STUDIES OF SOME NEW EUPHORBIACEAE DITERPENES

A thesis submitted in partial fulfilment of the
requirements for the degree of Doctor of Philosophy
in the Department of Chemistry, University of Natal.

by

Joan Margaret McGarry M.Sc. (Natal)
University of Natal,
Durban.

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DECLARATION

I hereby certify that this research is the
result of my own investigation, which has not already
been accepted in substance for any degree, and is not
being concurrently submitted in candidature for any other
degree.

Signed.. *J. M. McGarry*..

J. M. McGARRY

I hereby certify that the above statement is
correct.

Signed.....

H. A. CANDY M.Sc. (S.A.)

Supervisor

Department of Chemistry,

University of Natal,

Durban.

ACKNOWLEDGEMENTS

SUMMARY

The heartwoods of two species of South African Euphorbiaceae have been chemically investigated.

From Cleistanthus schlechteri three new diterpenes possessing the hitherto unknown ent-isopimara-8(14),15-diene skeleton were isolated. By means of chemical and spectroscopic methods these were shown to be 3α-hydroxy-ent-isopimara-8(14),15-diene, I, 3α-hydroxy-ent-isopimara-8(14),15-dien-12-one, II, and $3\alpha,12\alpha$-dihydroxy-ent-isopimara-8(14),15-diene, III. A biogenetic sequence has been proposed in which it is suggested that these compounds are probably the precursors of the major diterpenoid, cleistanthol, IV, previously isolated from this source.

From the second species, Spirostachys africana, a new diterpenoid seco-acid, spirostachic acid, VIII, was obtained in addition to the beyerene derivatives previously reported. Mass spectral fragmentation patterns of the seco-acid and its methyl esters proved to be useful as a diagnostic tool in structure elucidations.

CONTENTS

INTRODUCTION

The Euphorbiaceae which constitutes one of the
largest and most widely distributed plant families has pro-
vided a source of interesting compounds for chemical
investigation. This thesis is concerned with the isolation
and characterisation of some new diterpenes obtained from
two species indigenous to Southern Africa.

The first and major section of this work deals
with pimarane-type diterpenes isolated from the heartwood
of Cleistanthus schlechteri (PAX) HUTCH. var. schlechteri,
while the second section describes a beyerene (stachene)-
type diterpene obtained from the heartwood of Spirostachys
africana Sond. (syn. Excoecaria africana Muell. Arg.).

It will be shown that the three new diterpenes
present in Cleistanthus schlechteri are 3α-hydroxy-ent-
isopimara-8(14),15-diene, I, 3α-hydroxy-ent-isopimara-
8(14),15-dien-12-one, II, and 3α,12α-dihydroxy-ent-iso-
pimara-8(14),15-diene, III, congeners of the major constituent
cleistanthol, IV, whose structure was recently elucidated
(1,2,3).

The heartwood of Spirostachys africana has been
the subject of a number of investigations. Earlier studies
(4,5) revealed that the heartwood contained an oleoresin,
but it was only in 1962 that three of the main diterpenoid
constituents were characterised as being the ketone, V,
named stachenone, the α-ketol, VI, and the diosphenol, VII
(6,7). During a renewed investigation of the light
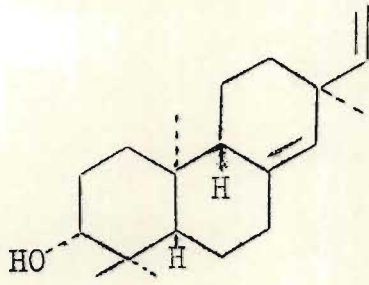
I
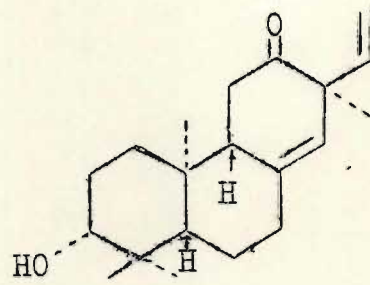
II

III
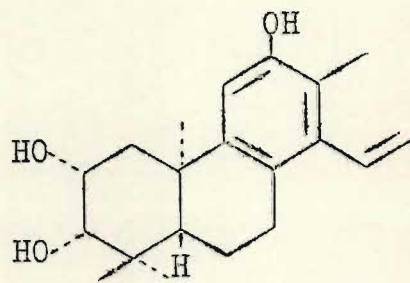
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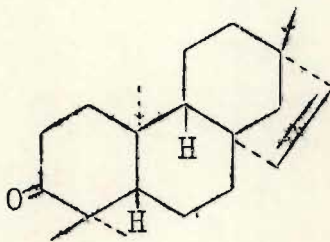
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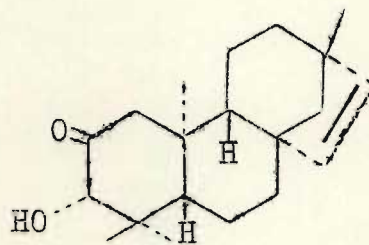
VI
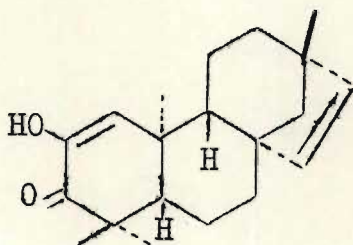
VII
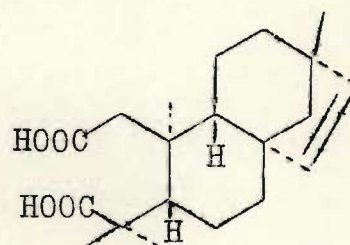
VIII

petroleum extracts of the heartwood a new diterpenoid,
named spirostachic acid was isolated and evidence leading
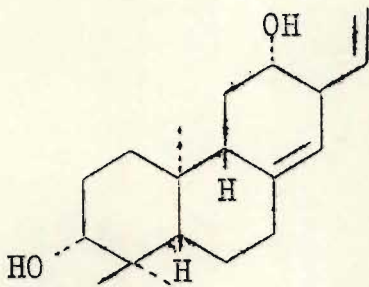to its formulation as VIII will be presented.

THE DITERPENOIDS

It is only in the past two decades with the
advent of chromatographic and physical methods allowing for
the purification and characterisation of naturally occurring
compounds that outstanding advances have been made in the
field of diterpenoid chemistry. Expansion has been so
rapid that it is necessary to provide a background to the
progress which has taken place, although many reviews have
been written on the subject (8,9,10,11,12,13). Particular
attention will be paid to diterpenoids isolated from the
Euphorbiaceae family and the results of their investigation
will be discussed in detail.

The diterpenoids comprise a widespread group of
compounds which are found mainly in plant resins. They are
derived essentially from the union of four isoprene units
to yield compounds containing twenty carbon atoms, thereby
satisfying the Biogenetic Isoprene Rule (14).

A number of basic polycyclic skeletons are now
recognised, but only a relevant few are illustrated (Figure
1) showing the nomenclature and numbering system employed.
The numbering of carbon atoms is based on that of the
steroid convention for rings A and B and not on the

FIGURE 1. SOME BASIC DITERPENOID SKELETONS
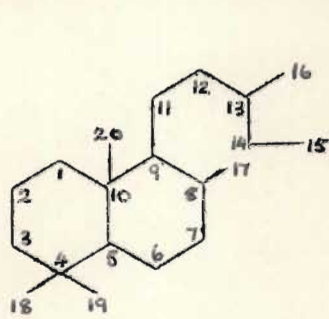
(a) Labdane
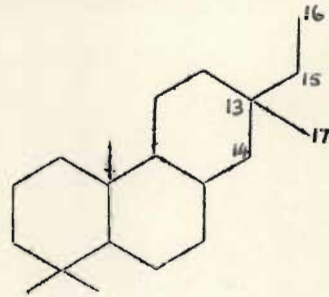
(b) Pimarane
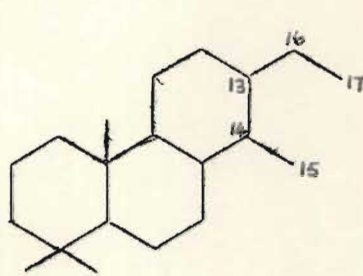
(c) Cassane
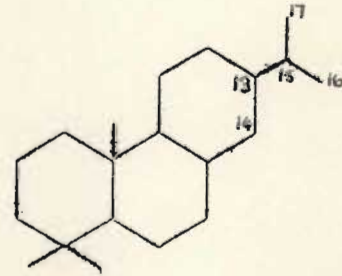
(d) Abietane
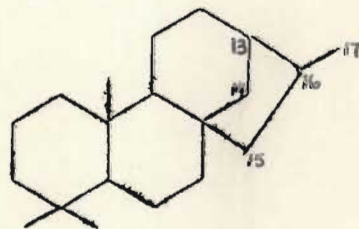
(e) Kaurane
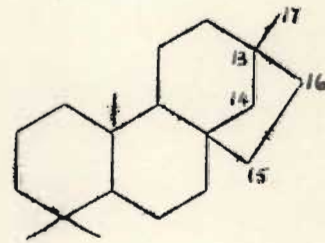
(f) Beyerane or Stachane
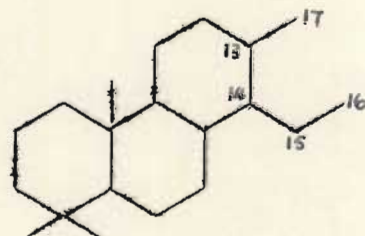
(g) Cleistanthane
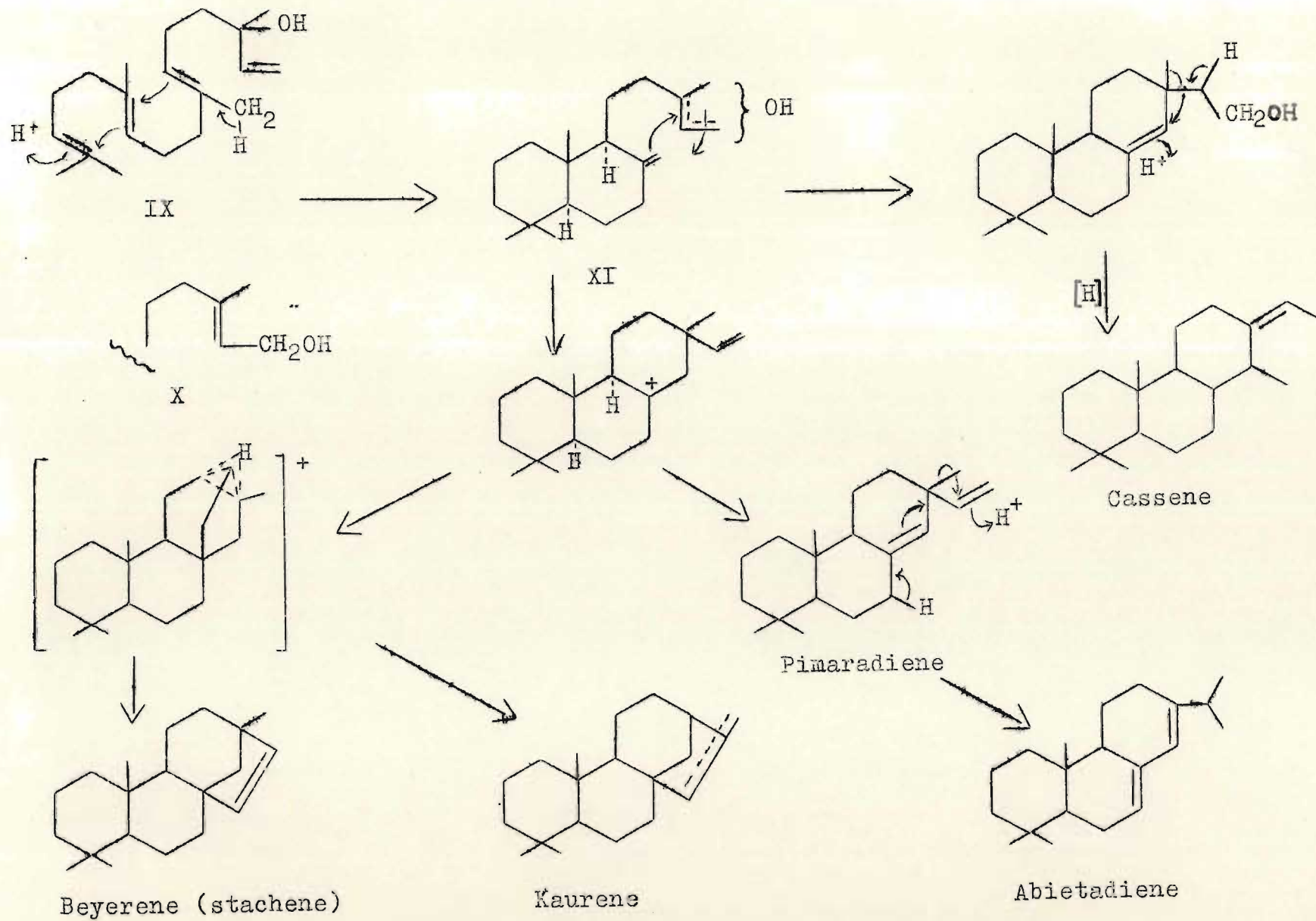
FIGURE 2   BIOGENESIS OF SOME   DITERPENOIDS

IX

X

XI

Cassene

Beyerene (stachene)

Kaurene

Pimaradiene

Abietadiene

phenanthrene skeleton as was previously the case (13).
The C-4 equatorial substituent is numbered 18 and the C-4
axial substituent, 19.

All di-, tri-, tetra- and pentacarbocyclic
diterpenoids may be derived in principle from geranyl-
geraniol, IX, or geranyl-linalool, X, via the intermediate
bicyclic alcohol, XI, of the labdane type. Hence the parent
skeletons shown in Figure 1 are readily synthesised through
a common biogenetic pathway as outlined in Figure 2. A
possible biosynthetic sequence leading to the new
cleistanthane skeleton will be discussed at the conclu-
sion of the section on Cleistanthus schlechteri.

DITERPENOIDS   OF   THE   LABDANE   TYPE

The evidence concerning the structures of manool,
XII, sclareol, XIII, and manoyl oxide, XIV, all of which
have the normal labdane skeleton, has been adequately
summarised (15,16,17).

Although there had been a large degree of uncer-
tainty over the absolute stereochemistry at C-13 in manool,
XII, and sclareol, XIII, direct configurational correlation
was achieved (18) by the conversion of sclareol into the
lactone of γ-hydroxy-γ-methylhexanoic acid, XV, which could
also be derived from R(-)-linalool, XVI.

The stereochemistry of manoyl oxide, XIV, at C-8
was confirmed by its hydrogenolysis to 8-hydroxy-labd-13-

ene, XVII, (19) while the assignment at C-13 was based on
the comparison of fragmentation patterns in the mass spectra
of manoyl oxide and its 13-epi compound, XVIII.

XII

XIII

XIV

XV

XVI

XVII

XVIII
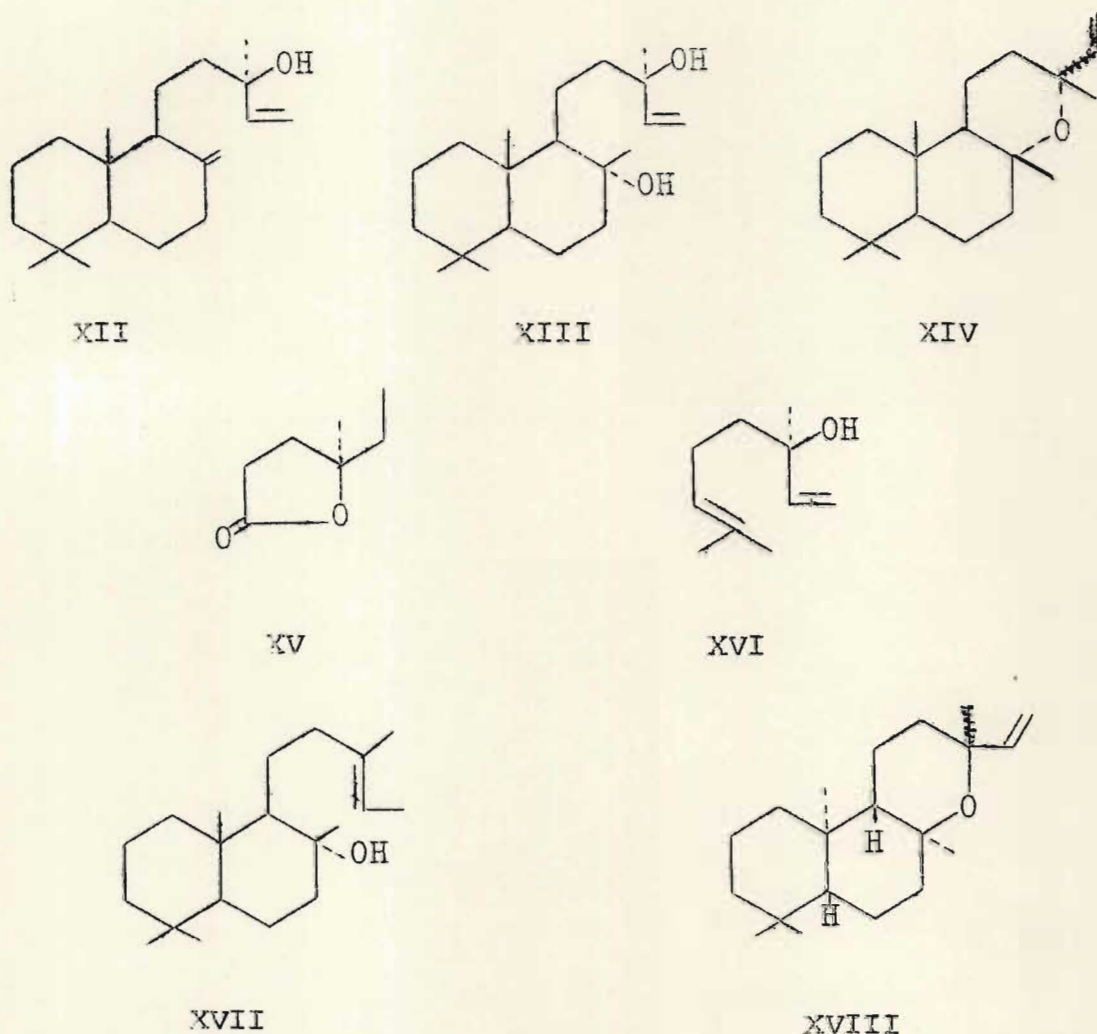
The occurrence of similar compounds has been
reported in some species of Euphorbiaceae. During the
investigation of Ricinocarpus muricatus (20,21) a number of
bicyclic diterpenes of the labdane type were isolated and
found to be related to eperuic acid, XIX, which was shown
to be antipodal to labd-8(20)-en-15-oic acid, XX, at all
its asymmetric centres except C-13 (22). The diterpenes

characterised were eperuane-8β,15-diol, XXI, eperuane-8β, 15,18-triol, XXII, 15,16-dihydroxyeperu-8(20)-en-18-oic acid, XXIII, eperu-8(20)-en-15,18-dioic acid, XXIV, 15-hydroxyeperu-8(20)-en-18-oic acid, XXV, and the $\Delta^{\alpha\beta}$-butenolide, XXVI.

The resin acids of Ricinocarpus stylosus were reported to contain the bicarbocyclic polyalthic acid, XXVII, (23,24,25) besides four new tetracyclic diterpenes which are discussed in a later section.

