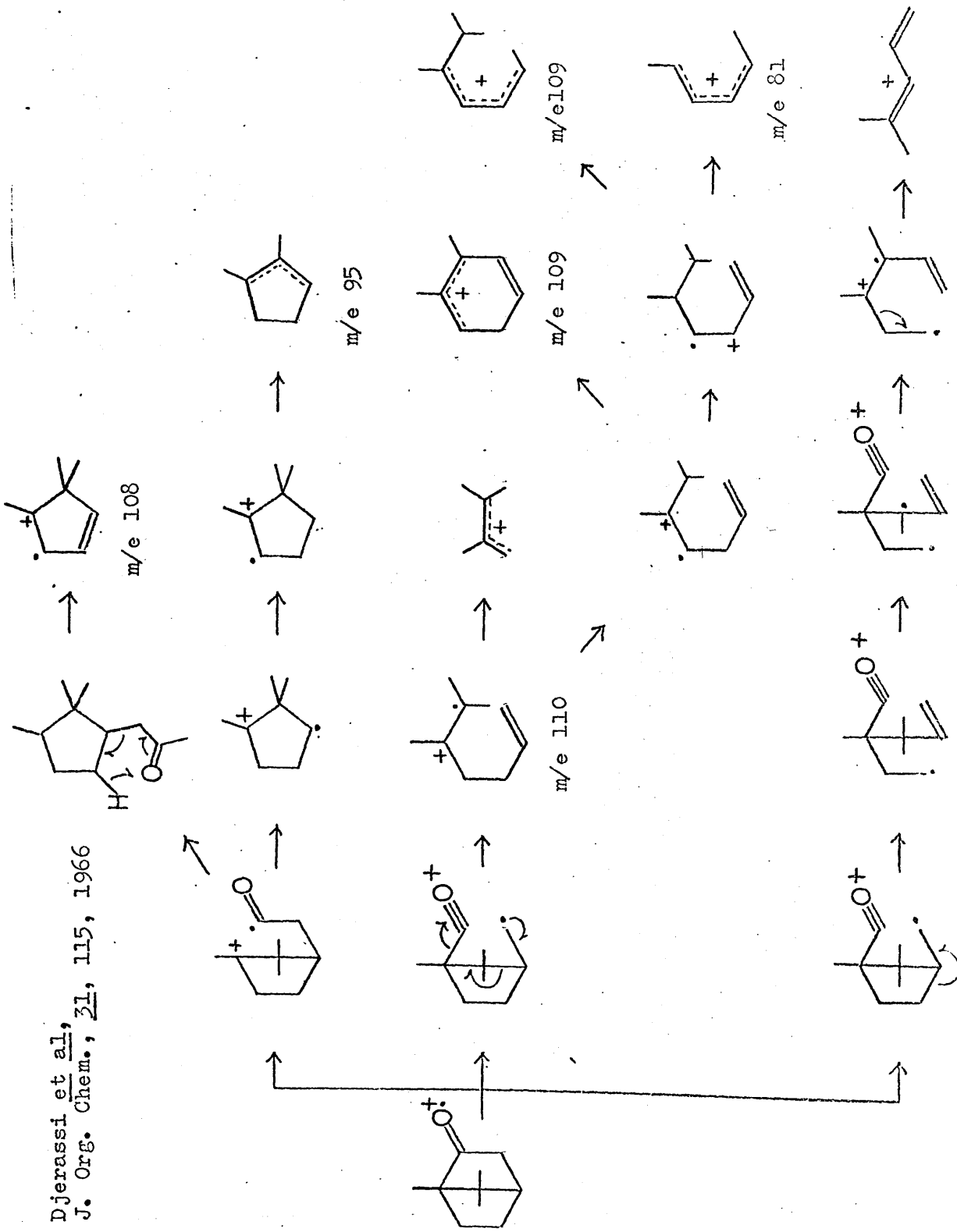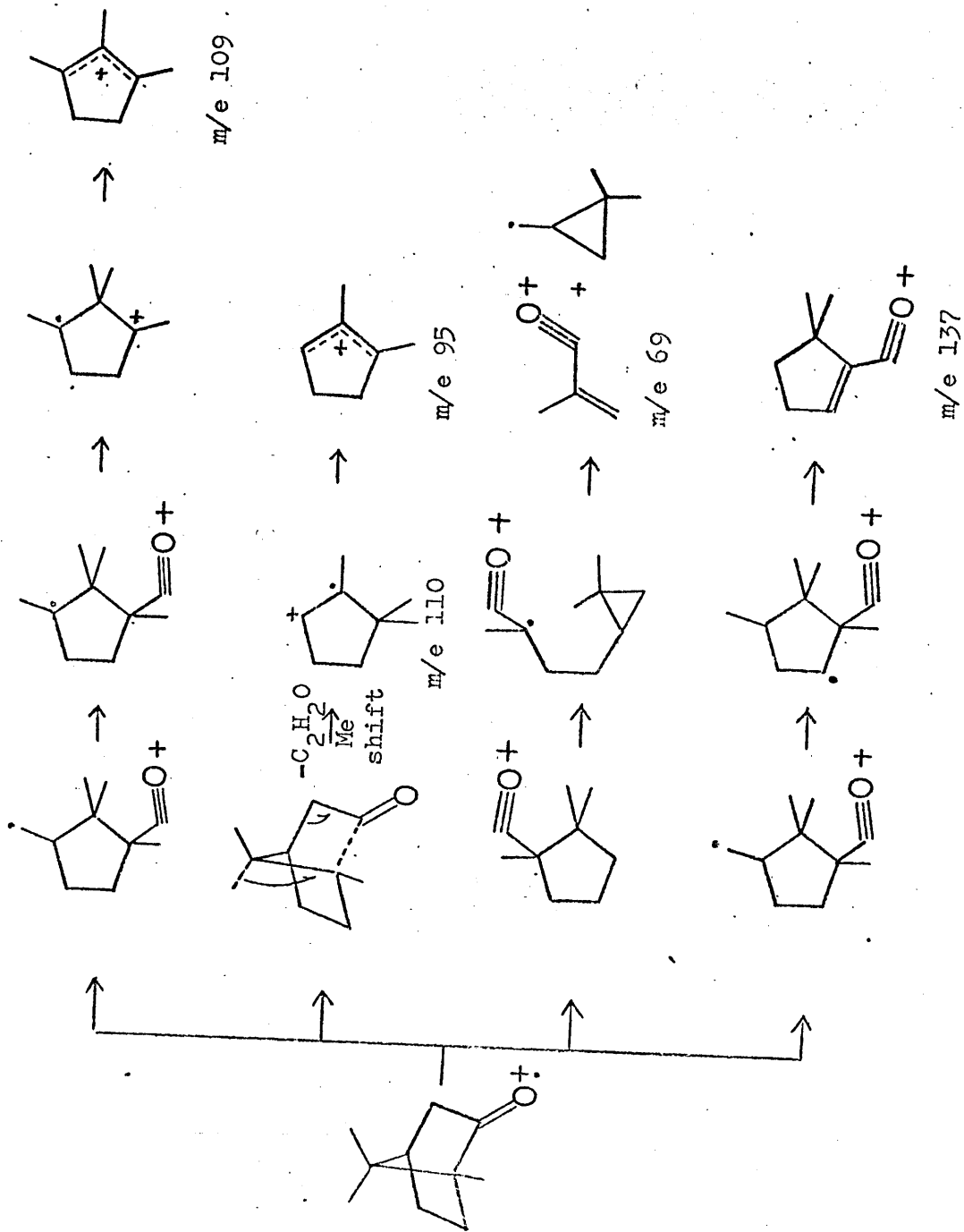
virtually superimposable; that of thiocamphenilone, the related

nor-compound,is also similar. The thione grouping is therefore

directing the fragmentation in a more regular and definitive

manner than the ketone grouping.

The principal peaks in camphor are due to hydrocarbon

fragments[44,45] (Fig. 23). Very few of these peaks have their

counterparts in the spectrum of the thione. In addition there are

no S-containing peaks in the mass spectrum of the thione

corresponding to O-containing peaks in the mass spectrum of the ketone.

It must therefore be assumed that many of the principal peaks

contain sulphur as a stabilizing hetero-species.

Several fragmentation schemes accounting for major ions in

the mass spectra of the thiones are suggested below, but in the

absence  of high-resolution mass spectral measurements and

deuteration studies, these schemes must remain purely speculative.

Djerassi[44] gives a pertinent warning about such hypothetical

mass spectral interpretations. Great care should be exercised in

interpreting the spectra of highly-fused or substituted

hydrocarbon nuclei lacking strong fragmentation-directing groups.

The thione grouping, however, is strongly-directing in its

properties. It is also to be expected that since the molecular

ion produced is both a cation and a free radical, and since many

terpenoids undergo carbonium  ion  and .free  radical

rearrangements  with  great  facility,  even  simple

Fig. 24

Fig. 25

PATH F

77, m/e 113

PATH G

78, m/e 85

PATH H

79, m/e 71(R=H)
m/e 85(R=Me)
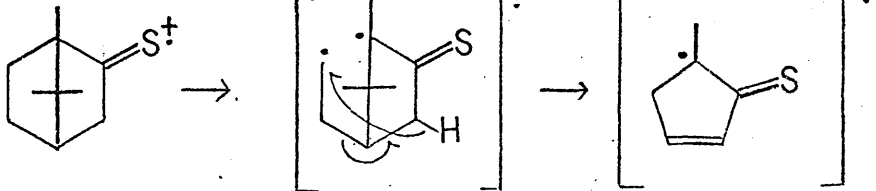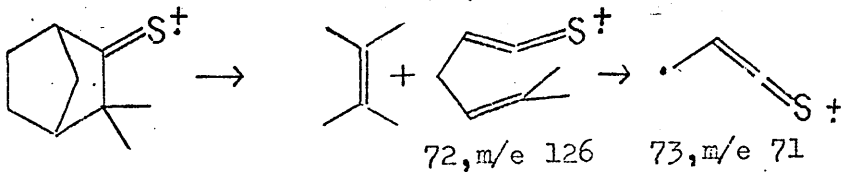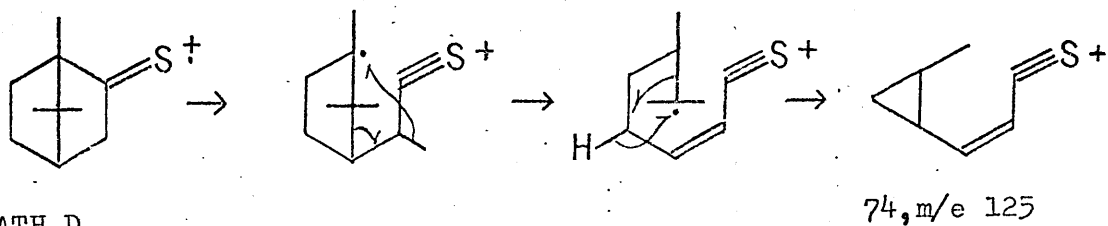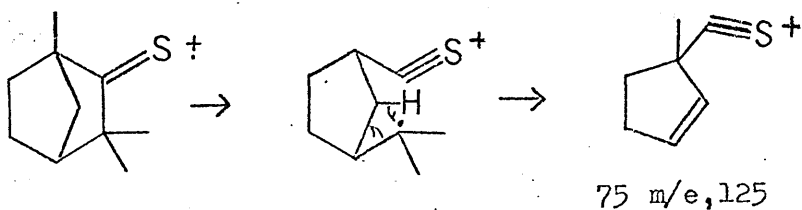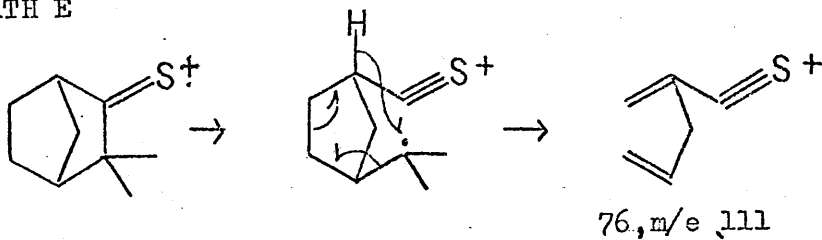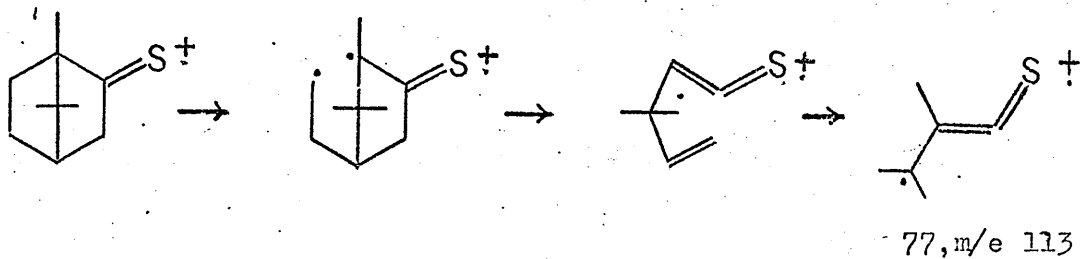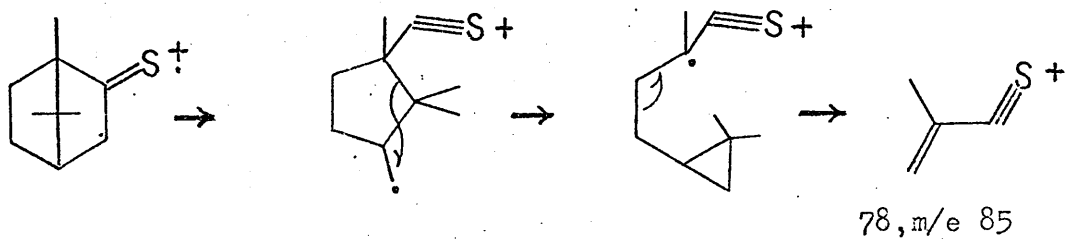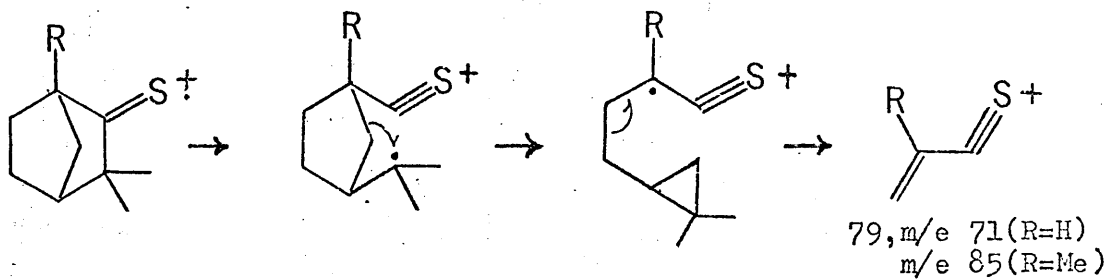
terpenoids will exhibit unusually complicated electron-impact fragmentation reactions.