From a new species of Beyeria a further four bicarbocyclic diterpenes were isolated and their structures formulated as the diol, XXVIII, the alcohol, XXIX, the acid, XXX, and the known 13 epi-(-)-manoyl oxide, XVIII (26).
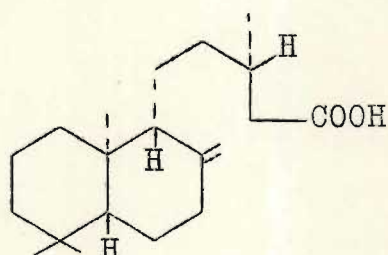
XIX
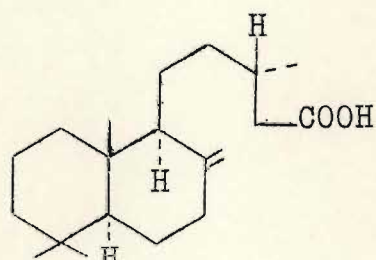
XX
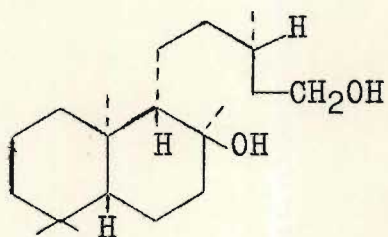
XXI
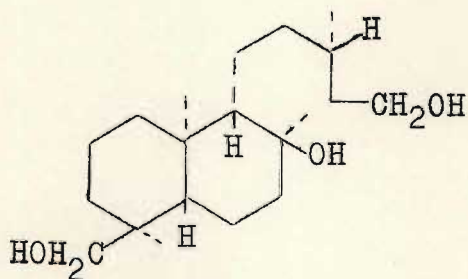
XXII
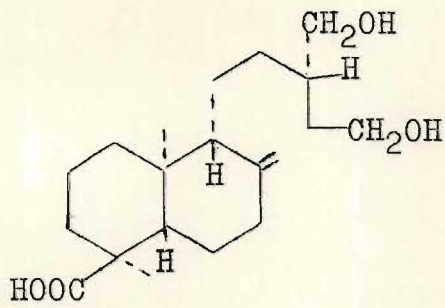
XXIII
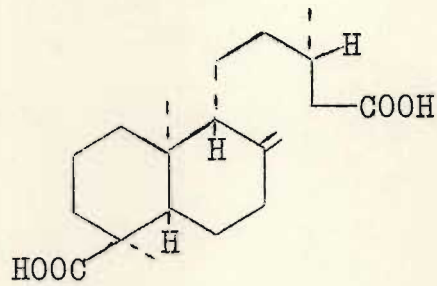
XXIV
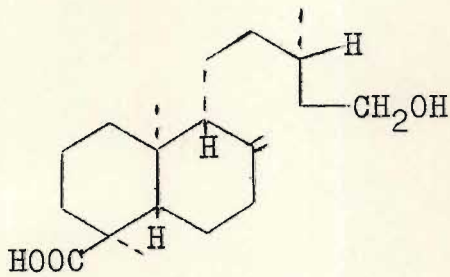
XXV
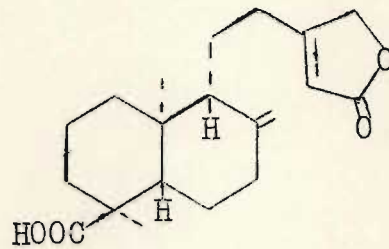
XXVI
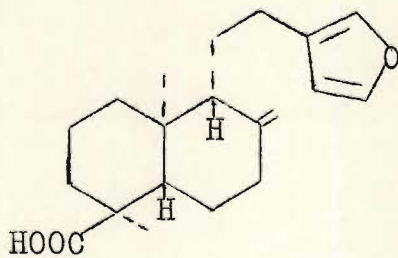
XXVII
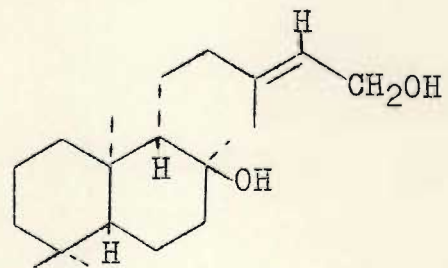
XXVIII
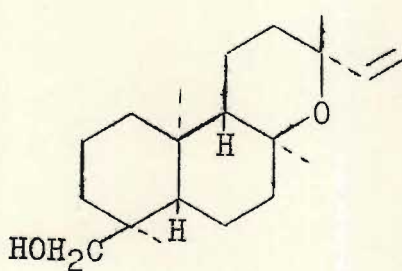
XXIX
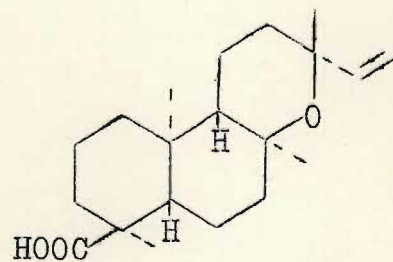
XXX

An epoxy-furanoid diterpene obtained from the
bark of Croton eleuteria (Cascarilla bark) was called
cascarillin A and for which the structure XXXI was proposed
(27). X-Ray analysis was performed on deacetylcascarillin
acetal iodoacetate and this allowed its structure and abso-
lute stereochemistry to be established as shown in XXXII
(28). From these investigations the previous structure
proposed for cascarillin (29), the bitter principle of
Cascarilla bark was revised to XXXIII.
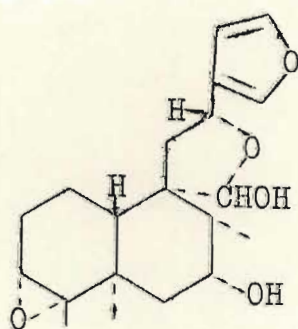
XXXI
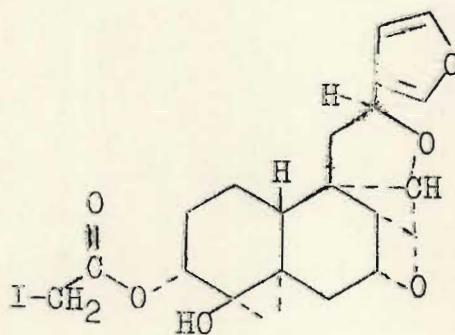
XXXII
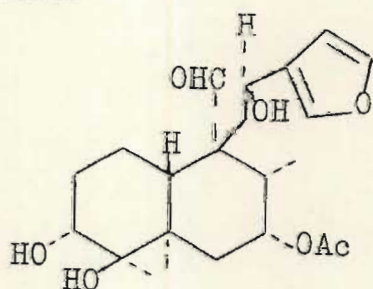
XXXIII

DITERPENOIDS OF THE PIMARANE TYPE

In order to eradicate the confusion caused by
the use of a variety of common names for some of the well-

known resin acids e.g. sandaracopimaric and isopimaric

acids, a system of nomenclature has been proposed in which

the basic skeletons are pimarane, XXXIV (a), and isopimarane,

XXXIV (b), which differ only in their stereochemistry at

C-13. This system will be adhered to throughout this thesis

to maintain uniformity and promote a better understanding

of the compounds under discussion.
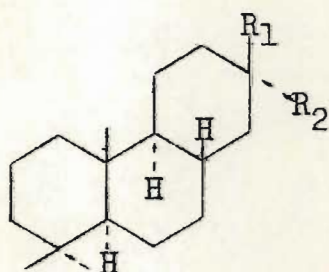
	R_1	R_2
XXXIV (a)	Et	Me
(b)	Me	Et

The resin acids - pimara-8(14),15-dien-19-oic

acid, XXXV, (pimaric acid), isopimara-8(14),15-dien-19-oic

acid, XXXVI, (sandaracopimaric acid) and isopimara-7,15-

dien-19-oic acid, XXXVII, (isopimaric acid) - have been the

subject of many investigations since the isolation of both

XXXV and XXXVII from the oleoresin acids of Pinus palustris

(30). They were shown to possess the same configurations

at three of the five asymmetric centres (31) whilst the

stereochemistry at C-9 and C-13 was left open to speculation.

By an acid-catalysed lactonisation of the 15,16-

dihydro derivatives of these acids (32) the asymmetry at

C-9 was destroyed and an infrared study of the products

revealed a small difference between them. This was taken

to indicate that the two acids therefore differed in their stereochemistry at C-13. Furthermore, after examining the yield ratios of the equilibrated lactones it was suggested that XXXV had its bulkier substituent axially orientated i.e. the 13β-configuration (32).

XXXV   XXXVI   XXXVII
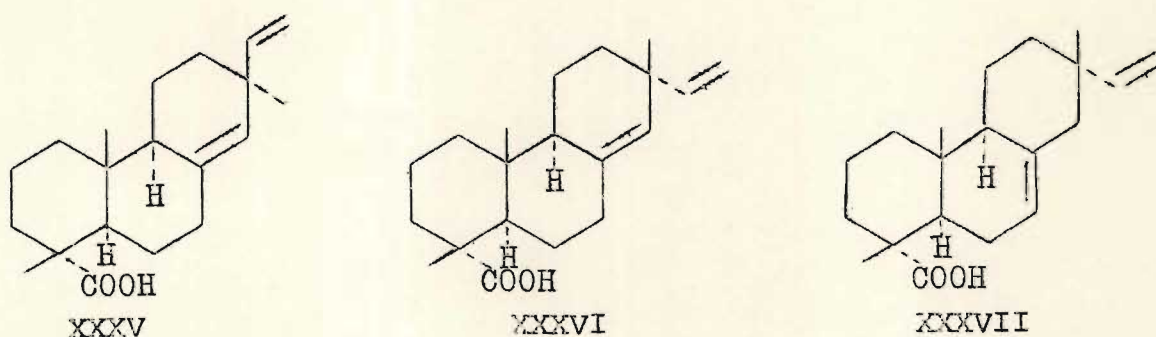
Independent researchers (33) confirmed the epimeric relationship at C-13 in both the resin acids, XXXV and XXXVII, while other investigators (34,35,36,37,38,39,40) favoured the idea that the compounds were also epimeric at C-9.

Edwards et al (41,42) then established by chemical correlation and optical rotatory dispersion data that the acid, XXXVI, was a C-13 epimer of XXXV. Furthermore, the synthesis of pimara-8(14),15-diene, XXXVIII, and iso-pimara-8(14),15-diene, XXXIX, and their comparison by means of gas-liquid chromatography with dienes derived from the corresponding acids substantiated the structures, XXXV and XXXVI, proposed for the acids (43,44).

Since the acids, XXXVI and XXXVII, were both

epimeric with XXXV at C-13, but not identical to each other, the difference was ascribed to their configurations at C-9. However, it was shown that XXXVII conformed to the expected trans-anti-trans system of fusion by its conversion into 13,13-dimethylpodocarpane, XL (45), and thus possessed a C-9 α-hydrogen. This suggested that XXXVII was not a C-9 epimer, but rather a double bond isomer of XXXVI.

XXXVIII   XXXIX   XL
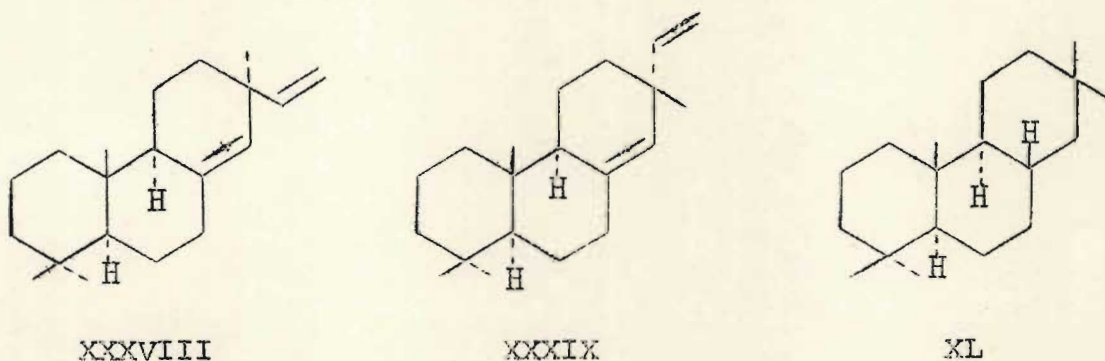
The stereospecific syntheses of Ireland et al (44,46,47) determined beyond doubt the C-7, C-8 position of the double bond in XXXVII and also defined the stereo-chemistry at C-13 in all three acids. They served to verify the conclusions put forward earlier (32) that pimara-8(14),15-dien-19-oic acid, XXXV, possesses 13α-methyl and 13β-vinyl substituents, and hence both XXXVI and XXXVII have the antipodal location of these groups.

Further chemical and optical rotatory dispersion evidence obtained by Edwards et al (48) also confirmed that isopimara-7,15-dien-19-oic acid could only be represented by structure XXXVII.

Investigations performed on the hydrogenation products of the pimaric acids indicated that the fully

reduced derivatives posed another interesting stereo-
chemical problem, this time at C-8. The relevant compounds
are pimaran-19-oic acid, XLI (a), and isopimaran-19-oic acid,
XLI (b).

		R_1	R_2
XLI	(a)	Et	Me
	(b)	Me	Et
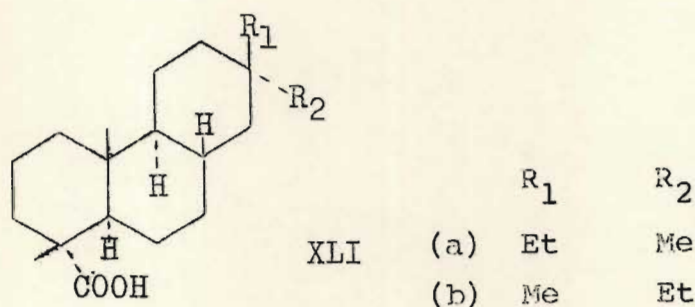
It had been discovered that during the hydro-
genation of XXXV the nuclear double bond underwent
migration to the $\Delta^{8,9}$ position before being fully reduced
over a platinum oxide catalyst in glacial acetic acid
medium (33). On the basis of the commonly accepted concept
that addition of hydrogen occurs from the less hindered
side of an unsaturated molecule in a cis fashion the
general prediction was that the tetrahydro derivatives would
have trans-anti-cis fused backbones i.e. the hydrogen at
C-8 would be α-orientated. This configuration was in fact
assigned to a tetrahydro pimaric acid produced on hydro-
genation under pressure at an elevated temperature (49) and
which possessed a melting point lower than that obtained by
hydrogenation under normal conditions. Other workers (50,
51) have shown the higher melting derivative to possess
the more stable trans-anti-trans configuration in which the

C-8 hydrogen would be β-orientated. The latter assignment was supported by n.m.r. evidence.

A similar chemical investigation performed on XXXVI and XXXVII caused the conclusion to be drawn that all the tetrahydro pimaric acids which had been obtained under standard conditions of hydrogenation possessed trans-anti-trans fused skeletons.

It had been proposed (52) that the hydrocarbon rimuene could be represented by the same structure as iso-pimara-8(14),15-diene, XXXIX, but this theory was shown to be erroneous by comparison of the two compounds (43). By means of chemical, spectrographic and synthetic research it has been conclusively proved that rimuene is not a $\Delta^{8(14),15}$-pimaradiene, but has the structure and stereo-chemistry as shown in XLII (53,54,55).

The partial synthesis of rimuane, XLIII, was instrumental in determining the absolute stereochemistry at every asymmetric centre, and hence in rimuene as well (56,57).
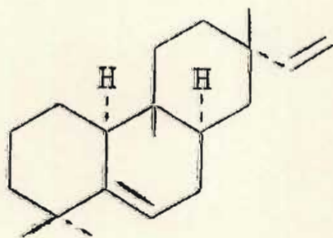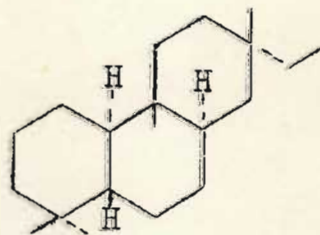
XLII   XLIII

A recent survey of the literature has revealed that only a few diterpenoids of the pimarane type have been isolated from the Euphorbiaceae.

From a species endemic to Western Australia, Beyeria brevifolia, a new acid was obtained and characterised as (15S)-15,16-dihydroxy-3,4-seco-ent-pimara-4(18),7-dien-3-oic acid, XLIV, (58). Its structure was assigned on the basis of extensive n.m.r. data and the conversion of the acid into 3,4-seco-beyerane, XLV, which was partially synthesised. The seco-acid was the first example of a bi-carbocyclic diterpenoid to be found in any species of Beyeria, which are better known for their tetracyclic diterpenoid constituents.

Three new diterpenes have been isolated from Croton oblongifolius L, an Indian species of Euphorbiaceae. The most recently reported (59a) compound, oblongifolic acid, has been formulated as (+)isopimara-7,15-dien-19-oic acid, XLVI (a), while the earlier congeners named oblongifoliol and deoxyoblongifoliol (59b,59c) are now considered to be structures, XLVI (b) and XLVI (c) respectively, rather than the C-8(14) double bond and C-13α-vinyl isomers previously postulated.

XLIV

XLV
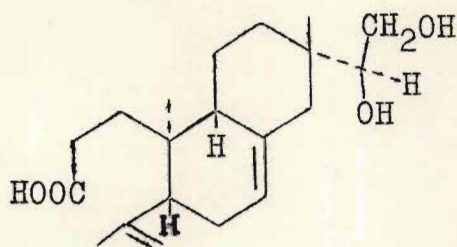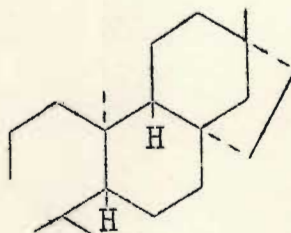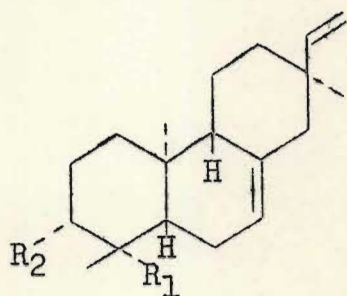
	R_1	R_2
(a)	COOH	H
(b)	CH_2OH	OH
(c)	CH_3	OH

XLVI

DITERPENOIDS   OF   THE   KAURANE   AND   BEYERANE   TYPES

It is generally accepted that tetracyclic di-
terpenoids arise by cyclisation of members of the pimarane
series (60). In particular cyclisation of pimara-8(14),15-
diene, XXXVIII, may proceed via a carbonium ion intermedi-
ate illustrated in the scheme below, to produce either of
two similar diterpenoid skeletons viz. hibaene, XLVII
and kaurene, XLVIII (61,62).