Thiocamphor and thiofenchone both lose 29 mass units from the molecular ion, and thiocamphenilone, 28. One possible mechanism involves initial $\beta$ -fission, followed by hydrogen transfer and ethyl cleavage as shown in path A. The resultant cation(71) is a resonance-stabilized sulphonium species. In thiocamphenilone a Retro-Diels Alder process may be invoked to account for P-28 (72) which possibly is the progenitor of $^m/e$ 71 (73) (path B).

The loss of 33 (HS) is another minor process in thiocamphor and thiofenchone, but in thiocamphenilone it produces an ion at $^m/e$ 121 of 55% abundance relative to the base peak.

The loss of 43 (isopropyl) is evident as a major process in all three thiones. Possible mechanisms, outlined in pathways C, D and E involve initial $\alpha$ -fission followed by hydrogen transfer and formation of a stabilized sulphonium cation (74,76).

The abundant sulphonium ions at $^m/e$ 113 in thiocamphor and thiofenchone involve loss of $C_4H_7$. This must involve either extensive skeletal rearrangement or a pathway such as F leading to the thioketene cation(77).

The only fragment(78) corresponding to an oxygenated fragment is at $^m/e$ 85 (and $^m/e$ 71(79) in thiocamphenilone). By analogy with the Wolinski fragmentation[45], pathways G and H may be invoked.

The inherent dangers of speculation are exemplified by the explanation of Reed[46] of the fragmentation of camphor. None of

his conclusions were substantiated by Djerassi's work[44].

It is hoped, however, that the fragmentation-directing properties of the thione group have been illustrated sufficiently to warrant further investigation.

Fig. 26

80

81

82

83
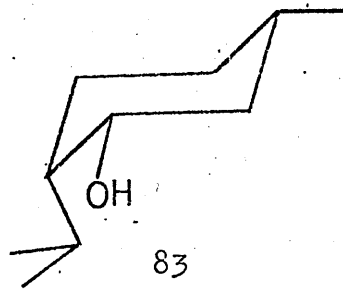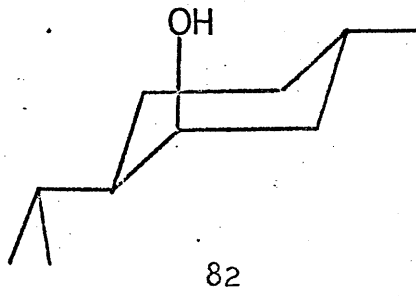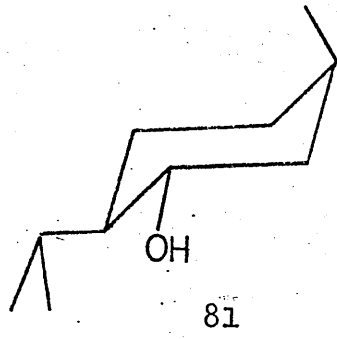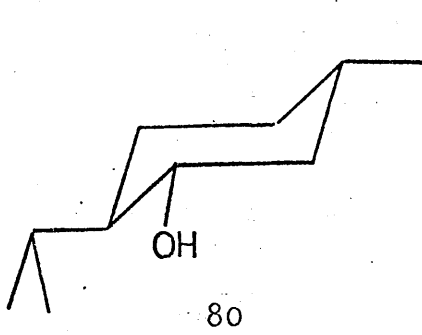
## GC-MS Properties of Trimethylsilyl Ethers of Epimeric Menthols

The use of mass spectrometry in the determination of relative epimeric configuration when both epimers are available is well known[47-51]. The empirical studies of Zaretskii et al[47-49] and of Biemann et al[51] have demonstrated that in epimeric alcohols and acetates, for example, the less crowded epimer gives a more intense molecular ion and conversely, the loss of water or acetic acid predominates in the more crowded ion. This is a consequence of the relative rates of steric decongestion which can be achieved through elimination from the energised radical cation. A germane study was that of Willhalm and Thomas[50] who examined the mass spectra of the epimeric menthols, and showed that the stereochemical isomers were readily distinguished. It follows that when models show an appreciable difference in congestion at the epimeric centre, mass spectrometry should allow confirmation of the stereochemical assignment.

It was of interest to examine the applicability of this procedure to combined gas chromatography-mass spectrometry, with the objective of differentiating between epimers in a mixture, without prior separation. The tendency of alcohols to undergo dehydration when subjected to GC-MS on the LKB 9000 instrument prevented their effective use as such. Accordingly, the trimethylsilyl ethers of menthol (80), isomenthol (81), neomenthol (82) and neoisomenthol (83) were prepared in the hope that the sterically-

enhanced epimeric difference would be manifested in significant fragmentation differences.

The reproducibility of the spectra obtained from consecutive examinations of the same epimer was observed to be a function of the following variable factors:

(a) Gas chromatography conditions: a balance had to be struck between rapid GC elution of the sample (since the source temperature rose unreproducibly during the run, slightly affecting ion abundances), and conserving optimum GC separation.

(b) Peak scan speed: too fast a scan resulted in loss of accuracy of relative ion abundances and too slow a scan resulted in a spectrum biased by concentration. 5-second scans were used.

(c) Scan timing: this factor, which was particularly susceptible to human error, resulted in very significant differences in spectra due to concentration changes.

(d) Thermal eliminations: this was minimized by lowering the separator temperature to $40^{\circ}$ and the source temperature to $170^{\circ}$. The lowest temperature to which the flash heater could be lowered without risk of impairing GC separation was estimated to be $170^{\circ}$. The filament current was also lowered.

(e) Injections on to the GC column were standardized by repetition till constant peak areas resulted. Again, the human factor was an important variable.

Under optimum conditions of operation in which these variables were controlled as far as possible, the reproducibility of the spectra was unsatisfactory (Table 3). It was concluded therefore, that GC-MS data from trimethylsilyl ethers of the epimeric menthols, from the LKB 9000 instrument, could not in this instance be used as a criterion of stereochemistry. It should be noted, however, that Sjövall[52] has found satisfactory regularities distinguishing hydroxy steroids epimeric at $C_{(3)}$ and $C_{(5)}$ using an LKB 9000 instrument. Similar regularities have been observed in other epimeric compounds in this department.[53]

TABLE 3.

Ranges of Abundances under Standardized Conditions Relative to Base Peak ($^m/e$ 143). - 3 Runs.

| Ion | Menthol | Isomenthol | Neomenthol | Neoisomenthol |
|-----|---------|------------|------------|---------------|
| 73 | 51.1-57.0% | 60.8-65.9% | 38.7-42.8% | 48.6-73.1% |
| 75 | 79.3-89.6 | 87.0-88.9 | 57.1-69.9 | 57.4-96.3 |
| 81 | 17.9-19.1 | 22.7-23.6 | 17.8-20.0 | 14.8-22.9 |
| 95 | 11.9-15.0 | 11.4-13.3 | 9.5-11.5 | 11.1-14.1 |
| 138 | 21.8-25.5 | 16.7-16.9 | 16.0-19.3 | 14.8-17.4 |
| 213 | 8.2-9.1 | 6.0-6.5 | 5.2-6.6 | 5.9-6.3 |
| 228 | 5.0-5.6 | 5.2-6.1 | 6.0-7.9 | 4.6-6.8 |

4.3                    EXPERIMENTAL

NMR spectra were recorded as 0.35M solutions on a Perkin

Elmer R10, Varian HA 100 or on the Varian HA 220 spectrometer

(60 Mc/s., 100 Mc/s. and 220 Mc/s. respectively).  Tetramethylsilane

was used as internal reference.  Gas chromatography–mass

spectrometry was performed on the LKB 9000 instrument, using

1% SE-30 as stationary phase.  Ultraviolet and infra-red spectra

were determined using the Unicam SP 800 and SP 100 spectrophotometers

respectively.

## Enol Trimethylsilylation  (Typical Preparation).

Ketone ($\sim$ 40 mg) was dissolved in bistrimethylsilylacetamide

(100 μl) and a catalytic trace of p-toluenesulphonic acid

monohydrate ( $\ll$ 1 mg) added.  The solution was heated at 60-80$^{\circ}$ in

a small sealed vial for 6 hours.  The supernatant liquid was blown

off with a stream of nitrogen, and the derivative extracted from the

white residue with ether.  Preparative TLC afforded pure enol

trimethylsilyl ether, which very rapidly hydrolysed on exposure to

air.  Infra-red (liquid film) 1650 cm$^{-1}$ (M), 1250 cm$^{-1}$ (S),

840 cm$^{-1}$(MS).  NMR:$\tau$9.85    (9H, Si($\underline{CH}_3$)$_3$).

The trimethylsilyl enol ether of menthone exhibited the

following NMR data, which permitted structural assignment:

$\tau$6.95 (1H, septet, J = 8 cps; isopropyl methine proton),  9.07 (3H,

doublet, J $\sim$ 7 cps; secondary methyl),   9.10 (6H, doublet, J = 8 cps;

isopropyl methyls),   8.95 (9H, singlet; silyl methyls).

## Synthesis of Thiocamphor

Camphor (5 g) was dissolved in absolute ethanol (25 ml) and chilled to $-10^{\circ}$. Dry hydrogen sulphide and dry hydrochloric acid gas were bubbled through for 6 hours. The solution gradually acquired an orange colour, indicating thione formation. Water (100 ml) was added and the solution neutralised with aqueous sodium bicarbonate. Extraction with ether, drying over anhydrous sodium sulphate and concentration in vacuo at room temperature afforded crude thiocamphor (4.8 g) which had a penetrating camphoraceous-sulphur odour.