XXXVIII

XLVII   XLVIII
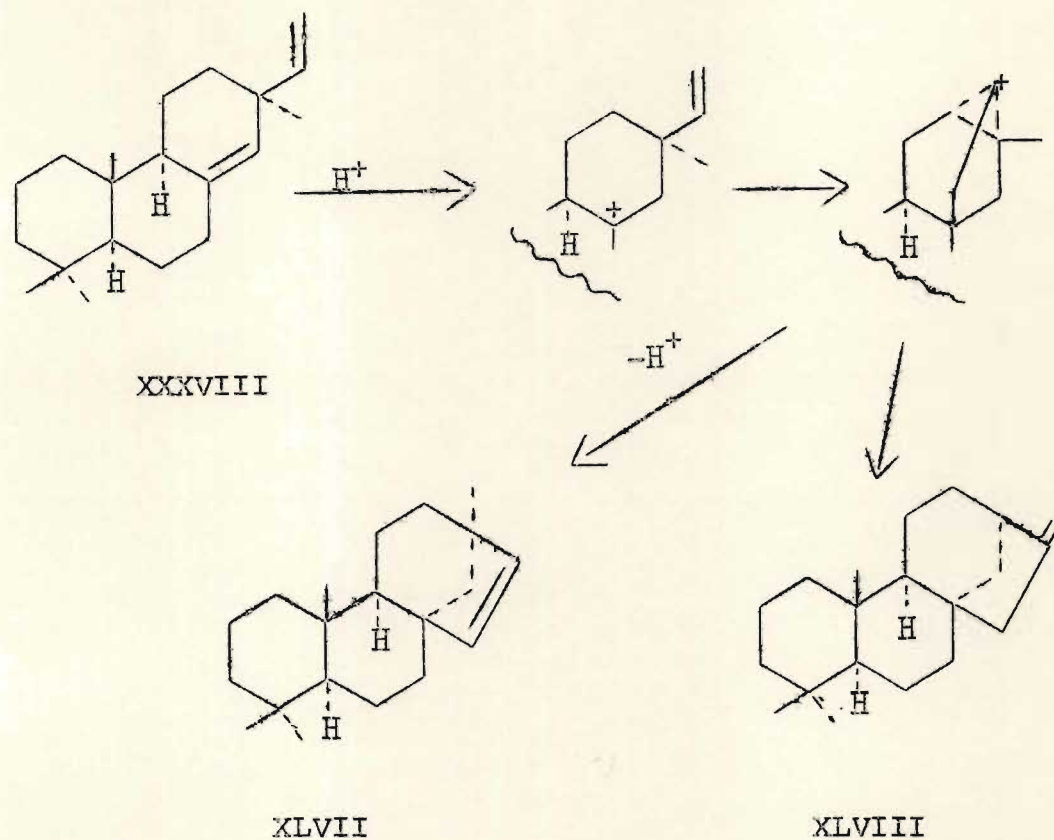
The structure proposed (63) for hibaene was con-
firmed by its total synthesis performed by Ireland _et_ _al_
(64). The relationship between hibaene and kaurene
prompted an investigation into the possibility of their
chemical interconversion. First attempts involved the
conversion of isopimara-7,15-diene, XLIX, into isohibaene,
L, by means of hydroboration and oxidation-reduction reac-
tions. A consideration of steric requirements and n.m.r.
studies of the synthetic intermediates established that
isohibaene, L, was indeed the C-8, C-13 epimer of hibaene,
XLVII (62).
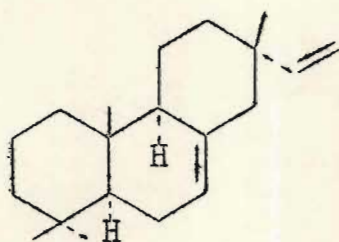
XLIX
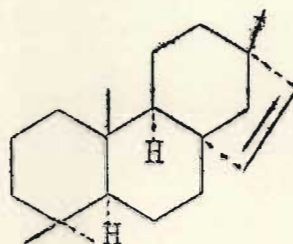
L

At this stage the presence of two hibaene-like diterpenes were reported in two species of Euphorbiaceae. A ketone called stachenone was isolated from the oleoresin of *Spirostachys* *africana* Sond. (7) and assigned the structure V, but no distinction was specified between the possible C-9 and C-8, C-13 isomers. A close study of the n.m.r. data and the chemical conversion of stachenone, V, to the hydrocarbon beyerene (stachene), LI, proved this compound to be enantiomeric with hibaene, XLVII (62).

Horn *et* *al* also reported (7) the presence of two more diterpenoids possessing the same hydrocarbon skeleton and formulated their structures as VI and VII.
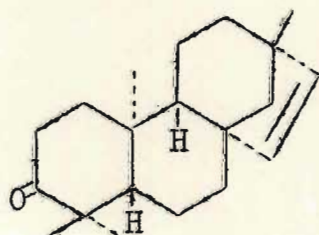
V
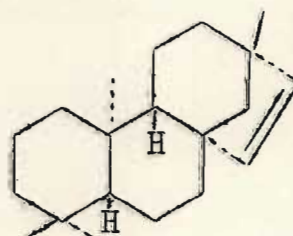
LI
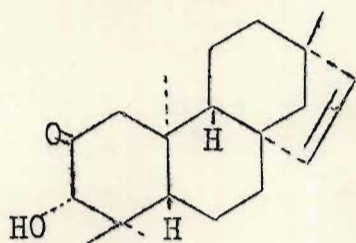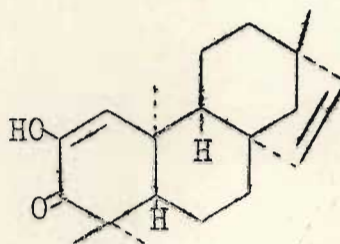
VI   VII

An Australian Euphorbiaceae, <u>Beyeria</u> <u>leschenaultii</u>
(DC) Baill. var. drummondi (Muell. Arg.) was shown to con-
tain a diterpenoid mono-cinnamate as its major constituent
(65). Alkaline hydrolysis of the substance yielded a tri-
hydroxy diterpene named beyerol, LII, whose structure and
stereochemistry was elucidated by Jefferies <u>et</u> <u>al</u> (66).
Thus the names stachene and beyerene refer to the same
hydrocarbon skeleton, LI.
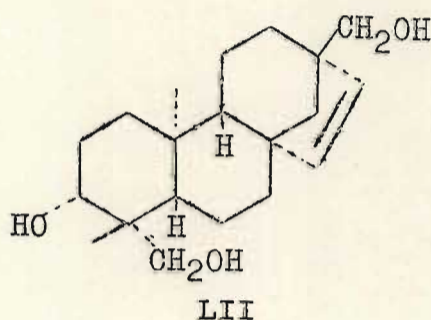
LII

Subsequently, a systematic survey of <u>Beyeria</u> and
other allied Australian genera was conducted. Ether
extraction of the leaves of a shrub tentatively identified
as <u>Beyeria</u> <u>brevifolia</u> (Muell. Arg.) Benth. afforded an
hydroxy keto-acid, an acetoxy-keto-acid and a triol. These
natural products were shown to be diterpenes possessing,

in contrast to beyerol, the 16α-(-)-kaurane [(-)-β-dihydro-kaurene] skeleton, LIII (62,65,66). By means of selective manipulation of the three oxygenated functions evidence was accumulated which allowed the structures and stereochemistry of the above-mentioned diterpenes to be formulated as LIV (a), LIV (b) and LV respectively (67).

Further examination of ether-soluble neutral compounds isolated from Beyeria leschenaultii (68) revealed the presence of the known triol, LV, together with a new diterpene shown by its relationship with the triol to be (-)-kaur-16-en-3α,19-diol, LVI.

Concurrent with the work on Beyeria species the hard resin coating of Ricinocarpus stylosus Diels was examined (23,24,25,69). Besides the bicyclic polyalthic acid, XXVII, four new kauranoid diterpenes were isolated. They were identified as 16α-(-)-kauran-17,19-dioic acid, LVII (a), 19-hydroxy-16α-(-)-kauran-17-oic acid, LVII (b), (-)-kauran-16α,17,19-triol, LVIII and (-)-kaur-16-en-19-oic acid, LIX.

Further results obtained on investigation of another species of Beyeria (70) disclosed the presence of a number of bicarbocyclic diterpenoids mentioned earlier and some tetracarbocyclic constituents. Two of the latter had been encountered in Ricinocarpus stylosus (69) and identified as LVIII and LIX, while the third compound, LV, had previously been reported from two species of Beyeria (67,68). The three new kaurane derivatives were formulated
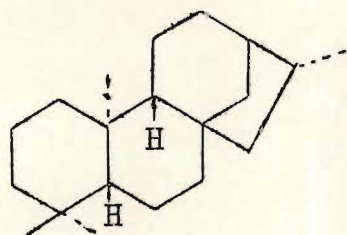
LIII
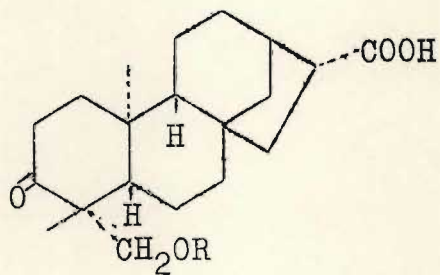
LIV   (a)   R = H

          (b)   R = $COCH_3$
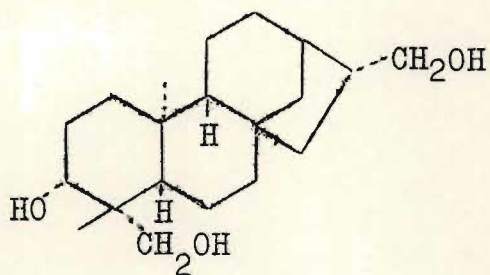
LV
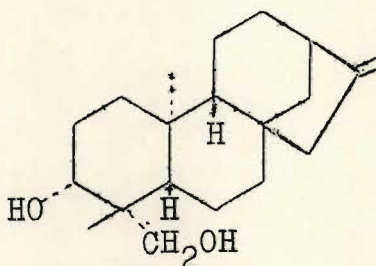
LVI
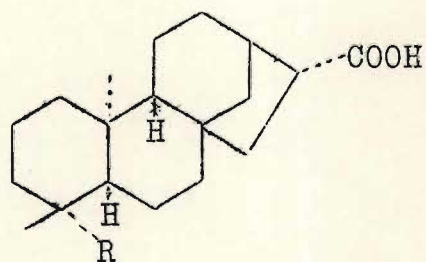
LVII   (a)   R = COOH

        (b)   R = CH_2OH
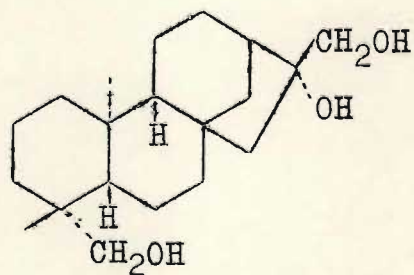
LVIII
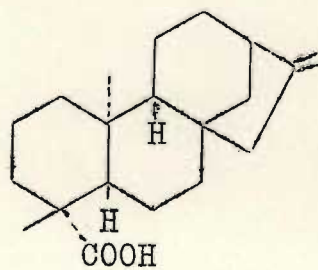
LIX

as the diol, LX (a), the hydroxy acid, LX (b) and the di-
hydroxy acid, LXI.

During the continued investigation of the acidic
components present in Ricinocarpus stylosus Diels two
further (-)-kaurane derivatives were identified (71).
One was the known 16,17-dihydroxy-16-(-)-kauran-19-oic acid,
LXI, first isolated from a Beyeria sp. nov. (70). The un-
known acid proved to be the first example of a 1α-hydroxy-
(-)-kaurane derivative (72) and an examination of the
chemical and n.m.r. data indicated that the compound was
in fact 1α,19-dihydroxy-16α-(-)-kauran-17-oic acid, LXII.

The acidic fraction of the ether extract of a
Beyeria sp. nov. on silicic acid chromatography yielded a
secobeyerene (73) which was characterised as 17-hydroxy-3,4-
secobeyer-4(18),15-diene-3-oic acid, LXIII, This was the
first reported natural occurrence of the 3-carboxy-3,4-
seco system in the diterpene series.

Yet another three diterpenes were isolated from
the leaf resin of a different variety of Beyeria
leschenaultii (74). Two of the substances were new and
possessed the beyer-15-ene skeleton while the third was the
previously reported 3,4-secobeyerene, LXIII. The major
constituent was a keto acetoxy alcohol whose structure
was elucidated as LXIV, while the remaining diterpene was
formulated as LXV.
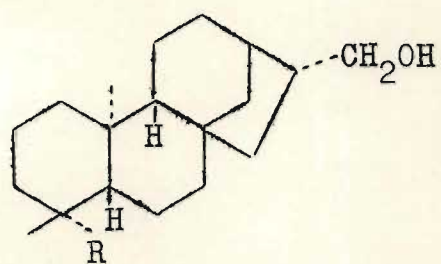
LX   (a)   R = CH$_2$OH
      (b)   R = COOH
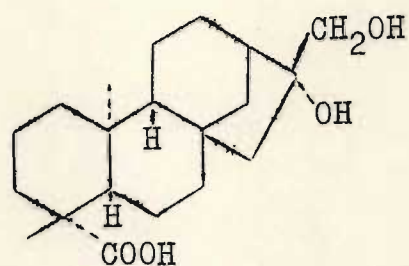
LXI
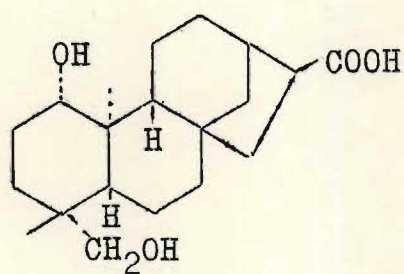
LXII
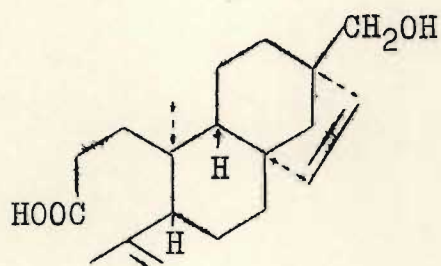
LXIII
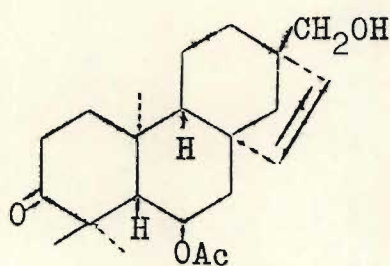
LXIV
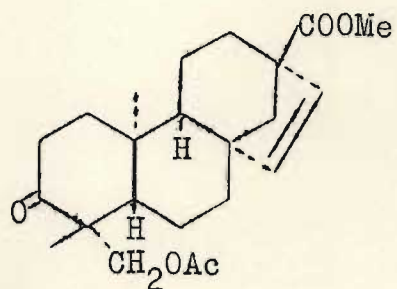
LXV

Beyeria latifolia, a species which abounds in the
southerly districts of Western Australia was found to con-
tain three new diterpenes of the (-)-kaurane type (75).
Their structures were established as 16β-(-)-kaurane-3α,16,
17,19-tetrol, LXVI, 16β-(-)-kaurane-3α,16,19-triol, LXVII
and 16β-(-)-kaurane-3α,16,17-triol, LXVIII, the latter two
being isolated as their acetates.

The diterpenes LXVI and LXVIII were also found to
occur in Beyeria leschenaultii together with the known
kaurane derivatives, LVII (b) (69) and LX (b) (70). The
structure of a new diterpene which had been isolated as its
methyl ester was deduced to be 12β,17-dihydroxy-16α-(-)-kauran-
19-oic acid, LXIX (76).

Examination of the leaf resin of Beyeria brevi-
folia (Muell. Arg.) Benth. was continued (67) and yielded
the known diterpenes beyerol, LII (62,66) and 16,17-di-
hydroxy-16β-(-)-kauran-19-oic acid, LXI (70), besides a
new seco-ent-pimarene diterpenoid, XLIV, mentioned in·the
previous section.
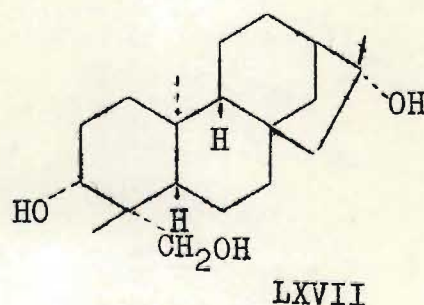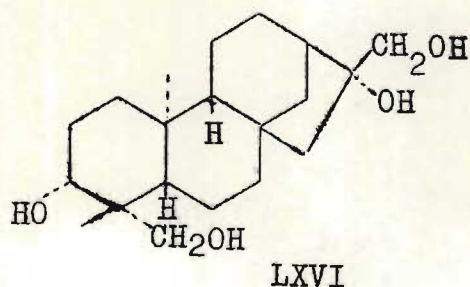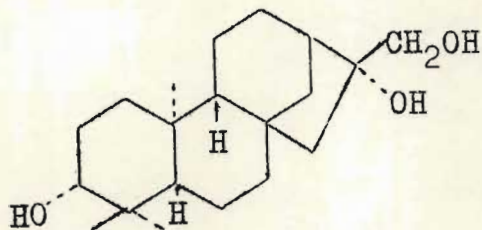
LXVI

LXVII
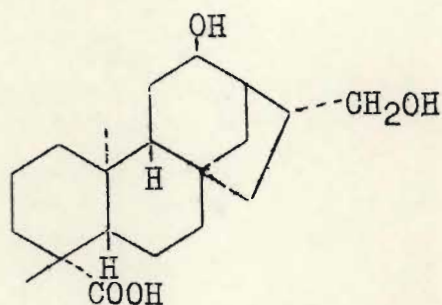
LXVIII   LXIX

The predominance of such a large number of di-
terpenoids of the (-)-kaurane series in particular, and of
the beyerene type in so many Australian species of
Euphorbiaceae prompted an attempt at the interconversion of
the two skeletons (77).   (-)-Kaurene, LXX, itself undergoes
partial isomerisation under acid conditions to give iso-
kaurene, LXXI, without any skeletal rearrangements taking
place (69,78), but 3α,19-ethylidenedioxy-beyer-15-ene, LXXII,
on acid treatment either failed to rearrange or resulted in
a complex mixture.

A chemical sequence was then devised in which
oxygen was introduced into the C-15 and C-16 positions by
means of hydroboration (66) of the beyerene, LXXII, and the
corresponding 17-hydroxy compound, LXXIII.   The ketones so
formed on lithium aluminium hydride reduction yielded the
15-exo isomeric alcohol, LXXIV (a) and the 15β,17-diol,
LXXV, respectively. Assignment of the configurations was
based on the close correspondence of molecular rotation
differences in the derivatives of both the 15-oxygenated
and the 15,17-dihydroxy compounds.

Reduction of the epoxide of the beyerene, LXXII, with lithium in ethylamine produced a mixture of isomeric alcohols, LXXIV (a) and LXXIV (b). Tosylation of the latter followed by treatment with sodium acetate and acetic acid yielded a hydrocarbon fraction containing the beyerene, LXXII, and the (-)-kaurene, LXXVI. This fraction was successfully chromatographed to furnish 3α,19-ethylidene-dioxy-(-)-kaur-15-ene, LXXVI thereby proving possible the interconversion of the beyerene and (-)-kaur-15-ene skeletons.

LXX   LXXI
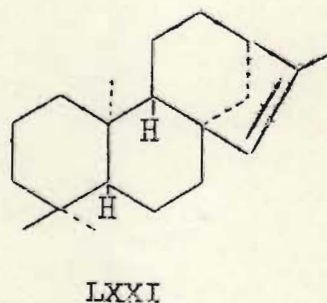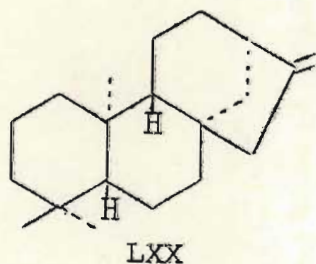
The frequent occurrence of kaurane-type diter-penoids found in many Australian species of Euphorbiaceae overshadows the number of beyerane-type diterpenes which have been isolated from the same family. Since it has been shown that a chemical conversion from beyerene to kaurene is possible it seems feasible that such a pathway is followed biosynthetically in the plant. In such an event it is possible that diterpenoids of the (-)-kaurane series constitute one of the major end products of biogenesis.

In contrast investigations performed on a
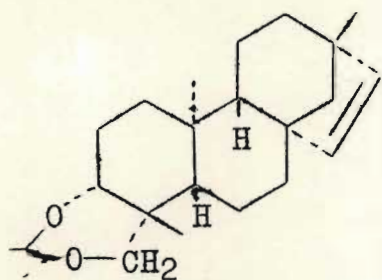
LXXII
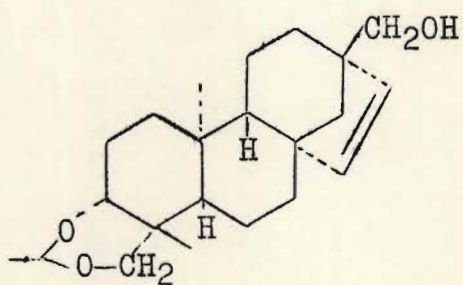
LXXIII
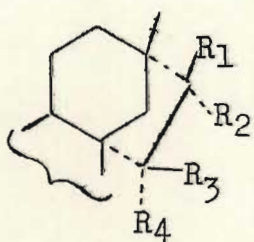
LXXIV

	R_1	R_2	R_3	R_4
(a)	H	H	OH	H
(b)	OH	H	H	H
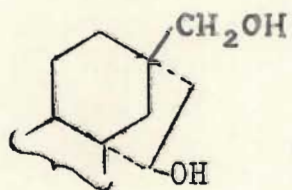
LXXV
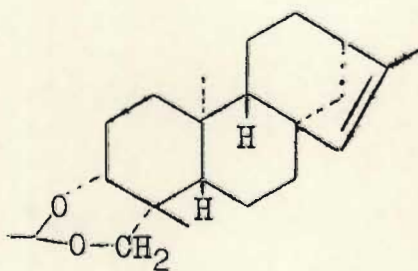
LXXVI

limited number of South African Euphorbiaceae have provided
little correlation of hydrocarbon skeletons, and further-
more no kauranoid diterpenes have been encountered. The
stachene type compounds isolated from Spirostachys
africana are, in fact, diterpenoids with the beyerene
skeleton, but the pimarenoids to be discussed have not pre-
viously been reported in any other Euphorbiaceae.

OXYGENATION PATTERNS

As is commonly found in triterpenoid and steroid
series an increasing number of diterpenoids have been re-
ported to possess oxygen functions at C-3. This is espec-
ially true in the case of diterpenes isolated from the
Euphorbiaceae.

The degree of oxidation at this position ranges
from a hydroxyl group which is predominantly present in the
d-configuration as in beyerol, LII, to a keto grouping as
in the kauranoid ketone, LIV (a) and to an acid as in the
seco-beyerene, LXIII.

However, only one example of oxygenation at the
C-1 position has been reported, viz. the kaurenoid, LXII,
while the ketol, II, is but the second example of oxygen-
ation at C-12 in alicyclic diterpenes, the first being
another kauranoid, LXIX. Oxygenation often occurs at the
C-19 position usually in the form of a secondary hydroxyl
or acid grouping, but it appears that oxygen functions at

C-7, C-11 or C-14 have yet to be encountered in the Euphorbiaceae diterpenes.

STEREOCHEMISTRY OF THE A/B RING JUNCTION

A characteristic feature of all the Euphorbiaceae diterpenoids is that they possess A/B <u>trans</u> ring fusions in which the C-5 hydrogen is in the β-configuration and the C-10 methyl group in the α-configuration.

This conforms to the usual A/B <u>trans</u> fused rings found in the triterpenoid series with the conspicuous difference that all the triterpenes isolated from this family possess the opposite but "normal" $5\alpha,10\beta$-stereochemistry common to naturally-occurring triterpenoids.

PRESENT   INVESTIGATIONS

PART 1.   THE   DITERPENES   OF   CLEISTANTHUS   SCHLECHTERI

Cleistanthus schlechteri (PAX) HUTCH. variety
schlechteri (family Euphorbiaceae) is a small tree which
grows to a height of about thirty feet.   It is found grow-
ing in the coastal forests of Zululand and extends north-
wards through East Africa to Kenya (79).   The tree has a
characteristic grey, longitudinally grooved bark and
possesses a light yellow sapwood with a clearly defined,
attractive tan-coloured heartwood.

Studies were confined to the heartwood which
appeared to provide the highest yields of constituents for
chemical investigation.   Extracts were obtained by soaking
the milled, air-dried wood overnight at room temperature in
light petroleum.   These extracts, after concentration, were
combined and furnished a viscous orange syrup from which
the three major components were separated as crystalline
products by means of column chromatography.   A thin layer
chromatogram revealed that these compounds were all less
polar than the known diterpene cleistanthol, IV, previously
isolated from the hot light petroleum extract.

1.1   3α-Hydroxy-ent-isopimara-8(14),15-diene, I.

The least polar (and least abundant) of the three

compounds isolated has been identified as 3α-hydroxy-ent-
isopimara-8(14),15-diene, I.

Preliminary colour reactions gave very little
indication of the class of compound under investigation.
No colour was produced under the conditions for the
Liebermann-Burchard test for triterpenes (80) and no detec-
table colour change was observed on the addition of a
neutral ferric chloride solution to a methanolic solution
of the substance, thereby suggesting the absence of phenolic
or enolic hydroxy groups. The characteristic yellow
colouration which developed when a few crystals were
treated with tetranitromethane was indicative of at least
one olefinic double bond (81).

The molecular formula, $C_{20}H_{32}O$ established by
elementary analysis and mass spectrometry indicated the
diterpenoid character of the compound, which was not sur-
prising in view of the previously reported isolation of
cleistanthol, IV, from the same source (1,2,3).

The Oxygen Function

The infrared spectrum of the diterpene, I,
(Figure 3) (in potassium bromide) displayed a broad hump
around 3325 $cm.^{-1}$ indicating the presence of an hydroxy
group. In general, the free O-H stretching frequencies of
alcohols in solution are found in the region between 3650-
3500 $cm.^{-1}$ (82). Thus the low frequency of absorption in

the spectrum of I implied some degree of intermolecular hydrogen bonding and polymerisation as expected for the solid state (82).

The other absorptions associated with alcohols are the O-H bending vibration, which usually occurs in the region from 1400-1300 cm^{-1} (83,84), and the C-OH stretching vibration which is generally found between 1205-1050 cm^{-1} (85). It has been shown that hydrogen bonding increases the frequency of the former absorption, and thus in the spectrum of I this band appeared at 1475 cm^{-1}. The latter absorption has been used to assign the configuration of hydroxy groups in the steroid and triterpenoid series (86, 87,88). However, as these bands occurred at 1045 and 1100 cm^{-1} it was not possible to determine the axial or equatorial configuration of the hydroxy group.

Acetylation of the diterpene, I, with acetic anhydride in pyridine afforded a monoacetate, LXXVII, which was shown by elementary analysis and mass spectrometry to have the formula $C_{22}H_{34}O_2$. The infrared spectrum of this acetate was similar to that of the parent alcohol except for the disappearance of the hydroxy bands and the appearance of an intense absorption at 1712 cm^{-1} due to the acetate carbonyl group and another at 1240 cm^{-1} arising from the ester C-O linkage.

This evidence established that the compound under investigation was a diterpene alcohol.
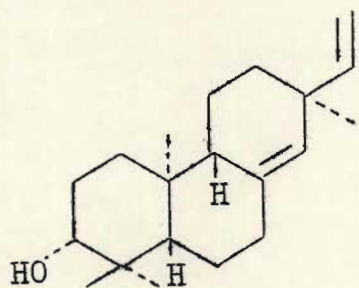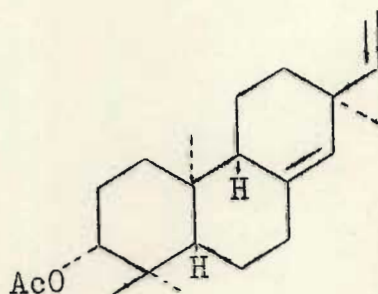
I   LXXVII

The Double Bonds

The positive tetranitromethane test had already
indicated the presence of at least one olefinic double
bond. A study of the C-H stretching region near 3000 $cm.^{-1}$
in the infrared spectrum of the alcohol, I, proved valuable
in determining the nature and degree of substitution of the
double bond(s). Investigations have shown (89) that stretch-
ing vibrations of compounds containing $RHC=CH_2$ groups are
found in the regions 3095-3075 and 3040-3010 $cm.^{-1}$. The
former band has been assigned to the terminal methylene
group and the latter to the =CHR group. A weak band at
3080 $cm.^{-1}$ in the spectrum (Figure 3) was consistent with
the presence of a terminal methylene group, but the lower
frequency band was not detected as it was probably obscur-
ed by the intense saturated C-H absorptions.

The second region associated with unsaturation
is that due to the C=C stretching vibration which is found
between 1680-1620 $cm.^{-1}$ (90). These bands are usually weak
but terminal double bonds absorb more strongly than those
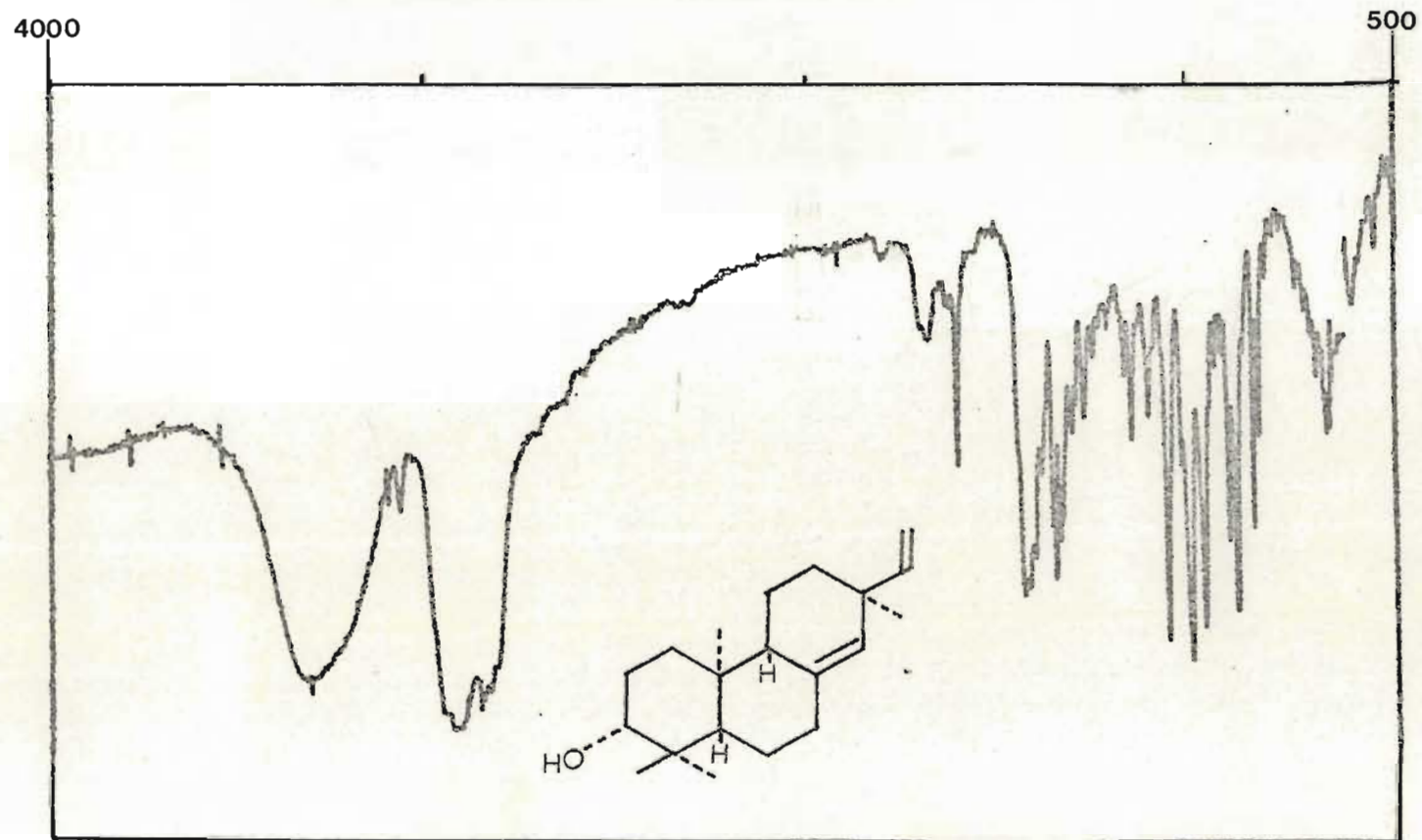
FIGURE 3   IR SPECTRUM OF 3α-HYDROXY-ent-ISOPIMARA-8(14),15-DIENE.
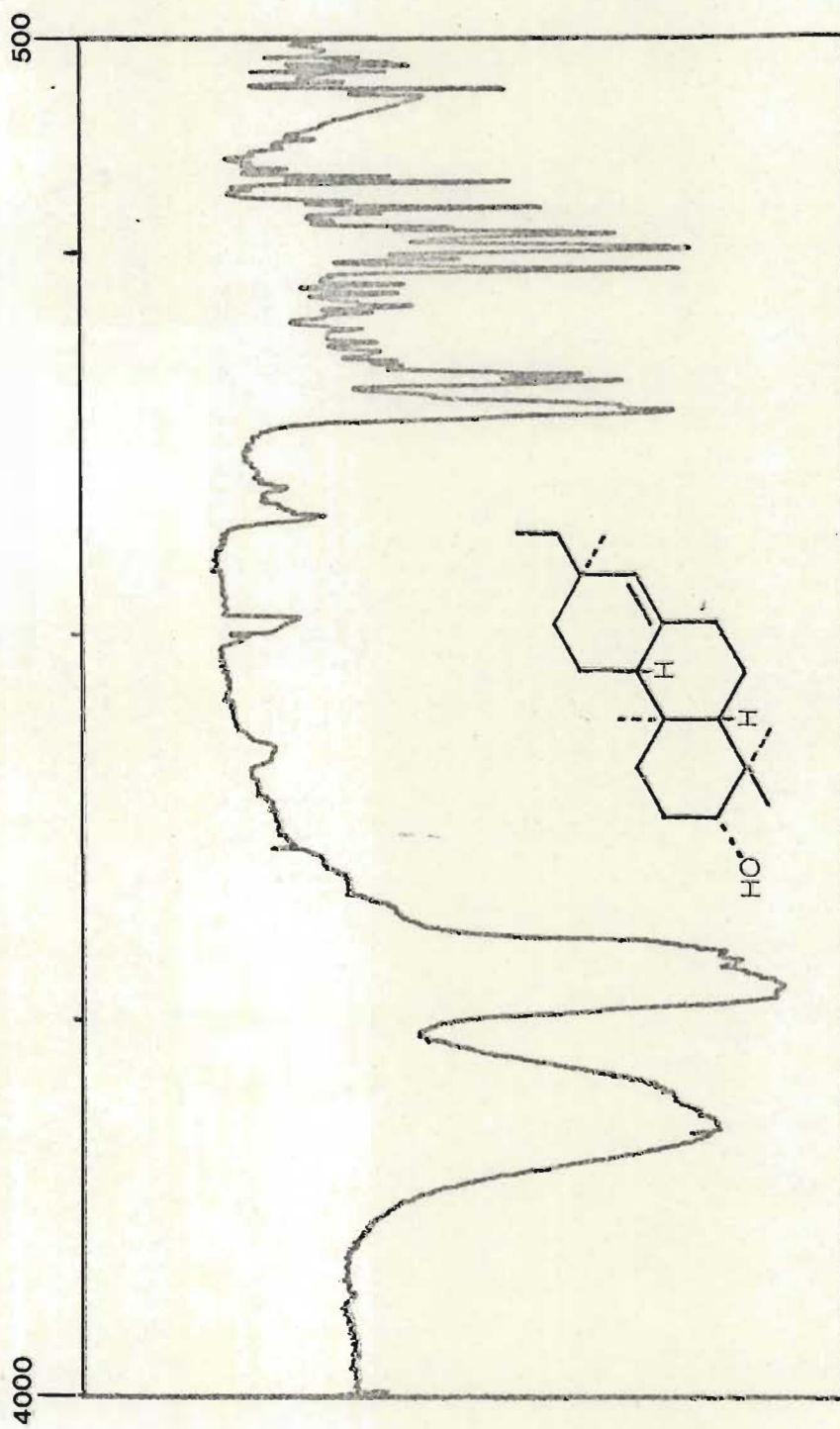
FIGURE 4   IR SPECTRUM OF 3α-HYDROXY-ent-ISOPIMAR-8(14)-ENE

within a ring. In the spectrum of I, a moderately strong band appeared at 1631 cm.$^{-1}$. The intensity of this band was consistent with a double bond being located outside a ring system.

In order to determine quantitatively the number of olefinic linkages in the alcohol, I, the compound was hydrogenated over a palladium on carbon catalyst in ethanolic medium at room temperature and atmospheric pressure. This resulted in the rapid uptake of one mole of hydrogen to give the 15,16-dihydro derivative, LXXVIII (a), whose molecular formula was shown to be $C_{20}H_{34}O$ by elementary analysis. In the infrared spectrum of this compound (Figure 4) the band at 3080 cm.$^{-1}$ was absent while the band at 1631 cm.$^{-1}$ underwent a marked decrease in intensity. This evidence implied that the terminal double bond had been reduced while a second double bond (probably endocyclic because of the low intensity of the band at 1631 cm.$^{-1}$) remained.

Acetylation of the dihydro derivative yielded the acetate $C_{22}H_{36}O_2$, LXXVIII (b) whose infrared spectrum was very similar to that of the parent acetate, LXXVII. It did confirm, however, the absence of the absorption at 3080 cm.$^{-1}$ but the band at 1631 cm.$^{-1}$ was obscured by the intense acetate carbonyl absorption.

Hydrogenation of the alcohol, I, over Adam's catalyst in glacial acetic acid resulted in the rapid uptake of one mole of hydrogen as previously observed, but a subsequent slow absorption of hydrogen continued.

Prolonged hydrogenation (24 hrs.) failed to produce a
pure tetrahydro derivative, but hydrogenation at 100
atmospheres and room temperature afforded the pure tetra-
hydro alcohol, $C_{20}H_{36}O$, LXXIX. The infrared spectrum of
this compound displayed no absorptions attributable to
double bonds, thereby confirming that the alcohol was fully
saturated. This therefore proved that the parent alcohol,
I, contained only two double bonds. The configuration of
the C-8 hydrogen was not investigated, but by analogy with
the results observed during the study of the tetrahydro-
pimaric acids by ApSimon et al (50) it would be expected
to be in the α-configuration so as to produce a product with
the trans-anti-trans fused skeleton.