The crude oil was adsorbed on to neutral alumina (Woelm, grade 1, 100 g) and eluted with light petroleum. An orange band was eluted and collected as a single fraction. Evaporation afforded thiocamphor (1 g) as a semicrystalline oil. Infra-red: 750 cm$^{-1}$(M), 1130 cm$^{-1}$(S), 1210 cm$^{-1}$ (S), 1280 cm$^{-1}$(S), 1300 cm$^{-1}$(S). Ultra-violet: 240 mμ ($\varepsilon = 11,500$), 490 mμ ($\varepsilon = 13$). (literature values [54]; 244 mμ ($\varepsilon = 11,500$), 493 mμ ($\varepsilon = 12$) ). Molecular ion (GC-MS), $^m/_e$ 168.

## Synthesis of Thiofenchone

Fenchone (1 g) was dissolved in xylene (10 ml) and phosphorus pentasulphide (2 g) added. The solution was refluxed for 20 minutes, becoming red as the thione formed. Chromatography of the total product on neutral alumina (Woelm, grade 1, 20 g), eluting with light petroleum, afforded thiofenchone (250 mg) as a red oil. Infra-red: 1100 cm$^{-1}$(S), 1180 cm$^{-1}$ (S), 1280 cm$^{-1}$(S). Ultraviolet: 242 mμ ($\varepsilon = 11,000$), 490 mμ ($\varepsilon = 12$). (lit., [54] 240 mμ ( 10,000);

488 mµ ($\epsilon$=11) ).  Molecular ion (GC-MS), $^m/e$ 168.

## Synthesis of Thiocamphenilone

Thiocamphenilone was synthesised in exactly the same manner as thiofenchone, in 50% yield as a highly volatile oil.  Analysis indicated the composition $C_9H_{14}S$ (Found, C, 69.73; H, 9.14; calcd. for $C_9H_{14}S$ , C, 70.1; H, 9.15%).  Infra-red: 1100 $cm^{-1}$(S), 1140 $cm^{-1}$(S), 1280 $cm^{-1}$(S).  Ultraviolet: 240 mµ ($\epsilon$ =11,000), 490 mµ ($\epsilon$ = 13).

## Trimethylsilylation of the Epimeric Menthols

To menthol (10 mg) in hexamethyldisilazane (50 µl) was added trimethylchlorosilane ($\sim$10 µl).  The solutions were warmed for 5 minutes and blown down in a stream of nitrogen.  The derivative was extracted from the white residue with light petroleum, and the solution used directly for GC-MS.  GC-MS conditions were standardized as described in the discussion.

## 5.1 THE THIOCARBONYL GROUP AS A DERIVATIVE IN NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY.

### INTRODUCTION

Two important aspects of the use of NMR in structure elucidation are the consideration of anisotropic shielding effects of functional groups and the use of solvent-induced chemical shifts. These procedures often facilitate the interpretation of the NMR spectrum of an unknown structure.

In order to increase the significance of these procedures it was predicted that in the case of certain carbonyl-containing compounds, derivatization of the functional group could be employed in such a manner as to reveal significant differences in the chemical shift of nearby protons. The corresponding effect on solvent induced chemical shifts was also of interest. An NMR study of the properties of the thiocarbonyl group, which in many instances is readily prepared from the corresponding ketone, was therefore initiated. It was hoped that consideration of the magnetic anisotropy, and of the benzene-induced solvent shifts of thiones, would provide data complementary to those obtained from the parent ketone.

It is proposed in this introduction to comment only briefly on the origin of magnetic anisotropy (since this is a physical problem of some complexity) and to outline the postulated

Fig. 27

Magnetic anisotropic effect.
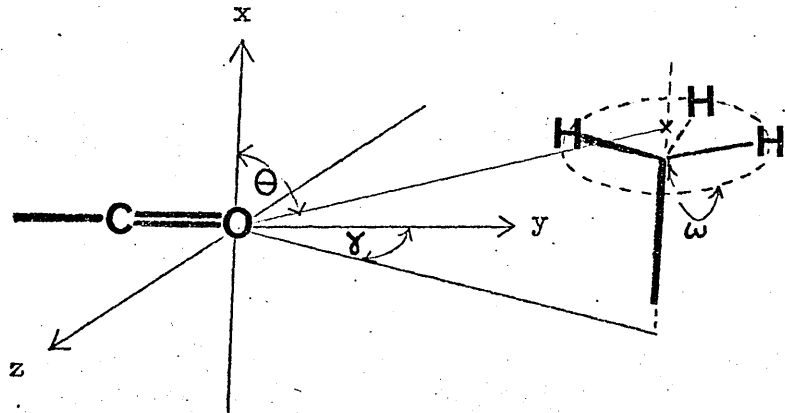


Electric field effect.
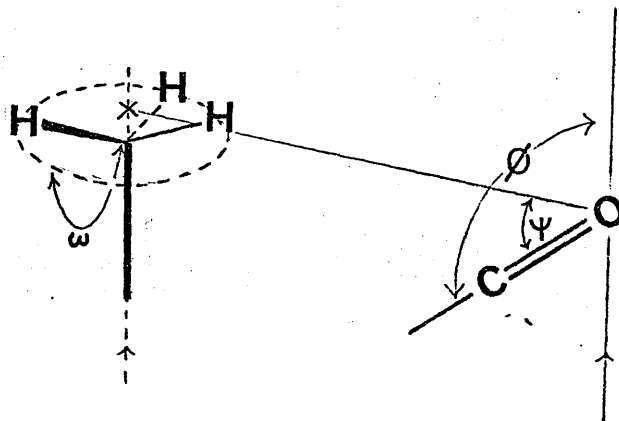
mechanisms by which aromatic solvents influence chemical shifts.

## Anisotropy of the carbonyl group

The magnetic shielding effect on a nucleus of a functional group such as the carbonyl group is dependent on their relative spatial disposition. This functional group anisotropy, which causes differential shielding, has been the subject of many empirical and theoretical treatments. Leading references to the phenomenon may be found in the series of papers by Mathieson et al[55] who have described mathematically the long-range screening effects of the C-H, C-C, C=C and C=O groups.

The total shielding effect of a carbonyl group upon a resonating nucleus is a composite of two important intramolecular factors, the magnetic anisotropic shielding and the electric field effect. The carbonyl group does not possess cylindrical or conical symmetry, therefore the magnetic anisotropic susceptibility has to be expressed in terms of three orthogonal susceptibility components (i.e. two anisotropies, $\Delta^{C=O}_{\chi_1}$ and $\Delta^{C=O}_{\chi_2}$). The relevant equation describing the contribution of magnetic anisotropic shielding to the chemical shift of a freely-rotating methyl group is given by

$$\sigma \text{ ppm.} = \frac{1}{3R3}\left[\Delta^{C=O}_{\chi_1}(1-3\cos^2\theta) + \Delta^{C=O}_{\chi_2}(1-3\sin^2\theta\sin^2\gamma)\right] \quad\text{———(1)}$$

where $\Delta^{C=O}_{\chi_1} = \chi_y - \chi_x$ and $\Delta^{C=O}_{\chi_2} = \chi_z - \chi_x$.

The x-axis coincides with the bond axis and the y-axis is the direction orthogonal to the nodal plane of the $\pi$-orbitals. The angles involved are defined in Fig. 27.

The contribution to shielding of a freely rotating methyl group due to the electric dipole is given by the equation

$$\sigma \text{ ppm.} = \frac{K}{3R^3} \left[ \cos \omega \left( 3\cos \beta \cos \Psi - \cos \theta \right) \right] \text{—————(2)}$$

in which $K = k\mu$ where k is a proportionality constant and $\mu$ is the dipole moment (in Debyes) of the carbonyl group. $\omega$ $(70^\circ 30')$ is the angle defined by the C–C axis of the methyl group and a C–H bond. The other angles involved are defined in Fig. 27.

When considering both magnetic and electric effects, the screening of the methyl group is defined as that component of the field at the centre of the circle of rotation of the hydrogen atoms, resolved parallel to one of the C–H bonds and averaged over $360^\circ$ of rotation.

To calculate for a given methyl group (or proton), the shift produced when a functional group is introduced into a molecule, both the substituted and unsubstituted molecules must be considered, such that the resultant shift

$$= \sum_1^N \sigma \text{ sub.} - \sum_1^N \sigma \text{ unsub.}$$

where $\sum_1^N \sigma$ is the sum of the screening effects of all N bonds in the molecule. However, since the net chemical shift difference between the two molecules is caused only by these bonds which differ in the substituted and unsubstituted case, only those bonds need be considered in the calculation i.e. the shift for any distant nucleus in proceeding from the alkane to the corresponding ketone comprises the algebraic sum of the screening constants for the displaced C–H

bonds and the introduced C=O bond. The relevant equation for computing the contribution due to the displaced protons is

$$\sigma \text{ ppm} = \left[\Delta\frac{x}{3R^3}(1-3\cos^2\theta)\right] + \frac{S^2}{R^5}\left[-\frac{(X_L+2X_T)}{2} + 5(X_L\cos^2\theta+X_T\sin^2\theta)\right.$$
$$\left. -\frac{35}{6}(X_L\cos^4\theta+X_T\sin^4\theta)\right] \text{_____(3)}$$

in which $X_L$ is the magnetic susceptibility of the bond in a direction along the bond axis, $X_T$ is the magnetic susceptibility in a direction at right angles to the bond axis, S is half the length of the induced dipole (taken as 0.25Å ) and $\theta$ is the angle between the symmetry axis of the proton and the direction of the group being shielded. The last three terms are correction terms which are used when R < 3Å.

Accordingly, Mathieson set up a series of equations with three unknowns, $\Delta^{C=0}_{X_1}$, $\Delta^{C=0}_{X_2}$ and K using the algebraic sum of (1), (2) and (3), for 15 mono-oxo-androstanes, using the shifts of the $C_{(18)}$ and $C_{(19)}$ methyl groups relative to androstane. Least squares analysis of the 30 equations gave values of $\Delta^{C=0}_{X_1} = -40.2 \times 10^{-30}$ cm$^3$ molecule$^{-1}$, $\Delta^{C=0}_{X_2} = -28.8 \times 10^{-30}$ cm$^3$ molecule$^{-1}$ and K = +0.8 \times 10^{-30}$D, when both magnetic and electric dipoles were assumed to be on oxygen. Corresponding values were computed, assuming the dipoles to be at other points along the C=O bond, but when both are assumed to be on oxygen the electric field tends to zero and can be ignored in anisotropic calculations, using the slightly modified values $\Delta^{C=0}_{X_1} = -39.7 \times 10^{-30}$ cm$^3$ molecule$^{-1}$ and

$\triangle \overset{C=0}{X_2} = -27.9 \times 10^{-30}$ cm$^3$ molecule$^{-1}$. These values were successfully used to predict many of the chemical shifts of methyl groups in a series of oxo-androstanes.

In principle, therefore, it should be possible in a carbonyl compound to modify the carbonyl group in such a way as to produce changes in anisotropy, observed as changes in chemical shift of certain protons in the NMR spectrum. The magnitude of the effect, if related to the relative spatial disposition of functional group and shielded/deshielded group, could be utilized in structural determinations. We have attempted to make a qualitative assessment of the anisotropic properties of the thiocarbonyl group. To our knowledge, these effects have not been reported.

## Aromatic Solvent-Induced Chemical Shifts

This technique is of great practical importance when applying NMR to the problems of natural product structure elucidation[56]. From the vast collection of empirical and semi-theoretical data it is possible in many instances to assign protons and conformations of suitable molecules. It is also possible to simplify spectral elucidation by resolving coalesced signals caused by coincidental isochrony.

The use in structure elucidation is well illustrated in the work of Bhacca and Williams[57] who showed in a series of steroidal ketones that the change from deuterochloroform to benzene produces important solvent shifts whose sign and magnitude are functions of the steric relationship of the relevant proton and the carbonyl group. In $5\alpha$- androstane the 18 and 19-methyl resonances are invariant in the change of solvent, but in a series of keto-derivatives[58], shifts dependent on geometrical relationships are observed. Generally, in the case of methyl groups adjacent to the carbonyl, axial methyls are shielded by 0.2-0.3 ppm. while equatorial methyl groups are deshielded by 0.05-0.10 ppm. Pertinent data are tabulated by Laszlo[58]. An important property of these solvent shifts is their additivity in the case of polyketones. The benzene shifts incurred by steroids can be calculated empirically as the sum of the separate shifts of the isolated carbonyl groups in the monoketones. An example of the use of the effect in conformational studies was provided in the study[59, 60] of the $\alpha$, $\beta$ and $\gamma$-substituted methyl cyclohexanones which exhibit benzene-induced solvent effects,

interpreted as caused by preferred conformations. The results
are self-consistent within each series of compounds. The chemical
shifts depend on the ease of formation of the postulated 1:1 complex
which in turn is dependent on the steric hindrance of the solvent
by the solute and therefore on its degree of substitution, according
to the authors. The potential use of the method in conformational
investigations is apparent.

There is much controversy about the nature of the effect.
No comprehensive theory has yet been advanced, but when problems
are approached through the use of a model, or by empirical
correlation, satisfactory results can often be obtained. Certain
authors[61] tend to favour as a model, a discrete 1:1 complex of a
transient nature. In the general case, benzene solvent molecules
are considered to be oriented by the electron-deficient site of a
local dipole, assuming that such dipole-induced dipole inter-
actions will lead as a first approximation to single transient 1:1
associations in which the benzene molecule will reside as far as
possible from the negative end of the molecular dipole. Benzene
is associated with the property of magnetic anisotropy, which in
the ring-current model will result in upfield shifts (shielding)
for protons above the aromatic ring and near the axis, and downfield
shifts (deshielding) for protons in the plane of, and near the
benzene ring. Zürcher[62] arrives at a similar interpretation.
Considering the 18- and 19- methyls in the steroid skeleton he
related the chemical shift increments with the algebraic sum of

a magnetic and electric term, (the anisotropic susceptibility and the dipole moment respectively) for the preferred staggered conformation of the methyl groups. The magnetic susceptibility term is independent of solvent to a first approximation and the electric (reaction field)[63] term does not vary significantly between deuterochloroform and benzene which have similar dielectric constants. One is compelled, therefore, to invoke a dipole-induced dipole association of solute and solvent, in which protons of the solute are specifically affected by the associating benzene molecule. This widely-accepted view was also described by Schneider[64] who assumed a dipole-induced dipole association which stabilises certain molecular orientations, so that solute protons are differentially shielded depending on their spatial relationship to the temporarily-ordered associating benzene molecule. He believes that the change in anisotropy due to the mechanism of complex formation is very small, the anisotropy of the solvent predominating in the process. Reaction field terms such as van der Waals forces are ignored. It is also demonstrated that probability controlled topological interactions such as the mixing of disc-shaped benzene with rod-shaped acetonitrile in a stereospecific manner are not the primary cause of solvent shifts.

In summary, such simple models of complex formation have resulted in remarkably accurate predictions[65] of solvent shift. Acceptable correlations of solvent shifts with solute molecules have been observed[64] after correcting for varying distances between the centre of gravity of the solute and of the benzene molecule,

supporting the postulated 1:1 transient complex. Dilution studies cannot demonstrate the existence of a 1:1 complex, but the apparent association constants thus measured for an assumed complex are in good agreement with the existence of a relatively weak complex.

There is, however, a growing body of opinion which questions the existence of a discrete 1:1 complex. Fort and Lindstrom in their recent publication[66] reveal that halogeno-adamantanes, which exhibit considerable solvent shifts, show no hint of complex formation in freezing point diagrams which routinely provide a facile indication of weak molecular interactions. They suggest, in common with Brown and Stark[67], that the data are more consistent with a slight geometrical ordering of the solvent about the solute molecule in a sort of 'cage construction', i.e. an indeterminate number of solvating molecules rapidly interchanging with the bulk solvent affords a geometrical effect. Measured equilibrium constants and enthalpies need have no real meaning therefore, except to indicate the magnitude of the interaction, since they are based on the presumably incorrect assumption of a 1:1 stoichiometry. Limitations of the Ronayne-Williams model were reported very recently. Matsuo[68] illustrated how certain anomalously-large solvent shifts cannot be accommodated in this model. Theoretical calculations of shifts (Johnson-Bovey treatment[69]) using this model are incompatible with experimental data. The author proposes a more general model in which van der Waals forces play a more significant part, and several solvent molecules simultaneously interact with the solute molecule. The shift is then due to the cumulative effect of these clustered solvent molecules.

This model is consistent with the fact that the observed enthalpy of the complex is of the same magnitude as the translational energies of the molecules involved, and interactions in this energy region are generally considered as being due to van der Waals forces.

Irrespective of the arguments about the origin of the effect, ignotum per ignotius, solvent-induced chemical shifts provide a powerful weapon in the armoury of the organic chemist. We have therefore examined and compared the solvent-induced chemical shifts of the carbonyl group and the corresponding thiocarbonyl derivative in a series of monoterpenoids.

5.2         RESULTS AND DISCUSSION

Anisotropy of Thiones

Compounds (66) and (68) were prepared according to the methods of Sen[70] and Mayer[71] respectively, and the products rigorously purified by column chromatography. Physical and spectroscopic data were consistent with literature values[71]. Thiocamphenilone, a new compound, was prepared and purified in the same manner as thiofenchone (68). Analysis, infra-red, ultraviolet and GC-MS spectral data were in accord with the monomeric structure (70).

These particular thiones were chosen for the following reasons:

(a) Simple preparations of thiocamphor and thiofenchone were described in the literature[70,71].

(b) They comprise a set of closely-related, rigid bicylic systems which are structurally similar.

(c) The methyl resonances of camphor, fenchone and camphenilone have been assigned in the literature[72,73].

(d) They are either non-enolisable or not appreciably enolised, unlike simpler alicyclic thiones which have been described[74].

(e) They are monomeric, unlike thioacetone, thiopropanone and similar simple thiones which exist in the polymeric form[74].

Fig.28
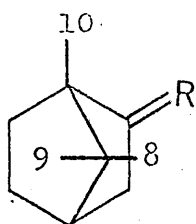


65, R=O
66, R=S
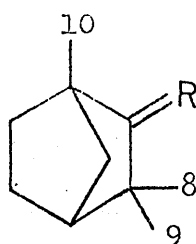
67, R=O
68, R=S
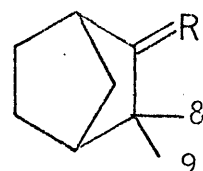
69, R=O
70, R=S

TABLE 4

Chemical Shift (CCl₄)

| | Me(8) | Me(9) | Me(10) | H(1) | $H^{exo}_{(3)}$ | $H^{endo}_{(3)}$ |
|---|---|---|---|---|---|---|
| Bornane | 9.17 | 9.17 | 9.17 | – | ~8.75 | ~8.75 |
| camphor | 9.15 | 9.03 | 9.13 | – | 7.80 | 8.30 |
| thiocamphor | 9.20 | 8.97 | 8.95 | – | 7.30 | 7.65 |
| fenchone | 9.01 | 9.01 | 8.91 | – | – | – |
| thiofenchone | 8.84* | 8.88** | 8.70 | – | – | – |
| camphenilone | 8.99 | 8.99 | – | 7.40 | – | – |
| thiocamphenilone | 8.87 | 8.87 | – | 6.60 | – | – |

*arbitrarily assigned.

TABLE 5

Distance ±(Å) of Functional Group to Shielded Protons

| | ME(8) | Me(9) | Me(10) | H(1) | $H^{exo}_{(3)}$ | $H^{endo}_{(3)}$ |
|---|---|---|---|---|---|---|
| Camphor | 4.00 | 5.24 | 3.14 | – | 2.80 | 2.80 |
| thiocamphor | 4.02 | 5.60 | 3.22 | – | 3.10 | 3.10 |
| fenchone | 3.40 | 3.40 | 3.14 | – | – | – |
| thiofenchone | 3.55 | 3.75 | 3.22 | – | – | – |
| camphenilone | 3.40 | 3.40 | – | 2.80 | – | – |
| thiocamphenilone | 3.55 | 3.75 | – | 3.10 | – | – |

± measured from heteroatom to geometrical centre
of rotation of methyl protons.

Fig. 29
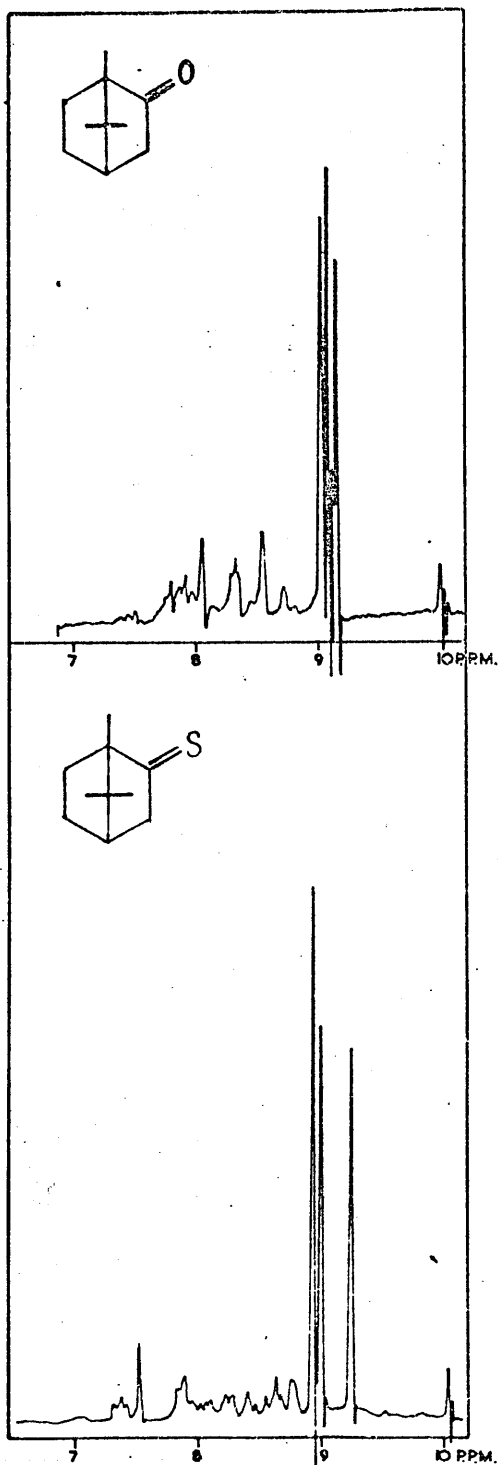


NMR spectra (60Mc/s.) of camphor and thiocamphor
in CDCl$_3$

It is assumed throughout this discussion that the thione $C_{(1)}-C_{(2)}-C_{(3)}$ angle is essentially similar to that of the ketone. In view of the rigidity of the bornane ring system we believe that any inherent tendency of the thione group to vary from the trigonal* $sp^2$ geometry will be negligible.

Preliminary inspection of the 60 Mc/s spectra of the three thiones immediately reveals that this functionality has a greater anisotropy than the carbonyl group. The relative anisotropic shielding and deshielding effects of the ketones and thiones are represented by the chemical shifts in Tables 6-8. The striking difference between ketone and thione is best illustrated, however, by Fig. 29 in which the methyl absorptions of camphor are completely dissimilar to those of thiocamphor. Comparison of the respective methylene and methine regions also indicate significant differences.

Assignment of the methyl resonances in thiocamphor was facilitated by the very recent paper of Baker and Davis[73] in which twenty-nine bornane derivatives were investigated by NMR; deuteration studies resulted in unequivocal assignment of the methyl groups and the pertinent observation that these methyls can invariably be

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*The chemistry of thiones[74] is dominated by reactions of the type $(R_2C=S, sp^2) \rightarrow$ product $(R_3C-S, sp^3)$ e.g. the extreme electrophilicity of the group and the tendency to dimerize and trimerize with formation of C-S $sp^3$ bonds. This is due to the decreased stabilization of $R_2C=S$ relative to $R_2C=O$; the overlap integral of the p-orbitals in $R_2C=O$ is greater than in C=S in which the S p-orbital is much more diffuse. This relative destabilization is also reflected in the bond energies[75] :
C=O, 152 Kc/M;    C=S, 103 Kc/M.