LXXVIII   (a)   R = H
           (b)   R = Ac   LXXIX
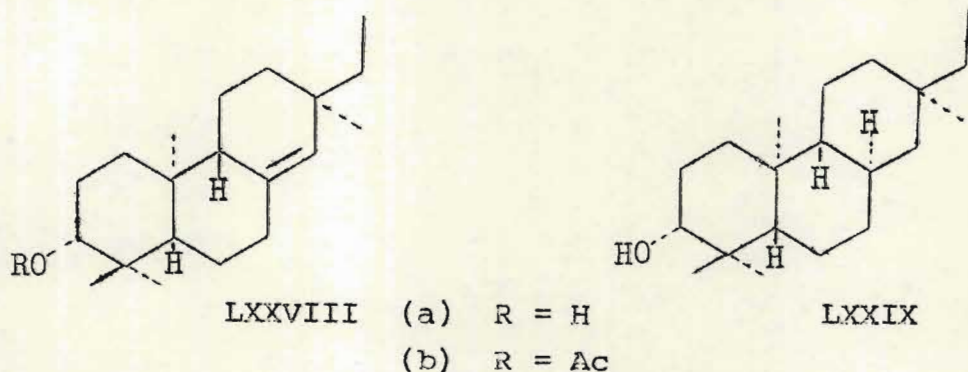
Ultraviolet Spectra

The ultraviolet absorption spectra of the
alcohol, I, and its derivatives did not exhibit any
characteristic bands in the region above 210 nm. thereby
establishing that the two double bonds discussed above were
not in conjugation.
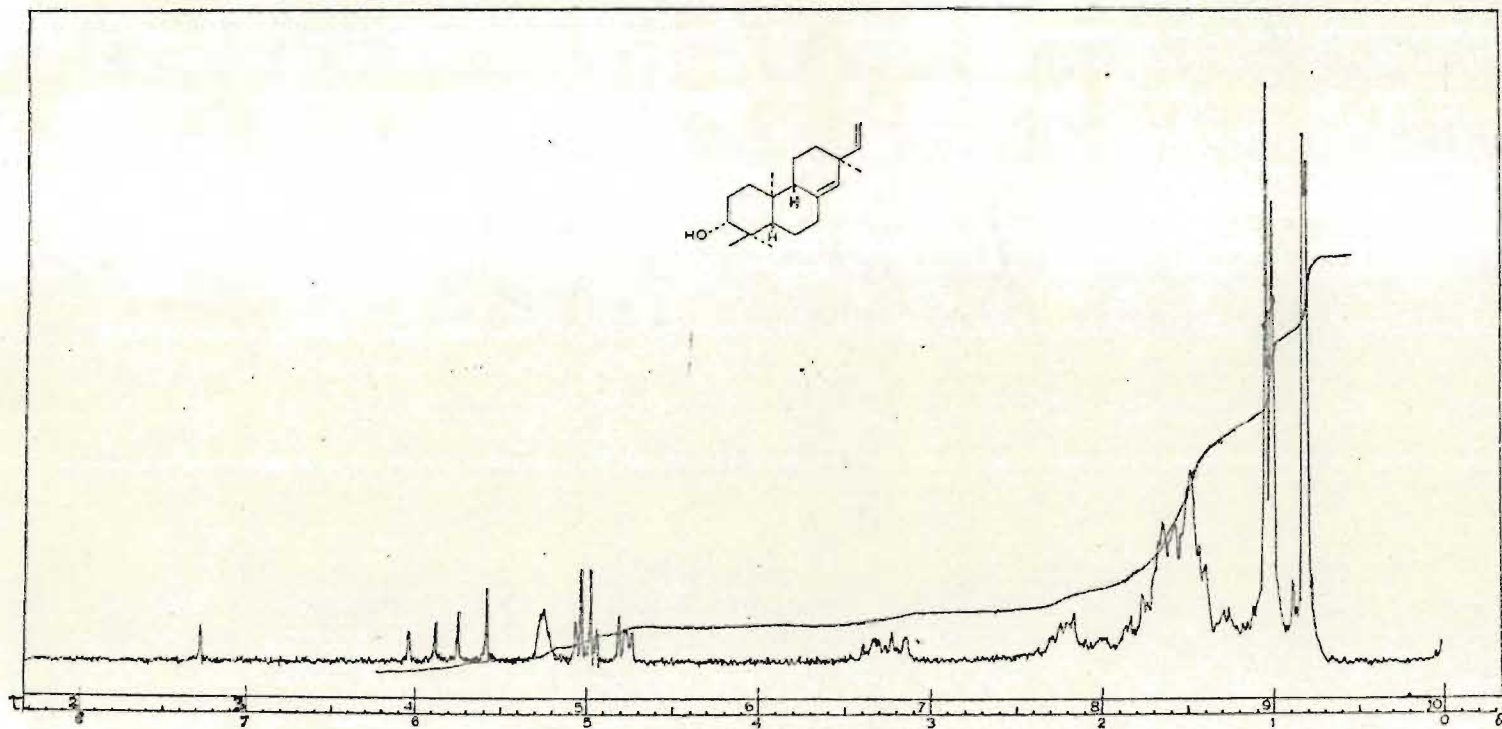
FIGURE 5   NMR SPECTRUM OF 3α-HYDROXY-ent-ISOPIMARA-8(14),15-DIENE

Nuclear Magnetic Resonance Spectra

The n.m.r. spectrum of the alcohol, I, proved
most informative in the determination of the basic skeleton,
the nature of the substituent groups, positions of unsatur-
ation and the configuration of the hydroxy group.

The spectrum (Figure 5) exhibited a quartet of
lines centred at $\tau 4.19$ due to one proton. This signal
was attributed to the C-15 hydrogen atom which forms the X
part of an ABX system, LXXX, in which the two non-equivalent
C-16 protons of the vinyl group constitute the AB portion
and resonate as a complex multiplet centred at $\tau 5.10$. It
has been determined that the <u>trans</u>-olefinic coupling constant
is usually greater than the <u>cis</u>-olefinic coupling constant,
and in the case of I the observed coupling constants were
$J_{AX}=18$ Hz and $J_{BX}=10$ Hz (91). The geminal coupling constant
was small (observed $J_{AB}=1.5$ Hz) as expected when the angle
(θ) between the protons is relatively large (about 120°).
This is in agreement with the relationship postulated by
Karplus (92,93) that the value of the coupling constant J
for geminate protons decreases from about 20 Hz at a dihedral
angle of 105° to 0 at a dihedral angle of 125°.
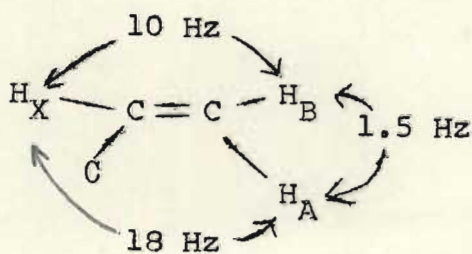
LXXX

A broad signal at ϒ 4.75 was assigned to the olefinic proton at C-14. It occurred as a slight doublet with an observed coupling constant of J= 2 Hz which may be attributable to allylic coupling with the axial C-9 proton. It has been established by empirical correlation that allylic coupling is greatest when the angle between the olefinic and allylic protons lies between $60^{\circ}-110^{\circ}$ (94,95). A study of a model of the alcohol, I, revealed that the angle between the C-14 and C-9 protons was approximately 90° and thus the observed coupling constant is in agreement with the theoretically predicted value.

The one proton multiplet at ϒ 6.73 was ascribed to the C-3 hydrogen atom adjacent to the hydroxy moiety. Again this proton may be theoretically regarded as the X portion of an ABX system, but the observed splittings indicated that coupling was not of the first order. The usual position for the signal due to such protons is between ϒ 5.5-6.5, but it has been found that an axial proton attached to a hydroxy substituted carbon atom resonates at higher field than in the corresponding epimeric situation (96). The position of this signal in the spectrum of I was consistent with the C-3 proton being axially orientated i.e. the hydroxy group was in the equatorial configuration. Furthermore, such an axial proton would be subjected to a large diaxial and a small axial-equatorial vicinal coupling which would lead to a broadening of the signal. In Figure 5 the broad pattern of splitting with

a half-band width of approximately 14 Hz served as a further indication that the C-3 proton was axially orientated.

The methylene envelope extended from τ 7.8-8.7, but no specific assignments could be made. Four strong signals due to the C-13, C-4 equatorial, C-4 axial and C-10 methyl groups resonated as singlets at τ 8.95, 8.99, 9.17 and 9.19 respectively.

Thus the above spectral data together with the molecular formula, $C_{20}H_{32}O$, were consistent with the di-terpene, I, possessing a tricarbocyclic skeleton having one hydroxy, one vinyl and four methyl substituents together with an endocyclic double bond. Assuming the presence of geminal methyl groups at C-4 and a third methyl group at C-10, the problem of locating the other substituents re-mained to be solved. Because the methyl signals in the n.m.r. spectrum all occurred as singlets and from the pattern of splitting of the vinyl signals, it was obvious that the fourth methyl group and the vinyl group must both be attached to a fully substituted carbon atom. This evidence strongly indicated a pimarane type skeleton, and therefore the remaining methyl and vinyl groups must be situated at C-13. Furthermore, in order to satisfy the n.m.r. requirements, the nuclear double bond can only be located between C-8 and C-14. It was also obvious that the hydroxy group could not be attached to any of these substituents, and must therefore be located on one of the

nuclear carbon atoms. Since hydroxylation on rings B and
C is rarely encountered in Euphorbiaceae diterpenes it was
probable that the hydroxy group was on ring A, the most
likely position being C-3.

Further Evidence of The Nature and Location of the
Hydroxy Group in I

In order to confirm the secondary nature of the
hydroxy group the alcohols I, LXXVIII (a) and LXXIX were
oxidised with Jones' reagent (97) to the colourless
ketones LXXXI, LXXXII and LXXXIII respectively. The
ketone, LXXXI, whose molecular formula was established as
$C_{20}H_{30}O$ was characterised by the formation of a 2,4-dinitro-
phenylhydrazone. This evidence therefore confirmed that I
was a secondary alcohol.
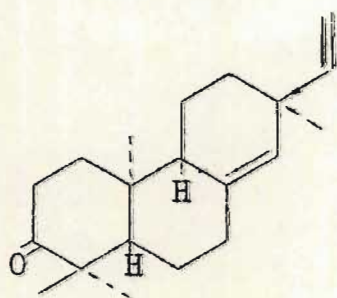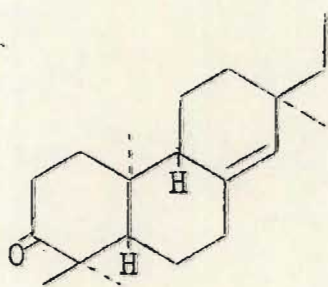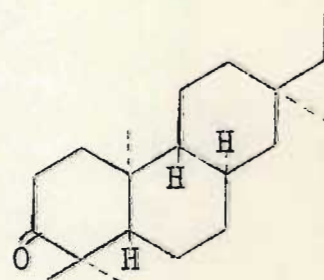
LXXXI   LXXXII   LXXXIII

The infrared spectrum of the ketone, LXXXI,
(Figure 6) exhibited no hydroxy absorptions, but the
expected carbonyl band occurred at 1697 cm.$^{-1}$. This
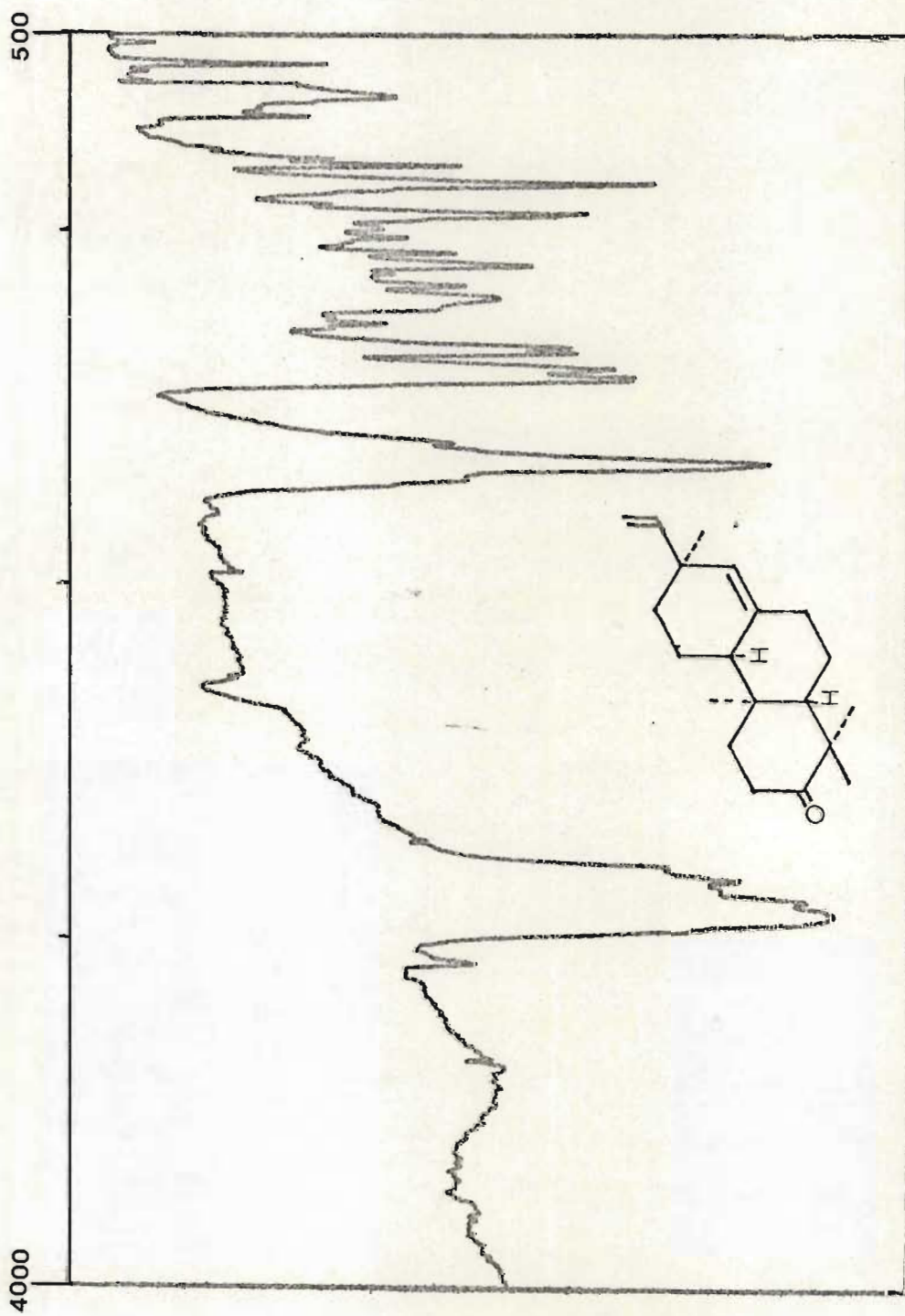
FIGURE 6   IR SPECTRUM OF ent-ISOPIMARA-8(14),15-DIEN-3-ONE
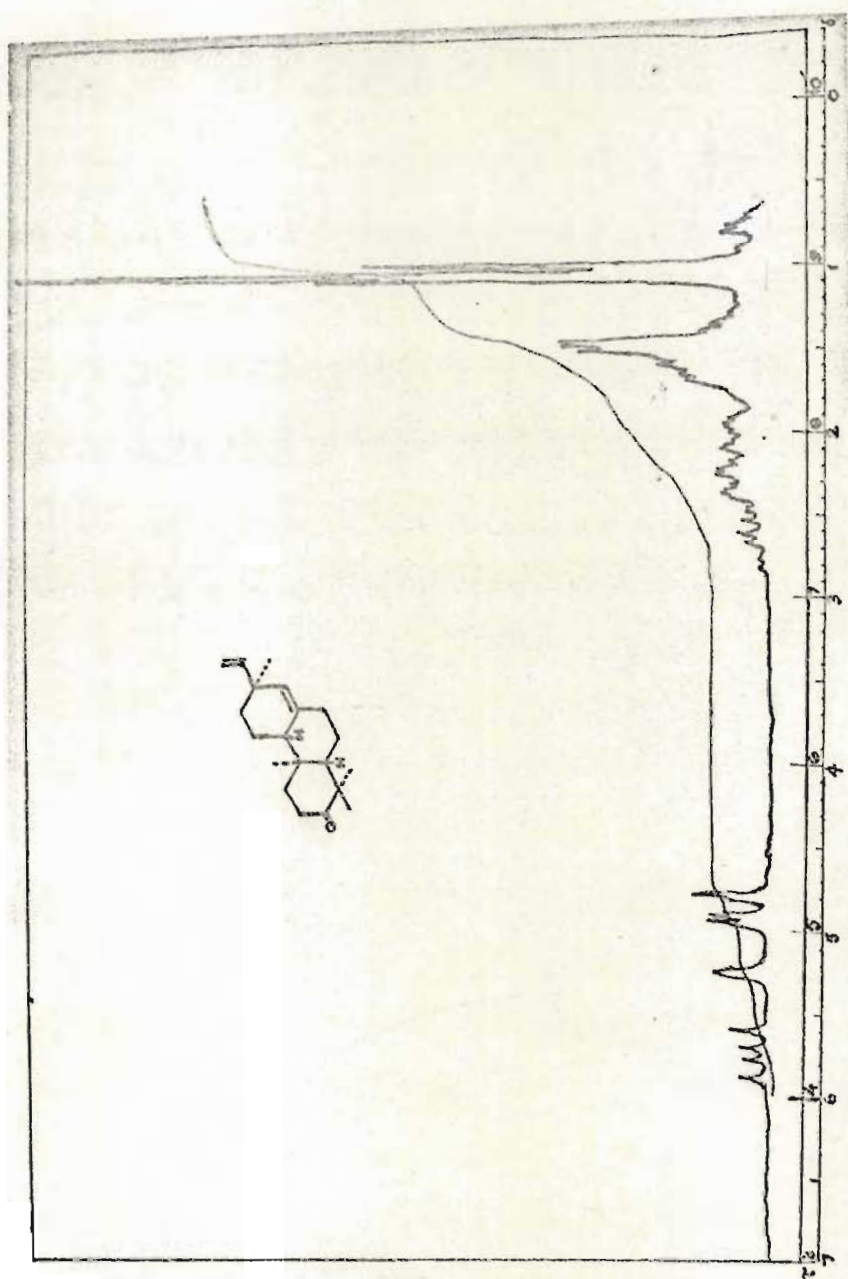
FIGURE 7   NMR SPECTRUM OF ent-ISOPIMARA-8(14),15-DIEN-3-ONE

value is lower than that measured for a solution spectrum due to association in the solid state. The absorptions at 3078 and 1630 $cm.^{-1}$ associated with the double bonds remained unchanged.

In the n.m.r. spectrum of the ketone, LXXXI, (Figure 7) the only major change observed was the disappearance of the signal due to the proton adjacent to the hydroxy group.

Finally, a very strong indication that the oxygen function was located at C-3 was provided by the positive reaction of the ketone, LXXXI, to the Zimmermann test which is characteristic for C-3 ketones (98). Thus it was concluded that I must be a C-3 diterpene alcohol.

The Basic Skeleton of I

The carbonyl group of the 3-ketone, LXXXI, was reduced by means of a Huang-Minlon reduction (99) via the intermediate hydrazone to produce the basic hydrocarbon, $C_{20}H_{32}$, LXXXIV. The infrared spectrum of this compound was typical of a hydrocarbon and exhibited the expected olefinic absorptions at 3080 and 1631 $cm.^{-1}$.

A survey of the literature revealed that the physical constants of this hydrocarbon were very similar to those reported for isopimara-8(14),15-diene, LXXXV (a), which had been isolated from pyinkado wood derived from a tree, Xylia dolabriformis (Leguminoseae) (100). An

authentic sample of the hydrocarbon was obtained for purposes of comparison. It was discovered that the infrared and mass spectra of LXXXIV and LXXXV (a) were superimposable, melting points and R_f values were almost identical, but a mixed melting point of the two compounds was depressed and their specific rotations, although similar in magnitude were opposite in sign.

Furthermore, two other constituents from Xylia dolabriformis were characterised as isopimara-8(14),15-dien-3-one, LXXXV (b) and isopimara-8(14),15-dien-3β-ol, LXXXV (c) and their reported physical constants were remarkably similar to those of the 3-ketone, LXXXI and the alcohol, I, respectively. Again the infrared and mass spectra of the corresponding pairs were found to be superimposable while their mixed melting points were depressed and their specific rotations of opposite sign.