Fig. 30

Projection of camphor along the $C_{(1)}-C_{(4)}$ axis



($\tau$9.04) Me  Me ($\tau$9.16)

9   8

7

deshielding    shielding
zone           zone

4

5   3

O
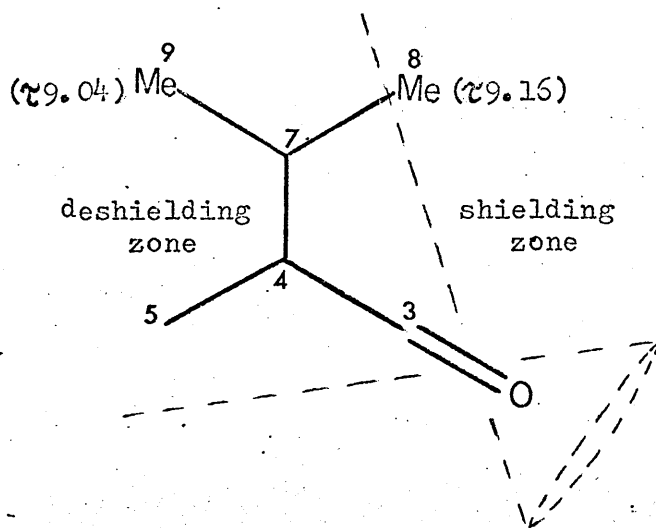
Projection of thiocamphor along the $C_{(1)}-C_{(4)}$ axis



($\tau$8.97) Me  Me ($\tau$9.20)

9   8

7

deshielding    shielding
zone           zone

4

5   3

S
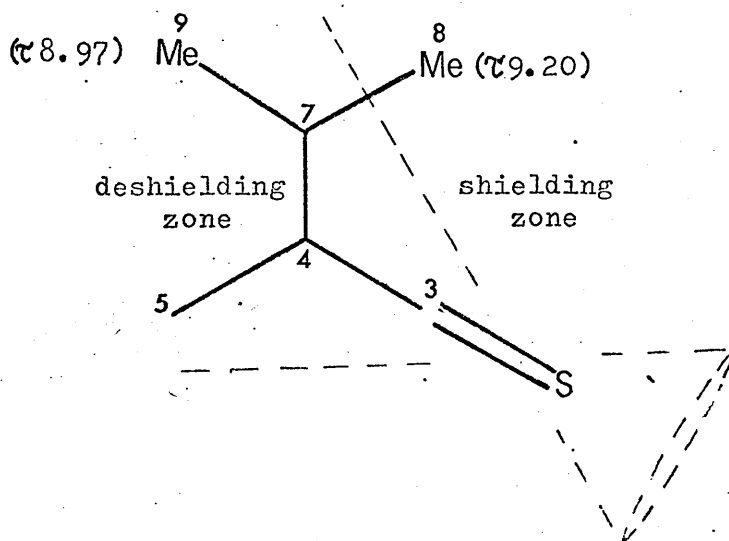
correlated on the basis of their line widths (and hence peak heights). In camphor the $C_{(10)}$ methyl absorption had the narrowest half-band width ( $\Delta W_{\frac{1}{2}} = W_{\frac{1}{2}} - W_{\frac{1}{2}} TMS = 0.18$ cps); the corresponding values for $C_{(8)}$ and $C_{(9)}$ were 0.84 and 0.78 cps. respectively. The causative factor was shown to be coupling through four $\sigma$ bonds having a W-configuration in the $C_{(8)} - C_{(9)}$ gem-dimethyl group. Since thiocamphor is a related bornane derivative we have accordingly assigned the methyl resonances on the basis of peak height, as shown in Table 8. Solvent shift data are in accordance with the assignments. Deuteration studies which would provide conclusive proof have not yet been performed.

A qualitative estimation of the relative anisotropic shielding propensities of ketone and thione can be obtained from Fig. 30 in which 2-dimensional representations of camphor and thiocamphor are presented. The Me(8), Me(9) and Me(10) absorptions of camphor are readily accommodated within the recently redefined cone of shielding and deshielding of the carbonyl group as shown in Fig. 30. The position of greatest shielding is assumed to be outside that section of the solid cone which surrounds the 2-fold symmetry axis of the carbonyl. Protons which lie within the solid section of the cone which points along the bond axis are deshielded. Hence, Me(9) in camphor at $\tau$ 9.04 is deshielded relative to its bornane analogue. Me(8), which lies within the shielding zone, is relatively shielded.

In thiocamphor a similar empirical cone of shielding can be

postulated to allow for the shielding of Me(8) which absorbs at $\Upsilon 9.20.$ Me(9) is similarly deshielded. The magnitudes of shielding and deshielding are apparently greater than in the corresponding ketones. Values obtained for thiofenchone and thiocamphenilone can also be accommodated within the postulated shielding/deshielding zones.

In order to evaluate quantitatively the anisotropic parameters of the thiocarbonyl group, a series of equations in three unknowns, $\Delta X_1^{C=S}$, $\Delta X_2^{C=S}$ and K would have to be solved. As a test of the applicability of the Mathieson treatment[55] to (2,2,1)- bicyclic systems, the chemical shifts of the methyl groups in camphor were calculated. The shift of Me(9) was correctly predicted, but those of Me(8) and Me(10) were found to differ from the experimental value by a significant amount ($\sim 0.1$ ppm). Similar disparities have recently been observed by Karabatsos et al[76]. Possible reasons, in this study, could involve the relief of steric compression which results in passing from bornane (from which the contribution of the two displaced protons are calculated) to camphor, and the error involved in estimating distances and angles from Dreiding models (Table 5). It was therefore deemed inadvisable to proceed with calculations on the thioketones (66), (68) and (70). A different series of thiones such as thio-oxo-androstanes may be more amenable to the technique.

A preliminary analysis of the NMR spectra of camphor and thiocamphor, which were superficially different, was undertaken

Fig. 31
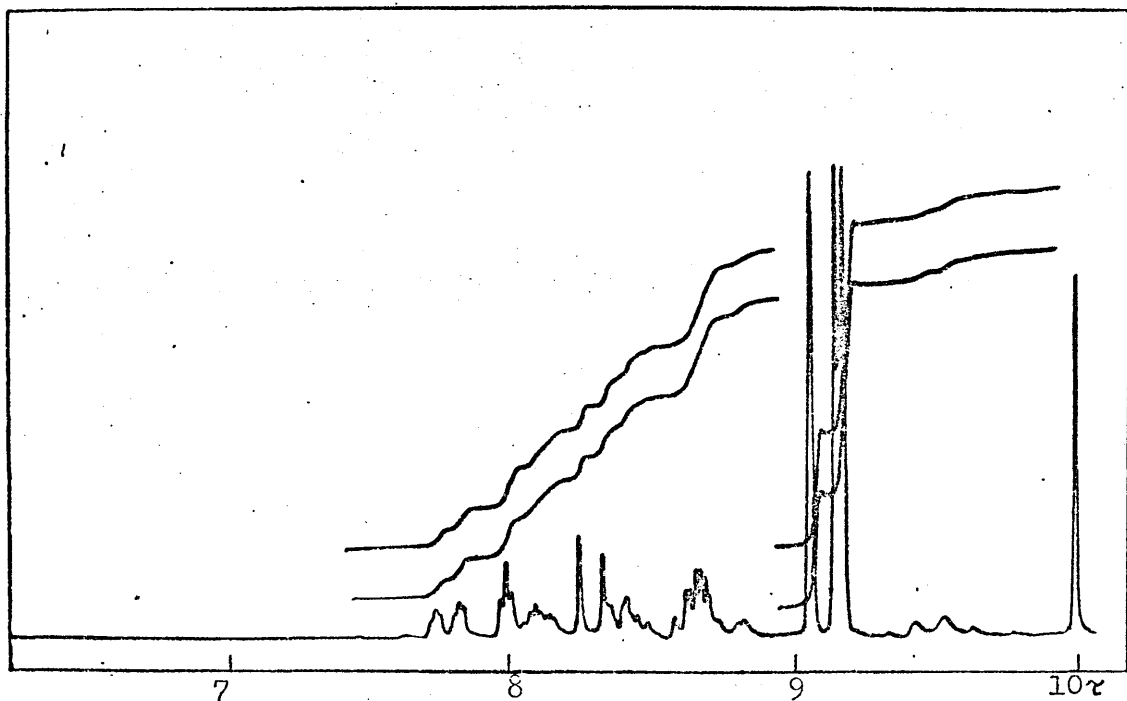


NMR spectrum (220Mc/s.) of camphor in CCl$_4$ (1000 cps. sweep width)
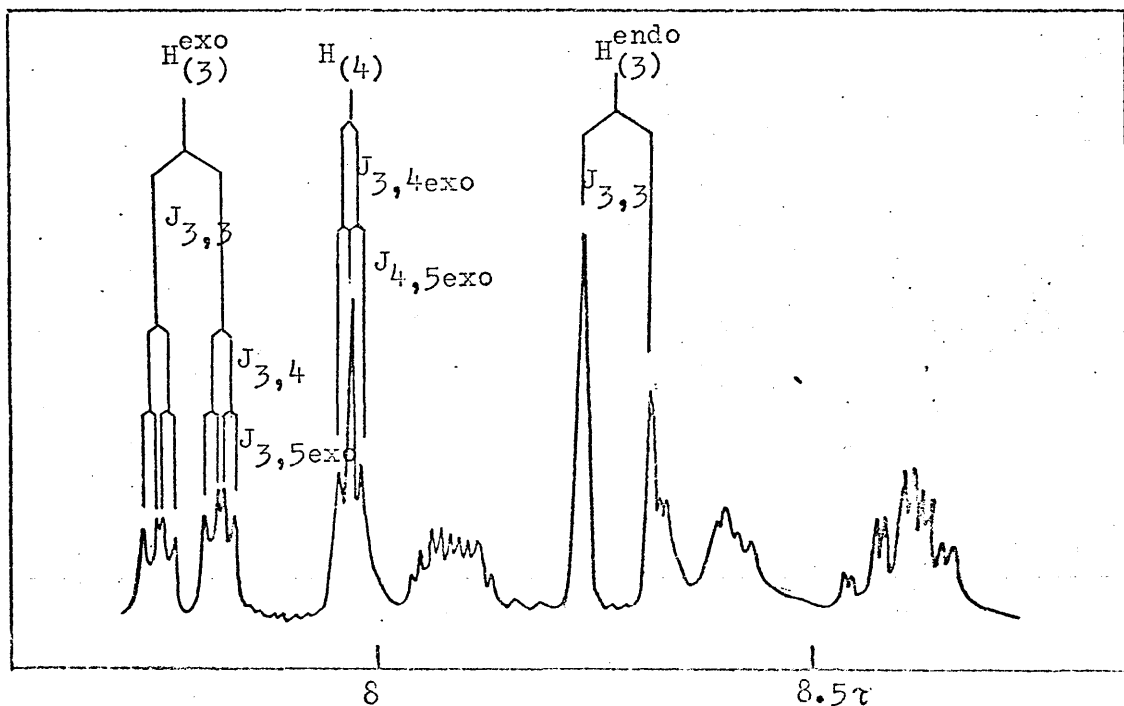


NMR spectrum (220Mc/s.) of camphor in CCl$_4$ (500 cps. sweep width)

Fig. 32
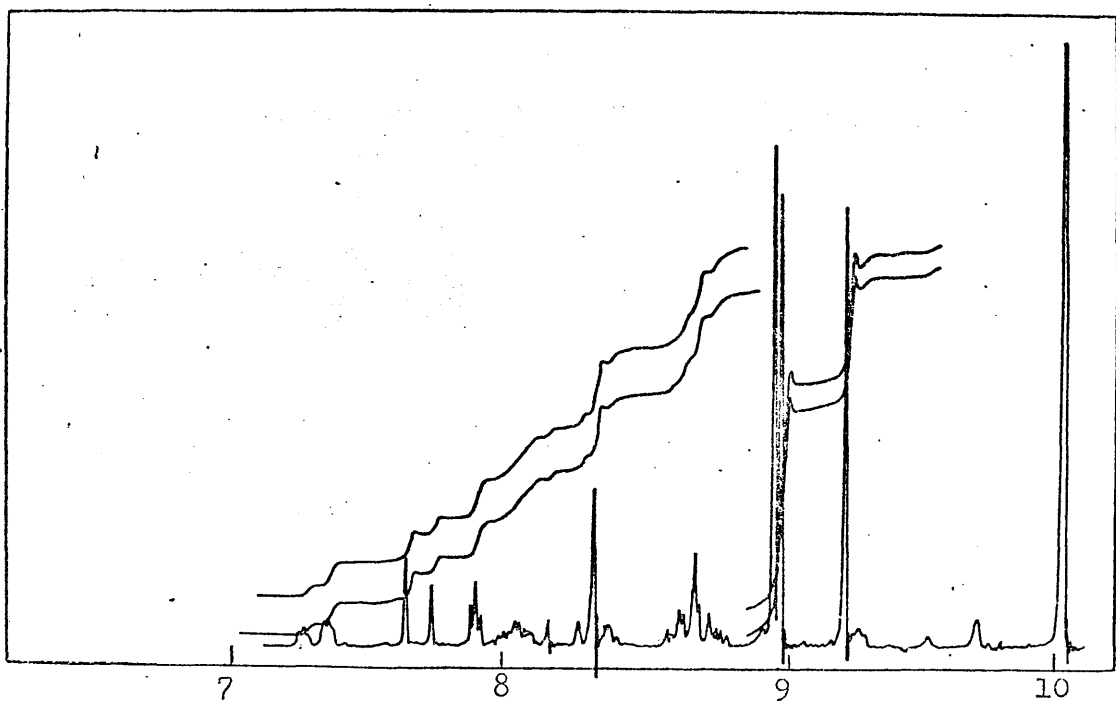


NMR spectrum (220Mc/s.) of thiocamphor in $CCl_4$ (1000cps. sweep width)
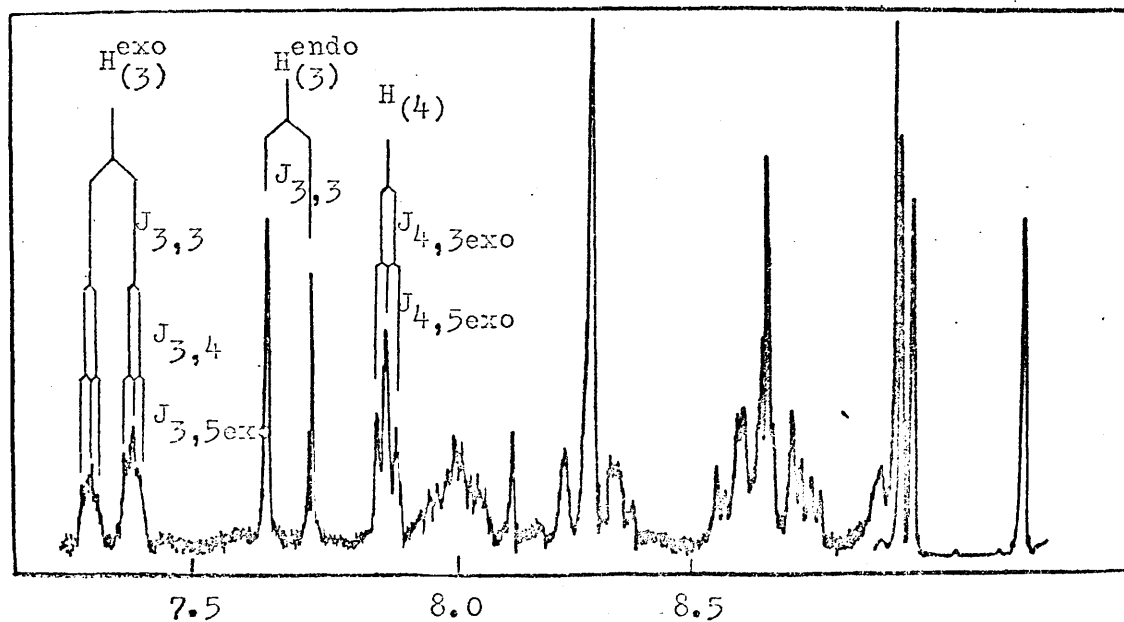


NMR spectrum (220Mc/s.) of thiocamphor in $CCl_4$ (500cps. sweep width)

in order that other proton resonances could be assigned. The differences in anisotropic effect between carbonyl and thione were especially emphasized when the methylene-methine proton regions of camphor and thiocamphor were examined at 220Mc/s.[+] (Figs. 31 and 32). The AB doublets (Jgem = -19 cps) due to $H_{(3)}^{exo}$ and $H_{(3)}^{endo}$ were apparent at $\tau$ 7.8 and $\tau$ 8.3 in camphor. $H_{(3)}^{endo}$ which subtends an angle of $90^{o}$ with $H_{(4)}$ is not further coupled. $H_{(3)}^{exo}$ on the other hand subtends an angle of $45^{o}$ with $H_{(4)}$ and is accordingly further split by 4 cps. $4\sigma$ coupling to $H(5exo)$, which is to be expected as a consequence of the 'W' relationship, causes further splitting of $H_{(3)}^{exo}$ ($J_{3exo,5exo}$ = 4 cps. ) The assignment of the $H_{(5)}^{exo}$, $H_{(5)}^{endo}$, $H_{(6)}^{exo}$ and $H_{(6)}^{endo}$ protons, which comprise part of a 7-spin system, is at present under study, using computational techniques.

Thiocamphor exhibits significant chemical shift differences in this region in the 220 Mc/s spectrum, although the signals are still well-separated from one another. $H_{(3)}^{exo}$ and $H_{(3)}^{endo}$ again comprise an AB system (Fig. 32) but with unusually high geminal coupling[*] of -22 cps. As in camphor, $H_{(3)}^{exo}$ is further coupled to $H_{(4)}$ ($J_{3exo,4}$ = 4 cps) and to $H_{(5)}^{exo}$ ($J_{3exo, 5exo}$ = 4 cps).

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In summary, the anisotropy of the thione function has been found to be markedly different from that of the ketone group. A qualitative examination indicates that the functionality may be of some potential use in structure elucidation by virtue of the added information which it provides.

## TABLE 8

|  | CAMPHOR | | | | | | THIOCAMPHOR | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
|  | Me(8) | | Me(9) | | Me(10) | | Me(9) | | Me(8) | | Me(10) | |
|  | $\tau$ | Solv. $\Delta CCl_4$ | $\tau$ | Solv. $\Delta CCl_4$ | $\tau$ | Solv. $\Delta CCl_4$ | $\tau$ | Solv. $\Delta CCl_4$ | $\tau$ | Solv. $\Delta CCl_4$ | $\tau$ | Solv. $\Delta CCl_4$ |
| Carbon tetrachloride | 9.16 | 0.00 | 9.04 | 0.00 | 9.14 | 0.00 | 8.97 | 0.00 | 9.20 | 0.00 | 8.95 | 0.00 |
| deuterochloroform | 9.18 | +0.02 | 9.05 | +0.01 | 9.10 | −0.04 | 8.98 | +0.01 | 9.22 | +0.02 | 8.92 | −0.03 |
| benzene | 9.41 | +0.25 | 9.37 | +0.33 | 9.12 | −0.02 | 9.29 | +0.32 | 9.43 | +0.23 | 8.91 | −0.04 |
| pyridine | 9.30 | +0.14 | 9.22 | +0.18 | 9.11 | −0.03 | 9.12 | +0.15 | 9.42 | +0.22 | 8.91 | −0.04 |
| 50% $CCl_4$ - phenol. | 9.41 | +0.25 | 9.18 | +0.14 | 9.30 | +0.16 | 9.20 | +0.23 | 9.41 | +0.21 | 8.93 | +0.03 |
| aniline | 9.49 | +0.27 | 9.40 | +0.36 | 9.20 | +0.06 | 9.30 | +0.33 | 9.44 | +0.24 | 8.94 | −0.01 |
| pyrrole | 9.43 | +0.27 | 9.33 | −0.29 | 9.22 | +0.08 | 9.25 | +0.28 | 9.44 | +0.24 | 8.98 | +0.03 |
| furan | 9.31 | +0.15 | 9.22 | +0.18 | 9.12 | −0.02 | 9.22 | +0.25 | 9.42 | +0.22 | 8.90 | −0.05 |
| thiophene | 9.40 | +0.24 | 9.32 | +0.28 | 9.14 | 0.00 | 9.23 | +0.26 | 9.40 | +0.20 | 8.92 | −0.03 |
| quinoline | 9.45 | +0.29 | 9.46 | +0.42 | 9.11 | −0.03 | 9.39 | +0.42 | 9.48 | +0.28 | 8.93 | −0.02 |
| anisole | 9.40 | +0.24 | 9.32 | +0.28 | 9.18 | +0.04 | 9.22 | +0.25 | 9.40 | +0.20 | 8.93 | −0.02 |
| chlorobenzene | 9.38 | +0.22 | 9.30 | +0.26 | 9.17 | +0.03 | 9.20 | −0.23 | 9.39 | −0.19 | 8.96 | +0.01 |

probable error $\pm$ 0.01 ppm.

TABLE 7.

| | FENCHONE | | | | | | THIOFENCHONE | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Me(8) | | Me(9) | | Me(10) | | Me(8) | | Me(9) | | Me(10) | |
| | $\tau$ | Solv. $\Delta$CCl$_4$ | $\tau$ | Solv. $\Delta$CCl$_4$ | $\tau$ | Solv. $\Delta$CCl$_4$ | $\tau$ | Solv. $\Delta$CCl$_4$ | $\tau$ | Solv. $\Delta$CCl$_4$ | $\tau$ | Solv. $\Delta$CCl$_4$ |
| Carbon tetrachloride | 9.01 | 0.00 | 9.01 | 0.00 | 8.99 | 0.00 | 8.84 | 0.00 | 8.88 | 0.00 | 8.70 | 0.00 |
| deuterochloroform | 8.99 | -0.02 | 8.99 | 0.02 | 8.88 | -0.11 | 8.83 | -0.01 | 8.87 | -0.01 | 8.69 | -0.01 |
| benzene | 9.09 | +0.08 | 9.11 | +0.10 | 8.91 | -0.08 | 8.91 | +0.07 | 9.00 | +0.02 | 8.69 | -0.01 |
| pyridine | 9.03 | +0.02 | 9.03 | +0.02 | 8.90 | -0.09 | 8.87 | +0.03 | 8.92 | +0.04 | 8.70 | 0.00 |
| 50% CCl$_4$ - phenol | 9.11 | +0.10 | 9.11 | +0.10 | 8.95 | -0.04 | 8.93 | +0.09 | 8.98 | +0.10 | 8.73 | -0.03 |
| aniline | 9.13 | +0.12 | 9.13 | +0.12 | 8.97 | -0.02 | 8.92 | +0.08 | 9.00 | +0.02 | 8.71 | +0.01 |
| pyrrole | 9.18 | +0.17 | 9.18 | +0.17 | 0.01 | +0.02 | 8.94 | +0.10 | 9.00 | +0.02 | 8.74 | +0.04 |
| furan | 9.03 | +0.02 | 9.05 | +0.04 | 8.90 | -0.09 | 8.87 | +0.03 | 8.94 | +0.06 | 8.69 | -0.01 |
| thiophene | 9.09 | +0.08 | 9.11 | +0.10 | 9.02 | +0.03 | 8.90 | +0.06 | 8.96 | +0.08 | 8.69 | -0.01 |
| quinoline | 9.06 | +0.05 | 9.08 | +0.07 | 8.84 | -0.15 | 8.95 | +0.11 | 9.01 | +0.03 | 8.69 | -0.01 |
| anisole | 9.10 | +0.09 | 9.10 | +0.09 | 8.94 | -0.05 | 8.91 | +0.07 | 8.98 | +0.10 | 8.71 | +0.01 |
| chlorobenzene | 9.01 | +0.08 | 9.09 | +0.08 | 8.93 | -0.06 | 8.92 | +0.08 | 8.98 | +0.10 | 8.72 | +0.02 |

probable error $\pm$ 0.01 ppm.