This evidence led to the conclusion that the hydrocarbons, LXXXIV and LXXXV (a), the ketones, LXXXI and LXXXV (b), and the alcohols, I and LXXXV (c) were in fact enantiomeric pairs. Proof of this relationship was established by means of optical rotatory dispersion. Curves were obtained (Figure 8) for the two ketones, LXXXI and LXXXV (b) under identical conditions. It was obvious that the former displayed a positive Cotton effect and the latter a negative Cotton effect (101). In agreement with the findings of Enzell et al (102) on the 8(14),15-pimaradienes both ketones exhibited the usual o.r.d. curves. Most significant
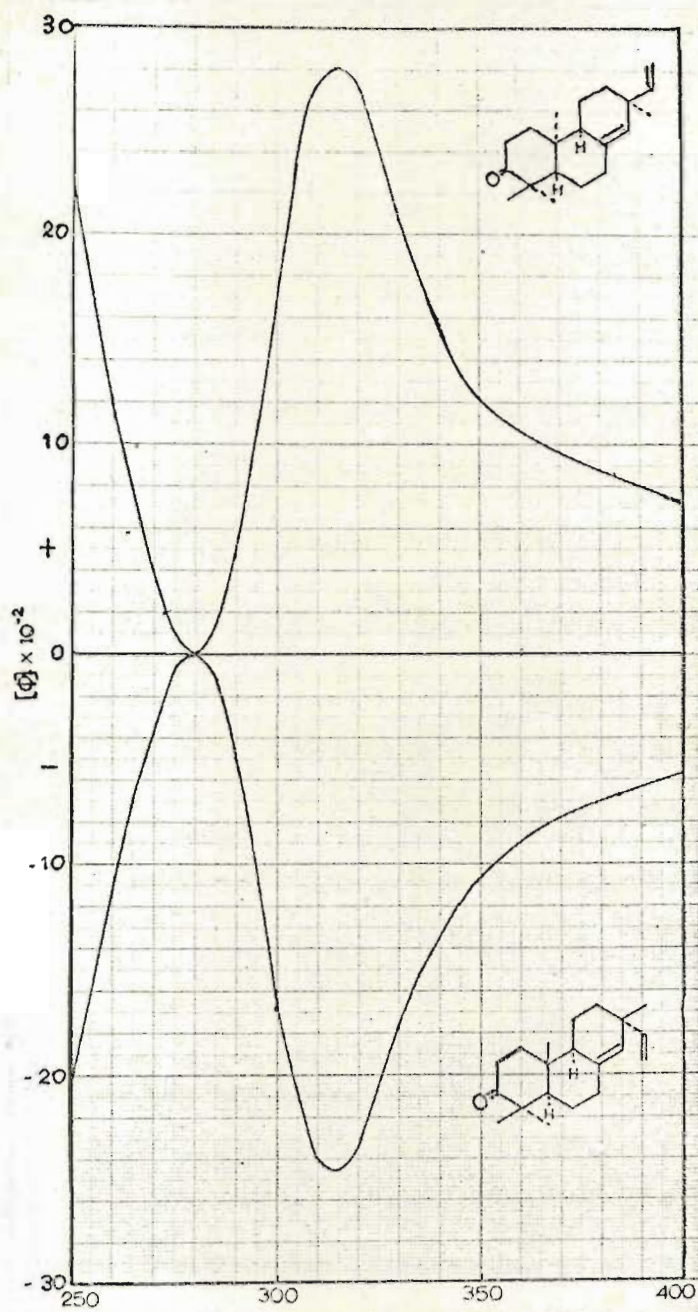
FIGURE 8   ORD CURVES OF ent-ISOPIMARA-8(14),15-DIEN-3-ONE

AND ISOPIMARA-8(14),15-DIEN-3-ONE

of all was the fact that the curves were antipodal since
the peaks and troughs coincided at the same wavelengths,
the values being $[\phi]_{315}+2810^{\circ}$, $[\phi]_{280}$ 0° for LXXXI and
$[\phi]_{315}-2460^{\circ}$ and $[\phi]_{280}$ 0° for LXXXV (b). Thus the ketones,
LXXXI and LXXXV (b) are clearly enantiomeric with the former
possessing the 5β-hydrogen, 10α-methyl, 9β-hydrogen and 13β-
vinyl substituents. These results are consistent with those
reported by Enzell et al (102) who found that the o.r.d.
curve exhibited a positive maximum in the case of a pimara-
diene with the C-13 vinyl group in the β-configuration and
a negative maximum when the vinyl group was in the α-con-
figuration.

From the foregoing evidence it therefore follows
that the alcohol, I, and the hydrocarbon LXXXIV are
enantiomers of LXXXV (c) and LXXXV (a) respectively. The
fact that I, possesses the 5β-hydrogen, 10α-methyl A/B
trans-fused ring system is consistent with the stereochemistry
of all the diterpenes previously isolated from the
Euphorbiaceae. As would be expected for such a normal
trans-anti-fused backbone the C-9 hydrogen is in the β-con-
figuration, but the accompanying C-13β-vinyl group is a
feature which has not previously been encountered. Thus
the alcohol, I, is the first recorded natural occurrence
of a compound possessing the ent-isopimaradiene skeleton.
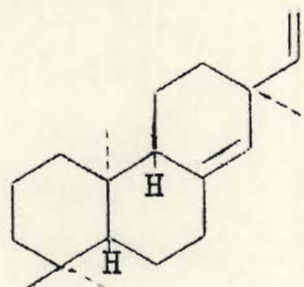
LXXXIV
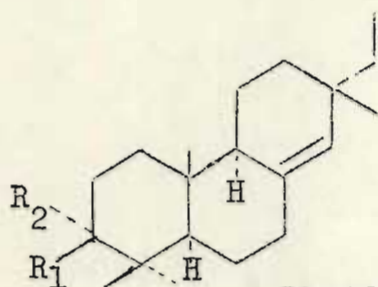
LXXXV

		R_1	R_2
(a)		H	H
(b)			O
(c)		OH	H
(d)		OAc	H

The physical constants and molecular formulae of the three enantiomeric pairs are tabulated in Table 1 which serves to emphasise the similarity of data between each pair except for the specific rotations which indicate an antipodal relationship. Constants for the acetate, LXXXV (d), and the hydrogenated alcohols, LXXXVI and LXXXVII, are also provided for comparison with those of the corresponding derivatives of the alcohol, I.
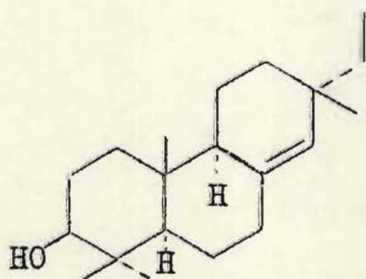
LXXXVI
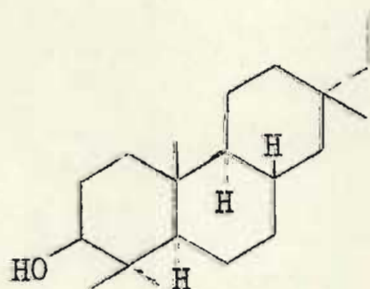
LXXXVII

TABLE 1

Compound	Formula	Mol. wt.	m.p.	$[\alpha]_D$
I	$C_{20}H_{32}O$	288	126–127°	+12.5°
LXXXV (c)	$C_{20}H_{32}O$	288	126.5–127.5°	–19.5°
LXXVII	$C_{22}H_{34}O_2$	330	104–105°	–7°
LXXXV (d)	$C_{22}H_{34}O_2$	330	102–104.5°	+4°
LXXVIII (a)	$C_{20}H_{34}O$	290	132–133°	–23.2°
LXXXVI	$C_{20}H_{34}O$	290	132–132.5°	+25.5°
LXXIX	$C_{20}H_{36}O$	292	135°	–46.8°
LXXXVII	$C_{20}H_{36}O$	292	134–135.5°	+21.8°
LXXXI	$C_{20}H_{30}O$	286	60°	+45°
LXXXV (b)	$C_{20}H_{30}O$	286	59–60°	–56°
LXXXIV	$C_{20}H_{32}$	272	38–39°	+7°
LXXXV (a)	$C_{20}H_{32}$	272	38–39.5°	–12.4°

Bromination Experiments

During the study of spirostachic acid, VIII, (to be discussed in Part 2) it was found that the mass spectra of 2,3-seco-dioic acids displayed characteristic fragments due to the loss of the C-1 — C-2 and C-3 — C-4 groupings. The application of these results had proved invaluable in the elucidation of the structure of cleistanthol, IV, (1,2, 3). With this view in mind, an attempt was made to convert the tetrahydroketone, LXXXIII, to the corresponding 2,3-seco-dioic acid.

The intended scheme was to obtain the seco-acid via the α-bromoketone, LXXXVIII, followed by hydrolysis and a Lemieux-von Rudloff oxidation. The latter two steps failed to produce the desired product but the intermediate bromination products proved to be interesting. Treatment of a benzene solution of the tetrahydroketone, LXXXIII, with bromine resulted in an initial slow uptake of bromine followed by a further more rapid uptake, which furnished a compound having the formula, $C_{20}H_{32}OBr_2$.
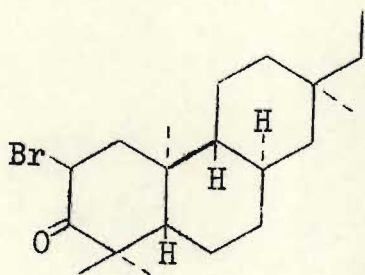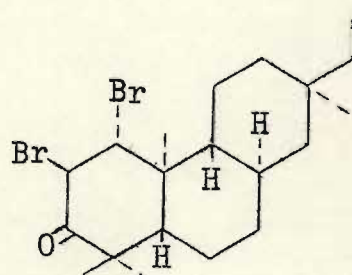
LXXXVIII   LXXXIX

The n.m.r. spectrum of the 1,2-dibromoketone,
LXXXIX, exhibited an AB system comprising two doublets at
$\tau 6.25$ and 6.92 (J_{AB}=16 Hz.). From the large coupling con-
stant observed for the C-1 and C-2 protons it was evident
that they were trans-diaxially orientated (104), and hence
the bromine atoms must have been trans-diequatorially
orientated.

The formation of the 1,2-dibromoketone, LXXXIX,
was probably initiated by the relatively slow process of
α-bromination of the tetrahydroketone, LXXXIII, giving rise to
the α-bromoketone, LXXXVIII(a), during which the elements of
hydrogen bromide were eliminated to give the α,β-unsaturated
ketone, XC. Further bromination of the C-1, C-2 double
bond would then produce the observed 1,2-dibromoketone,
LXXXIX, as outlined below.

LXXXIII   LXXXVIII(a)   XC   LXXXIX
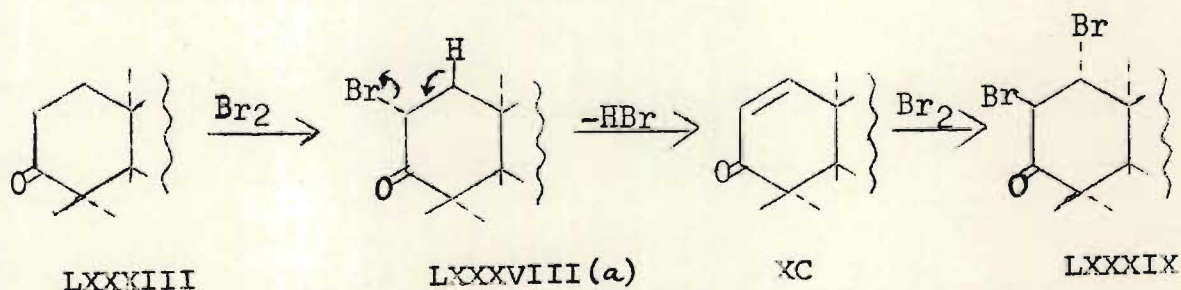
In order to inhibit the elimination of hydrogen
bromide in the sequence shown above, the bromination was
repeated in acetic acid medium in the presence of hydro-
bromic acid (76). This afforded both the mono- and the di-
bromo- compounds which were separated by means of preparative
thin layer chromatography. The α-bromoketone, LXXXVIII,
was shown by accurate mass measurements to possess the

molecular formula, $C_{20}H_{33}OBr$, whilst its n.m.r. spectrum indicated that the bromine was equatorially orientated due to the relatively low field signal of the C-2 hydrogen at τ4.99 (105,106). This is not unexpected since in the case of the analogous keto-steroids the equatorial bromide is the thermodynamically more stable isomer, although the axial bromide may be formed initially (107). Furthermore, for a 2-bromo-4,4-dimethylcyclohexanone the preferred conformation is the chair-form in which the bromine is equatorial (108). A comparison of the infrared spectra of the tetrahydroketone, LXXXIII, and the ∝-bromoketone, LXXXVIII, showed an increase in frequency of the C=O stretching vibration from 1695 $cm.^{-1}$ to 1720 $cm.^{-1}$ which confirmed the equatorial orientation of the C-2 bromine (109).

Mass Spectra

The mass spectra of some pimarane diterpenes have been briefly studied by Audier et al (103) who found that in general, the major peaks in the spectra could be explained by fragmentations analogous to those reported for the bicyclic diterpenes (110). However, all Audier's work was performed on the unsaturated pimaradiene skeletons and in no instance were the spectra of the hydrogenated compounds analysed.

The mass spectrum of the alcohol, I, (Figure 9) exhibited a molecular ion peak at m/e 288 which was in

agreement with the molecular formula $C_{20}H_{32}O$ determined by elementary analysis. It was noted that the fragmentation pattern was very similar to that of a published spectrum of isopimara-8(14),15-dien-3β-ol, LXXXV (c). Comparison of the mass spectrum of I with that of an authentic sample of LXXXV (c) isolated from Xylia dolabriformis (100) revealed that the pattern of fragmentation was the same in the two cases, but there were variations in peak intensities.

The spectra of I and its derivatives showed distinctly related fragmentation patterns and an attempt will be made to propose structures for the various ions which would provide added support to the evidence already presented for their structures. Unfortunately, the ion fragments have not been confirmed by labelling experiments or accurate mass determinations, but the proposals are based on reasonable and most probable cleavage mechanisms.

A notable feature in the spectrum of I was the intensity ratio of the M-18 ion (14%) to the molecular ion, M^{+} (36%), which resulted in a value less than unity. By analogy with the observations made during a study of the mass spectra of a series of monohydroxy steroids (111) this was added proof that the hydroxy group was in the equatorial configuration, since such alcohols dehydrate less readily under electron impact than their axial epimers.

The major peaks in the upper half of the spectrum of I were due to the molecular ion, M^{+}, and ions at M-15, M-18, M-27 and M-33. The M-15 fragment was almost certain-

ly due to the loss of the C-13 methyl group as this fragmentation involves the cleavage of a bond which is allylic to two olefinic double bonds. The M-18 ion which was of similar intensity to the M-15 ion, could be explained by the loss of water from the molecular ion, while the M-33 fragment probably arose by the loss of water from the M-15 ion. Both of these cleavages were strongly supported by the appearance of metastable ions at m/e 253 (288 → 270) and at m/e 238 (273 → 255) respectively. It was also possible that the M-33 ion could have been derived through the loss of a methyl group from the M-18 ion since this was suggested by the presence of a very weak metastable ion at m/e 241 (270 → 255) (Scheme 1).

SCHEME 1

M-15 (13.5%)

M-33 (14%)

M^+ (36%)

M-18 (14%)
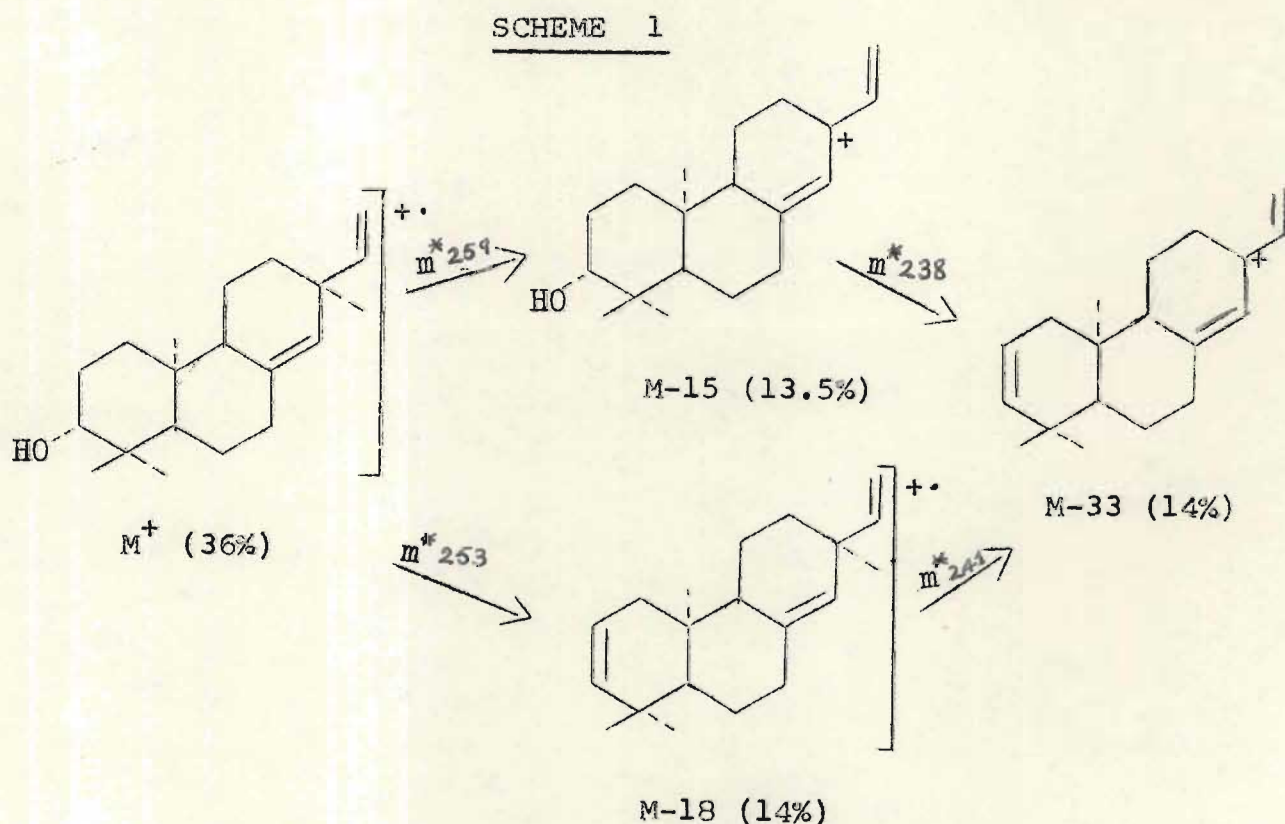
The M-27 ion was of lower intensity than the ions mentioned above and was probably due to the loss of the C-13 vinyl group. The low abundance of this ion is explained by the fact that its formation involves the cleavage of a vinyl bond albeit allylic to the 8(14)-double bond as shown below.