TABLE 6.

| | CAMPHENILONE | | | | THIOCAMPHENILONE | | | |
| | Me(8) | | Me(9) | | Me(8) | | Me(9) | |
| | τ | Solv. ΔCCl4 | τ | Solv. ΔCCl4 | τ | Solv. ΔCCl4 | τ | Solv. ΔCCl4 |
|---|---|---|---|---|---|---|---|---|
| Carbon tetrachloride | 9.01 | 0.00 | 9.01 | 0.00 | 8.87 | 0.00 | 8.87 | 0.00 |
| deuterochloroform | 8.97 | -0.04 | 8.97 | -0.04 | 8.85 | -0.02 | 8.85 | -0.02 |
| benzene | 9.12 | +0.11 | 9.12 | +0.11 | 8.96 | +0.09 | 8.99 | +0.12 |
| pyridine | 9.04 | +0.03 | 9.04 | +0.03 | 8.92 | +0.05 | 8.92 | +0.05 |
| 50% CCl4-phenol | 9.13 | +0.12 | 9.13 | +0.12 | 8.96 | +0.09 | 8.96 | +0.09 |
| aniline | 9.16 | +0.15 | 9.16 | +0.15 | 8.99 | +0.12 | 8.99 | +0.12 |
| pyrrole | 9.18 | +0.17 | 9.18 | +0.17 | 9.00 | +0.13 | 9.00 | +0.13 |
| furan | 9.06 | +0.05 | 9.06 | +0.05 | 8.92 | +0.05 | 8.92 | +0.05 |
| thiophene | 9.10 | +0.09 | 9.10 | +0.09 | 8.95 | +0.08 | 8.95 | +0.08 |
| quinoline | 9.11 | +0.10 | 9.11 | +0.10 | 9.00 | +0.13 | 9.00 | +0.13 |
| anisole | 9.11 | +0.10 | 9.11 | +0.10 | 8.97 | +0.10 | 8.97 | +0.10 |
| chlorobenzene | 9.11 | +0.10 | 9.11 | +0.10 | 8.97 | +0.10 | 8.97 | +0.10 |

# Fig 33



CAMPHOR AND THIOCAMPHOR

solvent shifts of $C_8$ and $C_9$.

CAMPHOR $C_9$
THIOCAMPHOR $C_9$
CAMPHOR $C_8$
THIOCAMPHOR $C_8$

PHENOL · PYRIDINE · FURAN · THIOPHENE · CHLOROBENZ. · ANISOLE · PYRROLE · BENZENE · ANILINE · QUINOLINE

Shift ($\tau$) relative to CDCl$_3$

Fig. 34
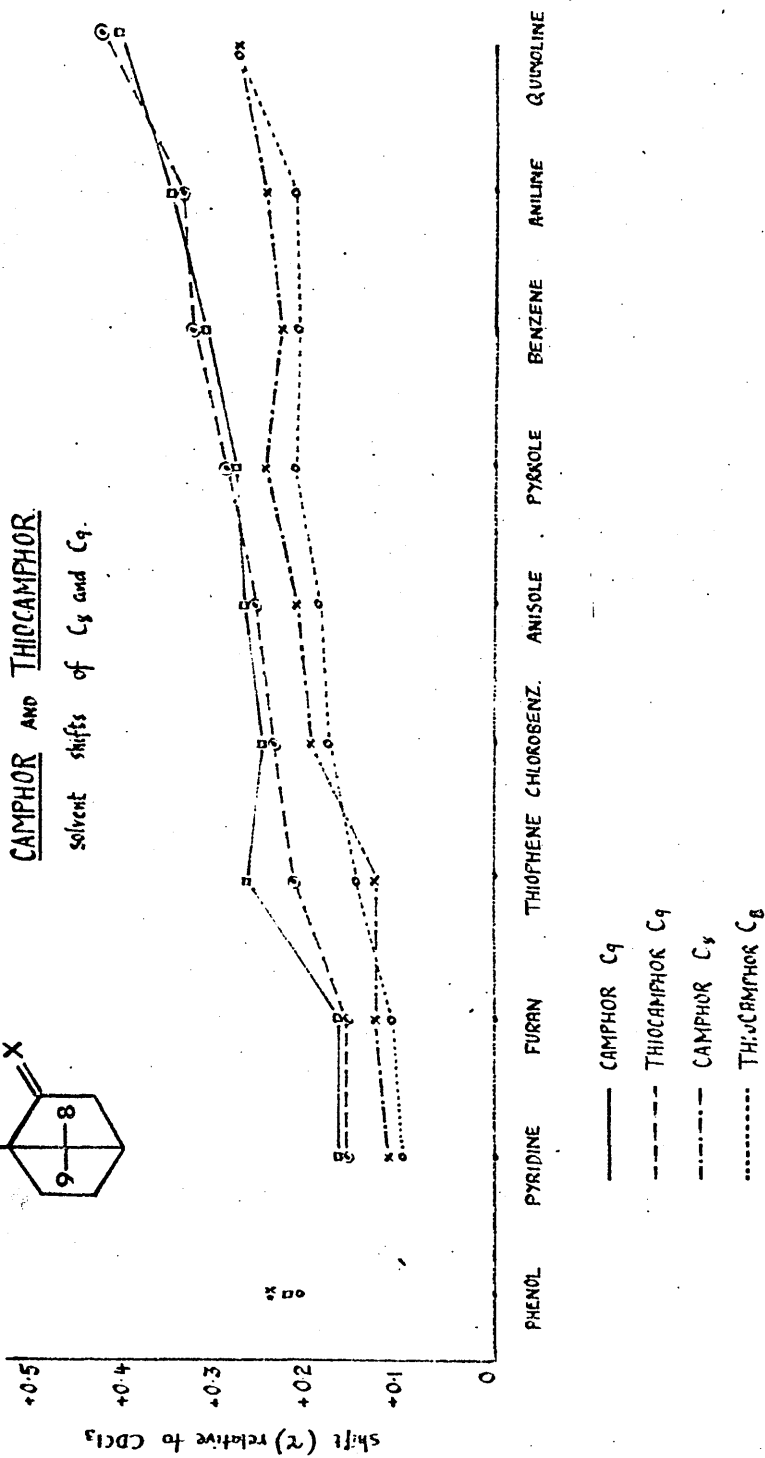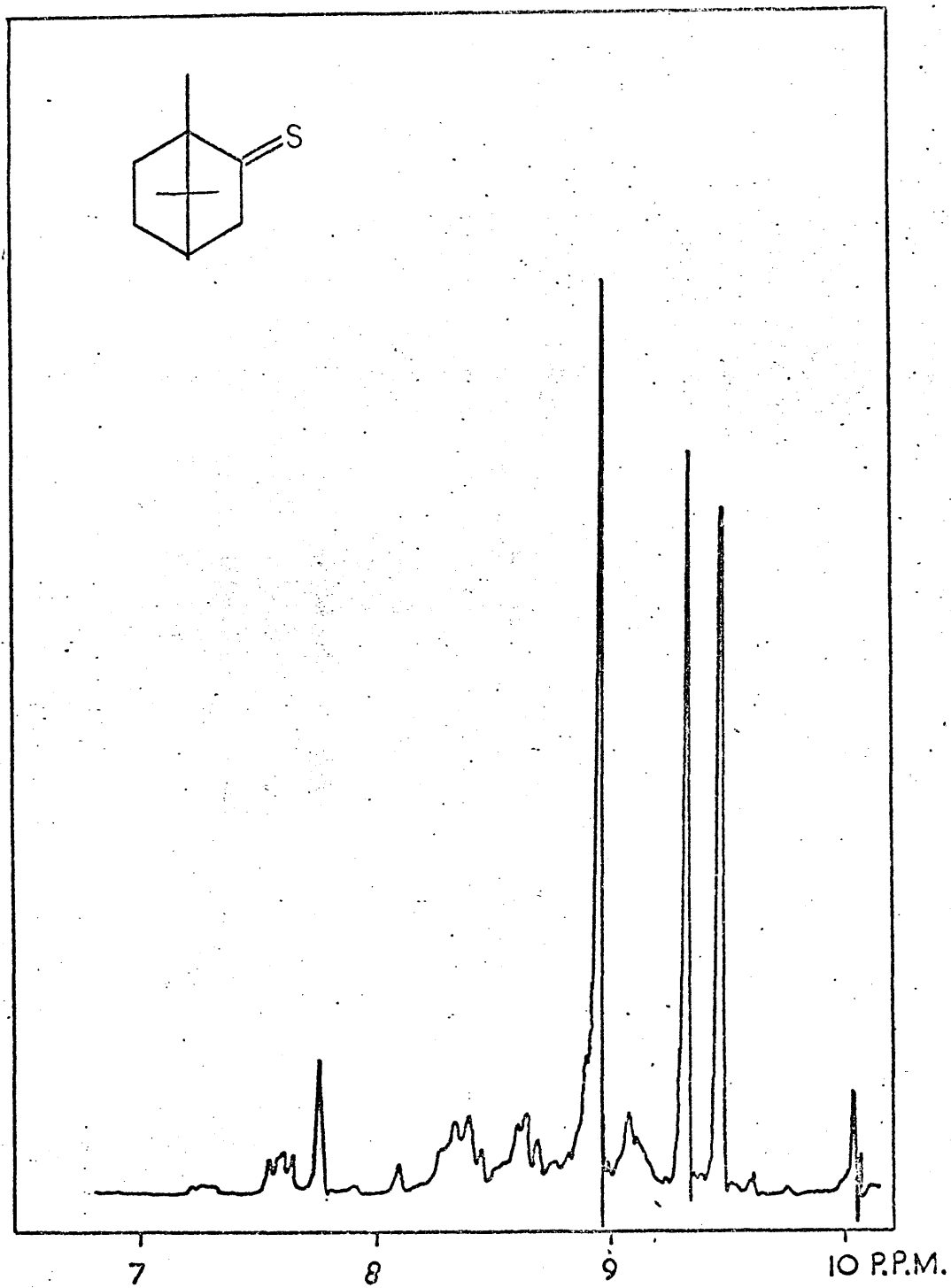
NMR spectrum (60Mc/s.) of thiocamphor in benzene

Aromatic-Solvent-Induced Chemical Shifts of the Thiocarbonyl Group.

The extensive literature on the aromatic-solvent-induced chemical shifts reflects the empirical value of the technique. Accordingly, an evaluation of the monoterpene ketone derivatives, thiocamphor, thiofenchone and thiocamphenilone has been performed.

60 Mc/sec. spectra of the ketones and their derivatives were run in carbon tetrachloride, deuterochloroform and a wide range of aromatic solvents. Chemical shift data are presented in Tables 6-8. Data have been presented[78] for camphor and fenchone in a similar series of solvents, but the thione group has not been examined. Typical solvent shifts for the thione function may be seen in Fig. 34 (cf. Fig. 29) in which the signals due to Me(8), Me(9) and Me(10), $\tau$ 8.98, 9.22 and 8.92, respectively, shift to 9.29, 9.43 and 8.91 (assignments according to peak heights). The solvent shifts observed for the thiones are of the same order of magnitude as those of the corresponding ketones.

Fig. 33 indicates a striking parallelism in solvent shifts of corresponding methyl groups (e.g. $C_{(9)}$ camphor and $C_{(9)}$ thiocamphor) which could be of some utility in correlating methyl assignments in ketones and thiones. A methyl group in a thione, perhaps assigned on the basis of anisotropy considerations, should display solvent shifts in a range of aromatic solvents, parallel to those of the methyl group in the parent ketone.

It would also appear that the empirical rule postulated by Connolly and McCrindle[72] for ketones is applicable to thiones:

Fig. 35



applied

magnetic field
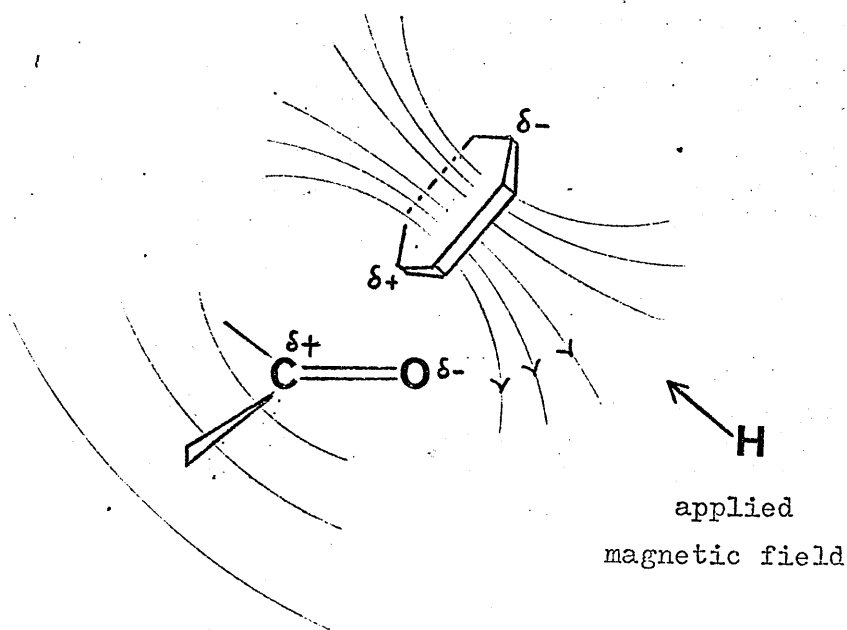
Diamagnetic ring current induced in a benzene molecule
solvating a carbonyl group. In a 1:1 complex the
benzene molecule would assume the position in which
repulsion due to interaction of the negative dipole of
oxygen and the π-cloud of benzene, and steric repulsion,
are minimized.

Fig. 36



Camphor — mole fraction benzene $(x_B)$ vs. $\dfrac{x_B}{\delta}$

slope $= \dfrac{1}{\delta_{AB}} = 0.0125 = \dfrac{1}{\text{limiting shift}}$

$\therefore$ limiting shift = 80cps.

intercept $= \dfrac{1}{K\delta_{AB}} = 0.04$

$\therefore K = 0.3 mf^{-1}$

$\dfrac{x_B}{\delta} \times 10^4$

$x_B$



Thiocamphor — mole fraction benzene $(x_b)$ vs. $\dfrac{x_B}{\delta}$

limiting shift = 70cps.

$K = 0.4$ mole fraction$^{-1}$

$\dfrac{x_b}{\delta} \times 10^4$

$x_B$
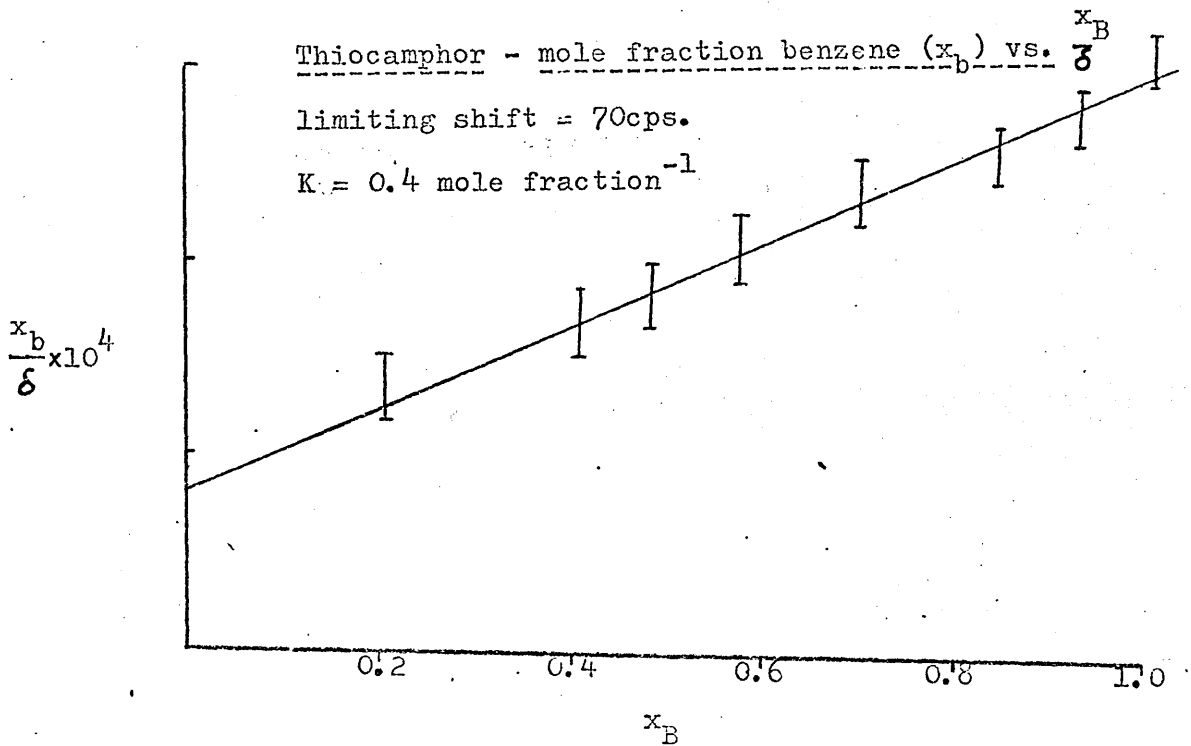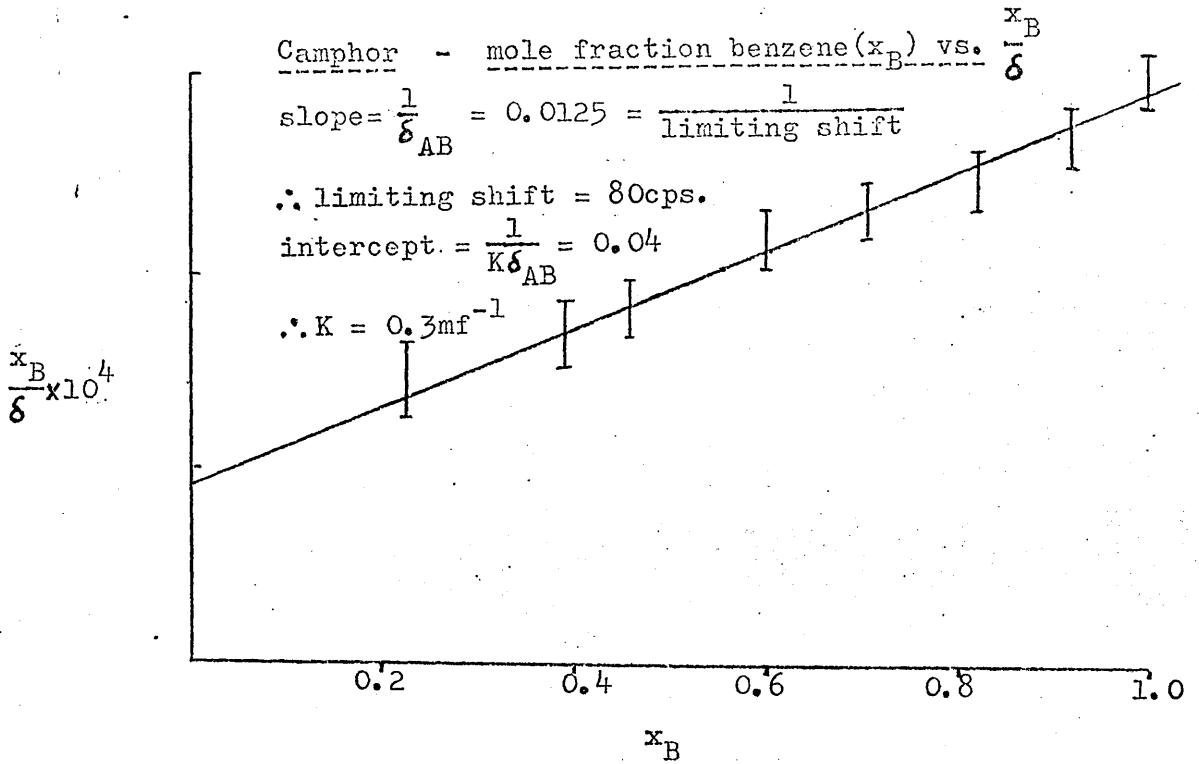
'if a plane is drawn at right angles through the carbon of the carbonyl group then methyl groups behind the plane are shielded, those in front of the plane are deshielded and those in the plane are relatively unaffected.' In camphor, for example, Me(10) is in the plane and exhibits only a small shift. Similarly in thiocamphor, Me(10) has a small shift ($\Delta^{C_6H_6}_{CCl_4}$ 0.04 cps).

Since the mechanism is assumed to involve dipole-induced dipole attractive forces, correlation of the magnitudes of the respective dipoles of the carbonyl and thiocarbonyl groups with observed shifts might be expected. In the 1:1 association model, benzene is expected to associate with the positive site of the functional group as shown in Fig. 35. The respective dipoles[79,80] of ketone and thione functional groups are 2.6 and 2.0D. These differences are not manifested as significant differences in solvent shifts (Tables 6-8 ).

If a time-averaged complex is assumed, then it can be shown[81,82] that the equilibrium constant Kass. can be obtained from the relationship

$$\frac{X_B}{\delta} = \frac{X_B}{\delta_{AB}} + \frac{1}{K\delta_{AB}} \underline{\hspace{3cm}}(1)$$

where $X_B$ = mole fraction of benzene, $\delta$ is the difference in chemical shift between 'inert' solvent and benzene which is due to complex formation, and $\delta_{AB}$ the difference in shift if complex formation is complete. High resolution chemical shift measurements were performed on both camphor and thiocamphor, as a function of mole fraction of benzene in carbon tetrachloride. $X_B/\delta$ was plotted against $X_B$ camphor and thiocamphor (Fig. 36) and Kass found to be 0.3-0.4 (mole

fraction)$^{-1}$ The limiting shifts were approximately 80 and 70 cps respectively. The dilution experiments cannot demonstrate unequivocally the existence of a 1:1 complex but the apparent association constants thus obtained for an assumed complex are in good agreement with the existence of relatively weak complexes.

In conclusion, the aromatic solvent-induced chemical shifts of thiones parallel those of the corresponding ketones, and are of a similar magnitude. The effect is of potential use in a parallel shift technique, in which proposed proton assignments may be corroborated by comparison of solvent shifts in both carbonyl and derived thione in a series of aromatic solvents. The equilibrium constants for the association with benzene are also of similar magnitude in both carbonyl and thione. From these equilibrium constants it is evident that a minority of the molecules are complexed (assuming the hypothetical 1:1 complex). Although the dipolar and bond length parameters of carbonyl and thiocarbonyl differ appreciably, no information about the mechanism of the effect can be safely derived.

Emphasis has been placed by many investigators on the attractive forces such as dipole-induced dipole and/or Van der Waals forces involved in the effect. Topological considerations which could result in local geometrical ordering of the solvent cage with resultant differences in cumulative shielding effects at different points of the solute molecule, have not been taken into consideration. There is a further factor which has not obtained much attention:

in any postulated dipolar 'complex' of a carbonyl group with
benzene, the electronic distribution of the carbonyl group may be
perturbed. This effect could conceivably be manifested intra-
molecularly as a change in anisotropic shielding at other points
in the molecule. The origin of the effect is obviously speculative,
but the empirical application has proved to be a powerful weapon
in structure elucidation.

5.3                    <u>EXPERIMENTAL</u>

All solvent shift data were recorded on a Perkin Elmer R-10
60 Mc/s spectrometer. Calibrations, using the cyclohexane singlet,
were performed before and after each series of measurements.
0.3M solutions were carefully prepared, and tetramethylsilane
added as internal reference. Accurate shift data for the calculation
of association constants were measured using expanded-scale
conditions. All solvents used in the compilation of Tables 6-8
were distilled before use.

Anisotropic chemical shift data were obtained on both the
Perkin-Elmer R-10 and Varian HA 220 spectrometers, from 0.3M
solutions, using tetramethylsilane as internal reference.

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