M⁺   M-27 (6%)
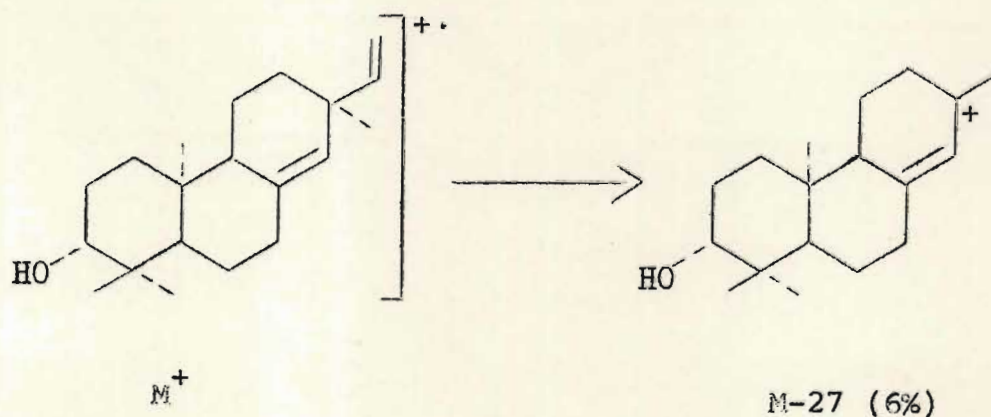
Acetylation of the hydroxyl group made very little difference to the spectrum (Figure 10) in which the molecular ion appeared at m/e 330. The M-18 and M-33 fragments were replaced by M-60 and M-75 ions due to the loss of the elements of acetic acid (by a McLafferty rearrangement) from the molecular ion and the C-13 methyl group from the M-60 ion respectively. The peaks in the lower half of the spectrum were almost completely unchanged by acetylation.

M⁺   M-60   M-75
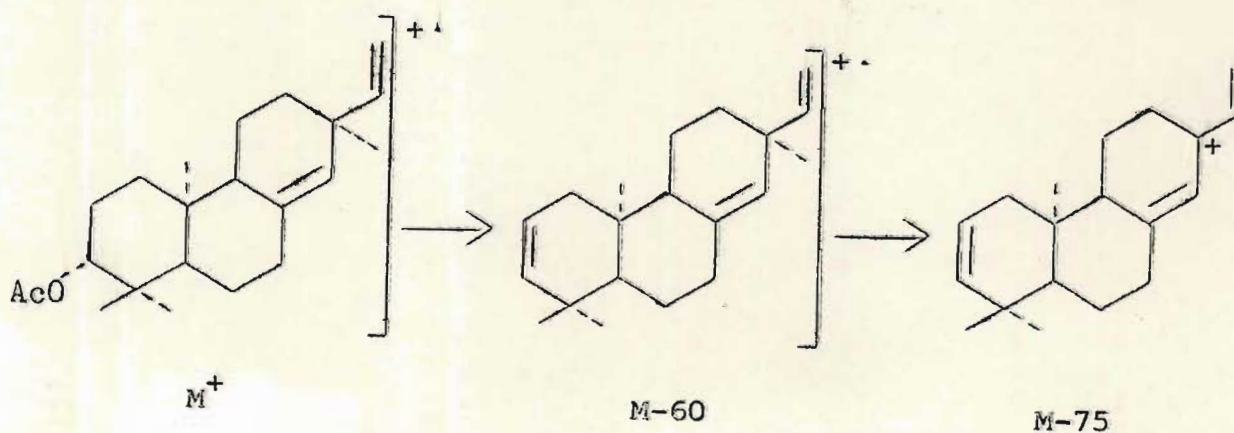
Hydrogenation of the vinyl double bond gave rise to a spectrum (Figure 11) in which the main differences were the appearance of the molecular ion at m/e 290 and of fairly intense peaks at m/e 261 and 243. The M-29 fragment probably arose from the loss of the C-13 ethyl group and corresponds with the M-27 ion discussed for the parent alcohol, I. The marked increase in intensity of this ion could be explained by the fact that its formation no longer involved the cleavage of a vinyl bond and was thus a more favoured process than that for the formation of the M-27 ion. This cleavage mechanism was supported by the appearance of a weak metastable peak at m/e 235. The M-47 fragment could be readily explained by assuming the loss of the elements of water from the molecular ion to give the M-18 fragment at m/e 272 (m^* 255.2, 290 $\longrightarrow$ 272) followed by the loss of the C-13 ethyl moiety (m^* 217, 272 $\longrightarrow$ 243). A small contribution to the M-47 ion could also have arisen from the reverse pattern of fragmentation i.e. loss of water from the M-29 ion.

In addition to being allylic to the 8,14-double bond the bulkier nature of the C-13 ethyl group obviously facilitated the cleavage of this substituent rather than the C-13 methyl group. This therefore accounts for the lower intensity of the M-15 peak in Figure 11. The M-33 ion was shown by the presence of a metastable ion at m/e 243 to result from the loss of the C-13 methyl group from the M-18 ion, and again the intensity of this peak was much

lower than that of the peak due to the M-47 ion (Scheme 2).

SCHEME 2
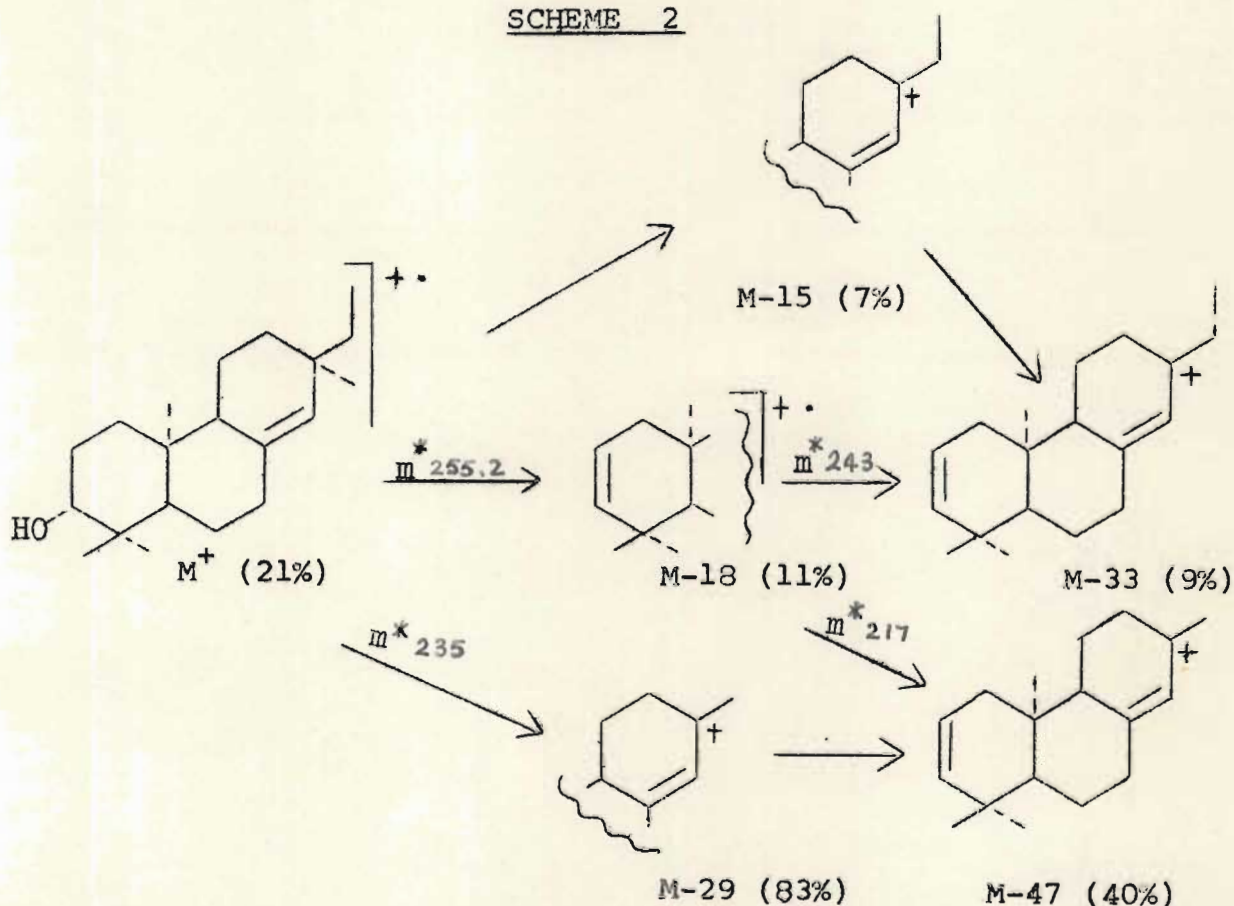
In the spectrum of the tetrahydro alcohol, LXXIX, (Figure 12) the significant features were the appearance of the molecular ion at m/e 292, increased intensities of the M-18 peak and peaks at m/e 191 and 163. Since the double bonds have been fully reduced there is less incentive for the cleavage of the C-13 substituents and hence a low abundance of the M-15 (7%) and M-29 (9%) fragments. The loss of water from the molecular ion is by comparison now a more favoured process resulting in the M-18 ion (32%) at m/e 274, and confirmed by a metastable ion at m/e 257. This ion then loses either the C-13 methyl group to yield the

M-33 ion (22%) at m/e 259 or the preferred bulkier ethyl
group to give the M-47 ion (41%) at m/e 245. The fragment-
ation pattern giving rise to the M-101 and M-129 ions was
probably initiated by a retro Diels-Alder rearrangement of
the M-18 ion (Scheme 3).

SCHEME   3

M   (54%)

$m^{*}257$

M-33   (22%)

M-18   (32%)

M-47   (41%)

M-101   (21%)   M-129   (45%)
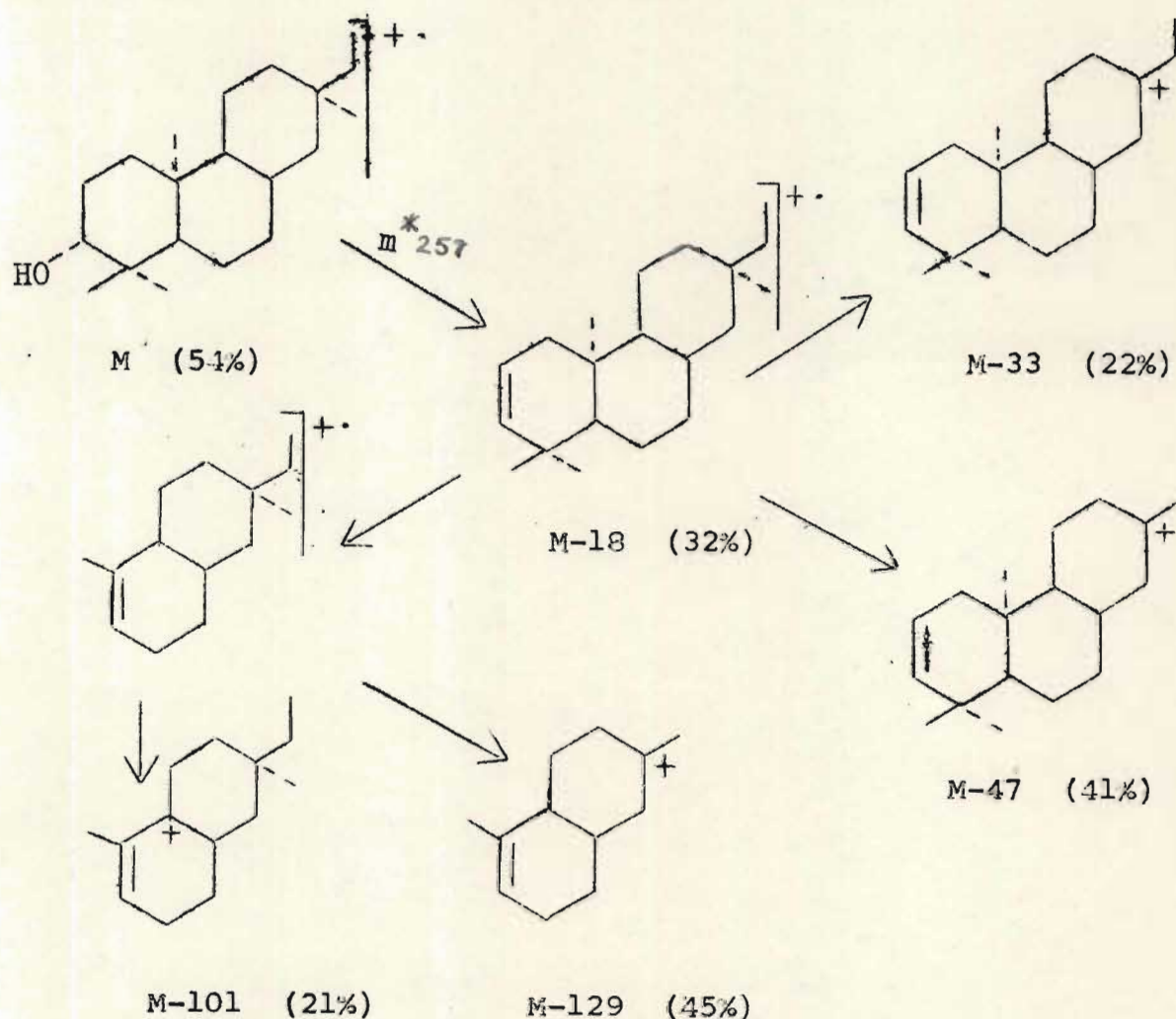
The base peak in the spectrum of the parent
alcohol, I, (Figure 9) occurred at m/e 135 (M-153). This
fragment was unchanged by acetylation or hydrogenation of

the vinyl double bond, thereby implying that this ion con-
tained neither the oxygen function nor the vinyl group.
Confirmation of this fact was obtained by an accurate mass
measurement of this peak which established the formula of the
ion to be $C_{10}H_{15}$. The formation of this ion could be explain-
ed by a process proposed by Enzell et al (110) for the
formation of the corresponding ion in the spectra of bicyclic
diterpenes possessing a C-8, C-20 double bond. It was also
possible for this ion to lose one of its C-4 substituents
together with a hydrogen atom to give the M-169 ion at m/e
119 as shown below. Enzell determined that the process was
favoured by increasing molecular weight of the C-4 sub-
stituents, and when these were either -COOH or -COOCH$_3$, the
latter ion frequently formed the base peak in the spectrum.

M-135 (7.5%)
m/e 153

M-153 (100%)
m/e 135

OR

M-169 (17.5%)
m/e 119
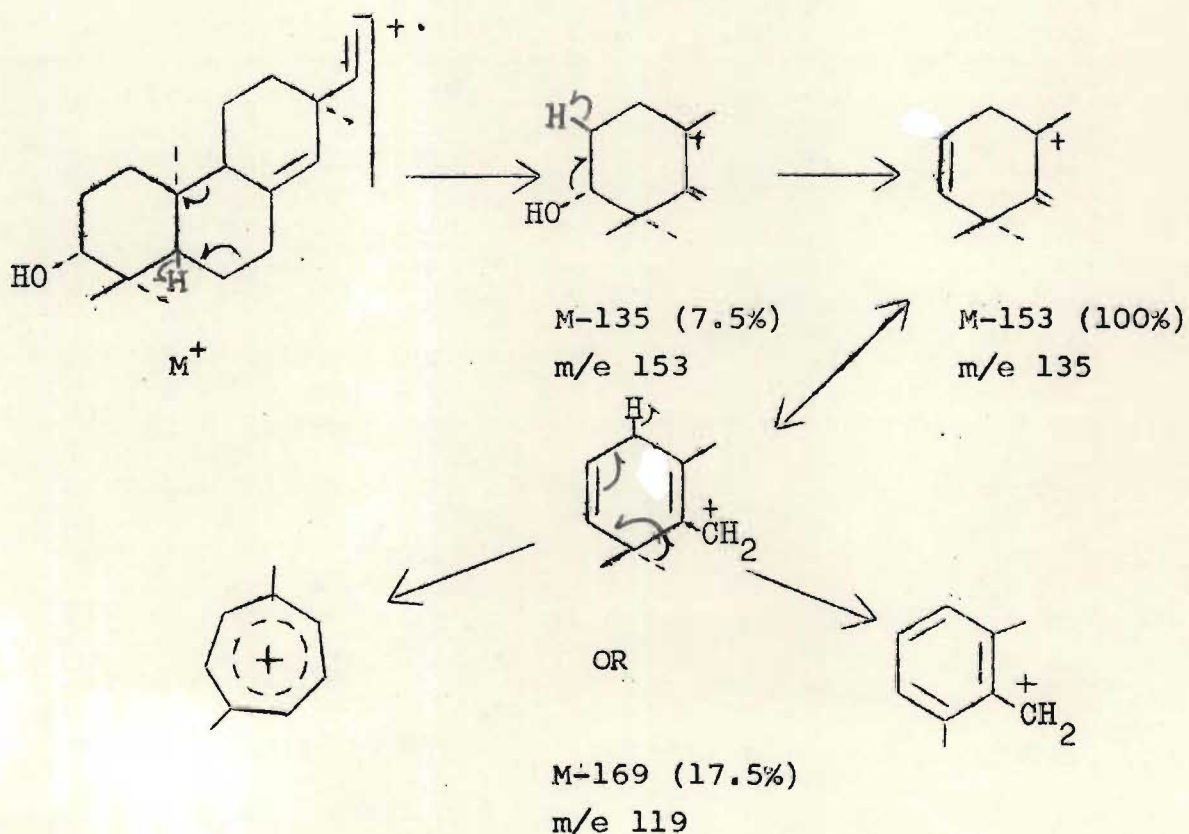
The relatively abundant ions at m/e 152 and m/e 134 could have arisen by a process outlined by Audier et al (103) for the formation of a fragment similar to the former ion in the spectrum of the hydrocarbon, LXXXV (a). Loss of water from this ion probably gave rise to the m/e 134 fragment, while cleavage of a methyl group from the latter would provide an alternative pathway to the M-169 ion.

M-136 (15%)
m/e 152

M-169 (17.5%)   M-154 (16%)
m/e 119   m/e 134
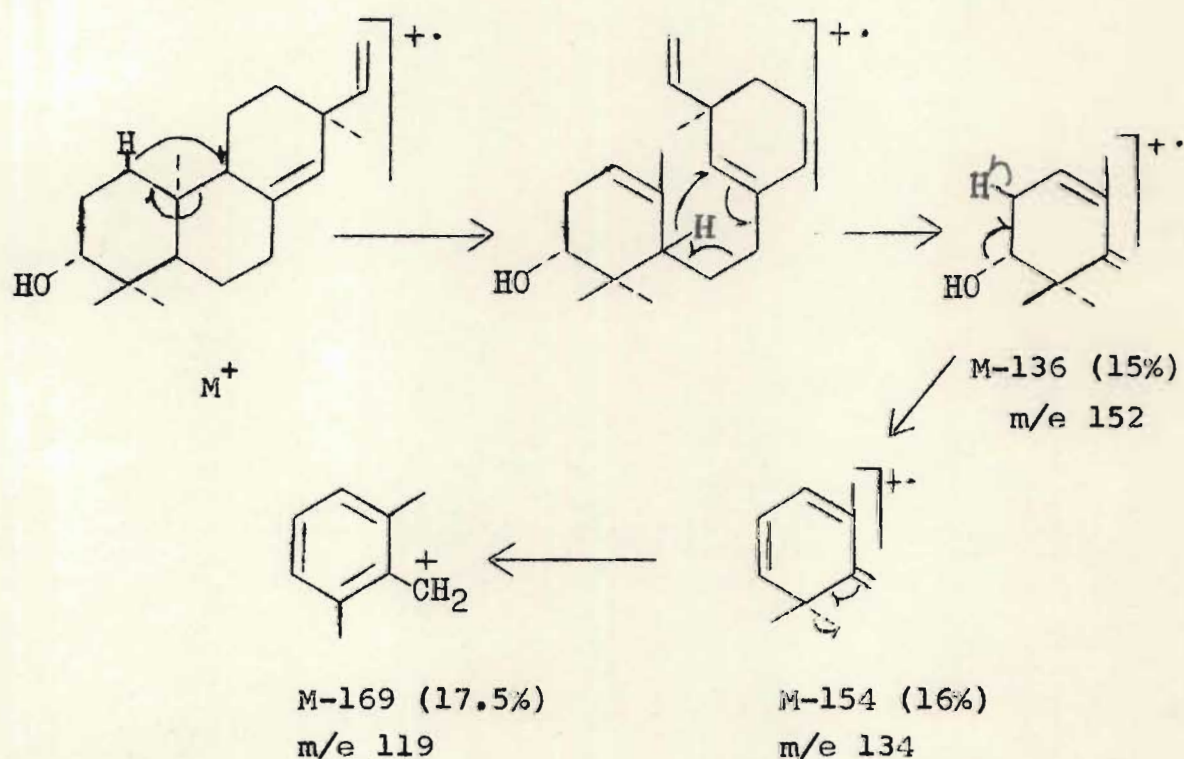
In the lower half of the spectrum the only significant peaks which underwent any change on hydrogenation of the vinyl double bond were those at m/e 148 in the spectra of I (Figure 9) and its acetate (Figure 10) which appeared at m/e 150 in the spectra of their dihydro derivatives. This fragment probably arose by the process outlined below involving the same intermediate proposed for the formation

of the M-136 ion at m/e 152.

M⁺ ⟶ ⟶ M-140 (12%)
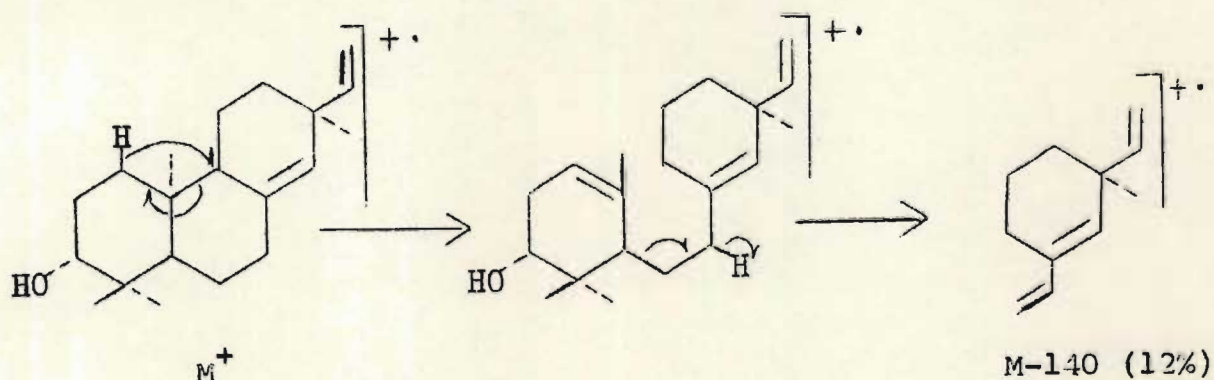
The peaks at m/e 136 and m/e 121 might have arisen
from the molecular ion or the M-18 ion by the process out-
lined below, which is essentially very similar to those pro-
posed for the formation of the m/e 152, 134, and 148 frag-
ments but involves a transfer of a hydrogen atom from C-11
to C-10 rather than from C-1 to C-9 as in the above cases.
It was considered most likely that the m/e 136 fragment was
derived from the M-18 ion for if it had arisen from the
fragmentation of the molecular ion a significant peak would
have been expected at m/e 154, followed by the loss of the
elements of water to produce the m/e 136 fragment. Loss of
one of the C-4 methyl groups from the ion at m/e 136 provided
an explanation for the fragment appearing at m/e 121.

M-18   m/e 136 (27%)   m/e 121 (19%)

M-155

M-140 (12%)

M-27 (6%)

M-167 (19%)

M-152 (27%)

M-153 (100%)

M-135 (75%)

M⁺

M-18 (14%)

M-169
(17.5%)

M-154 (16%)

M-136 (15%)

M-15 (13.5%)

M-33 (14%)

64
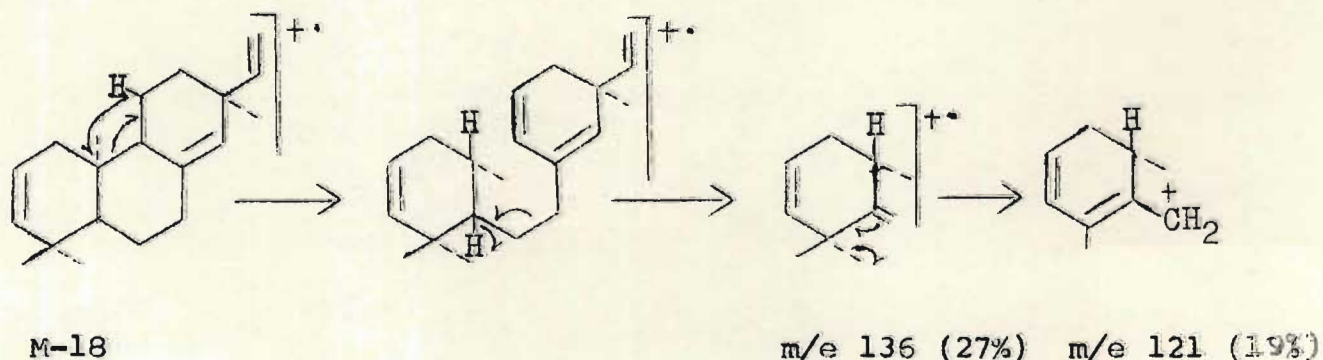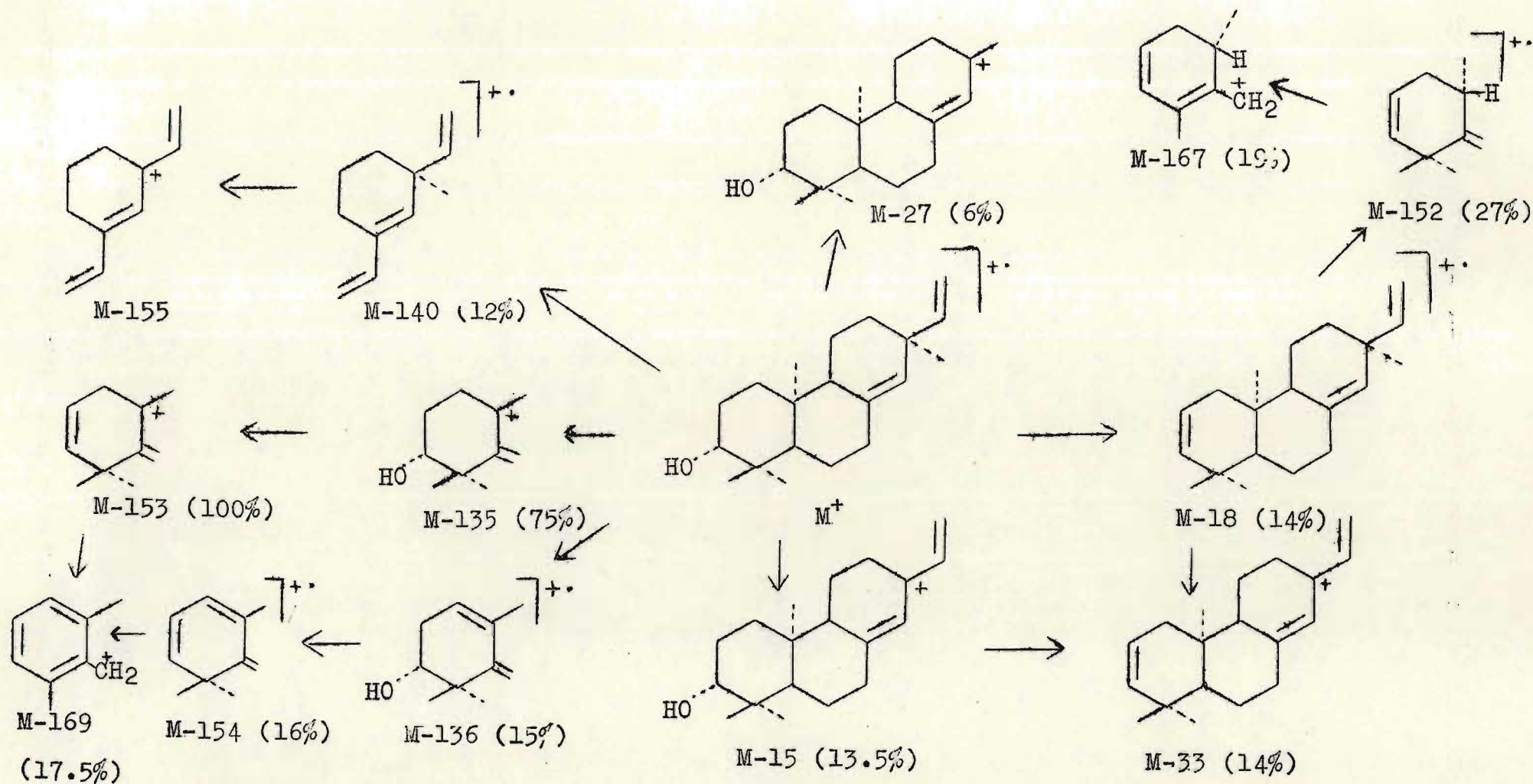
SCHEME 4

In the spectra of the 3-ketone, LXXXI, (Figure 13) and its dihydro- and tetrahydro- derivatives (Figures 14 and 15) the appearance of the molecular ions at m/e 286, 288 and 290 were all in agreement with the formulae $C_{20}H_{30}O$, $C_{20}H_{32}O$ and $C_{20}H_{34}O$ respectively.

Peaks in the upper half of each spectrum corresponded well with the fragmentations proposed for similar ions in the spectra of the C-3 alcohols. However, ions appearing in the lower halves of the spectra showed little correlation and were thus difficult to analyse. The fragment at m/e 135 no longer formed the base peak in these spectra as had been the case for the alcohols. Audier et al (103) outlined the process below for the formation of this ion in C-3 ketones, but this representation of the ion is open to question since it was observed that upon hydrogenation of the vinyl bond (Figure 14) the intensity of the m/e 135 fragment was still greater than that of the m/e 137 fragment.

M^+ m/e 135 (48%)
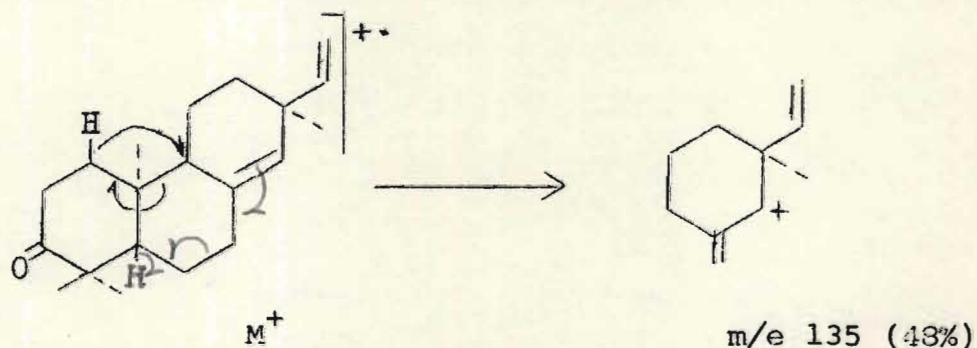
Comparison of the spectrum of ent-isopimara-8(14), 15-diene, LXXXIV, (Figure 16) with those of an authentic sample of its enantiomer and a published spectrum (103) revealed that the fragmentation patterns were identical.

The molecular ion formed the base peak in Figure 16, appearing at m/e 272 in agreement with the formula $C_{20}H_{32}$. The fragments were similar to those proposed for the alcohol, I, except that no ions involved the loss of the elements of water due to the absence of the hydroxy group. Prominent peaks at m/e 136 and 137 have been explained (103) and their mechanisms of cleavage are shown below.

M⁺

m/e 136 (47%)

M⁺

m/e 137 (91%)
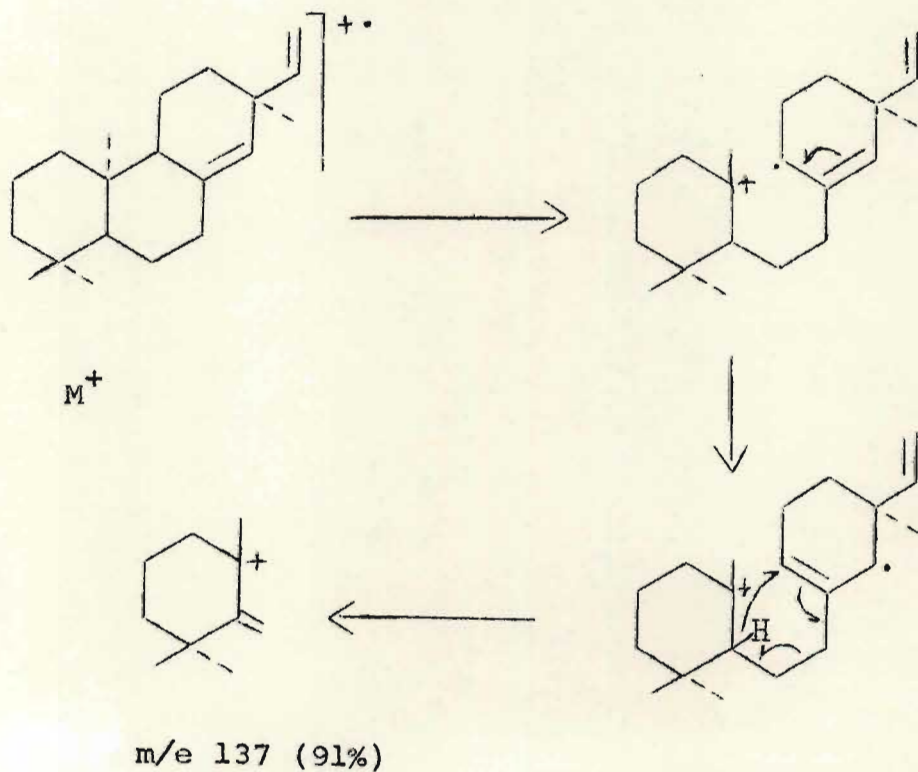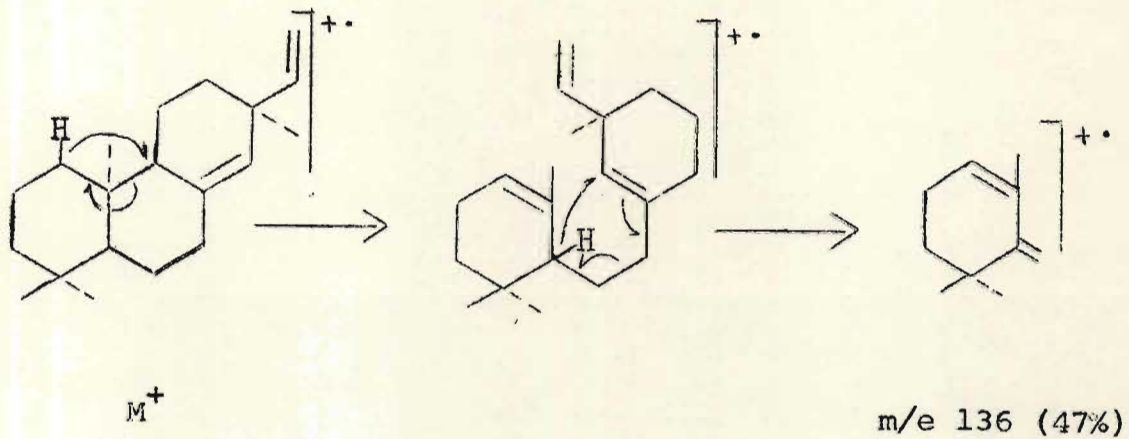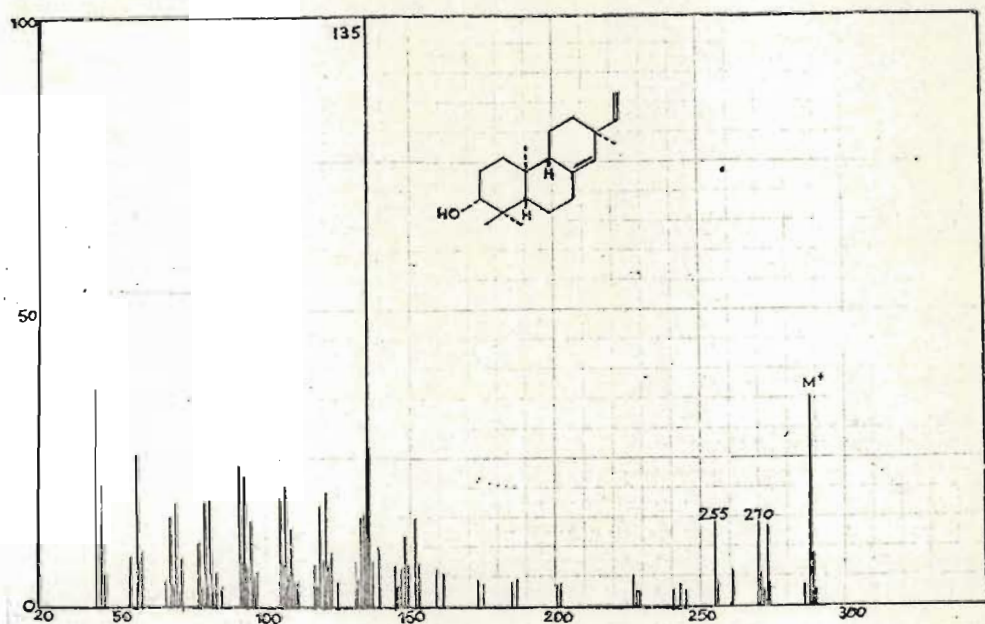
FIGURE 9   MASS SPECTRUM OF 3α-HYDROXY-ent-ISO-
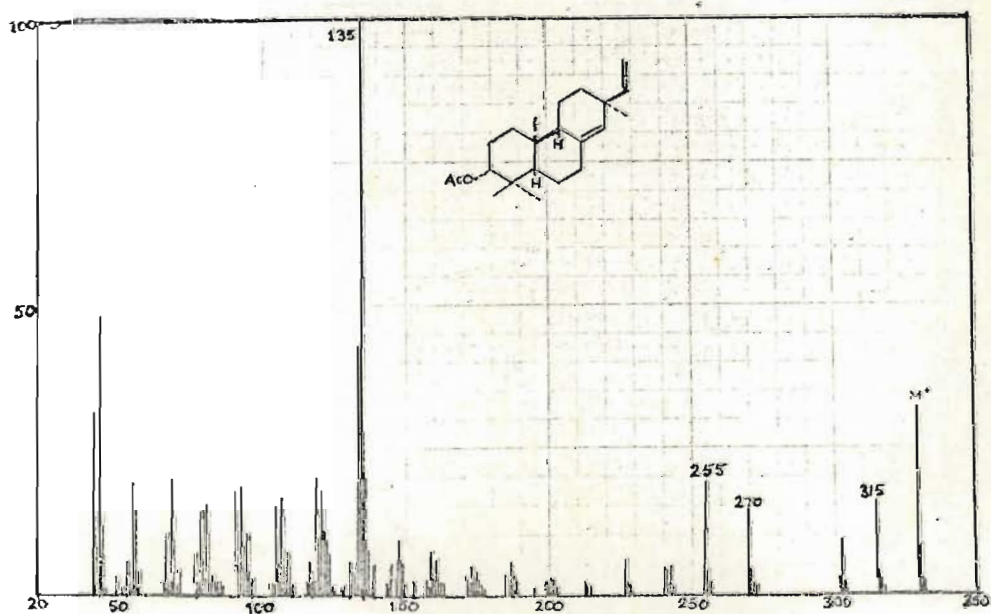
PIMARA-8(14),15-DIENE

FIGURE 10   MASS SPECTRUM OF 3α-ACETOXY-ent-ISO-
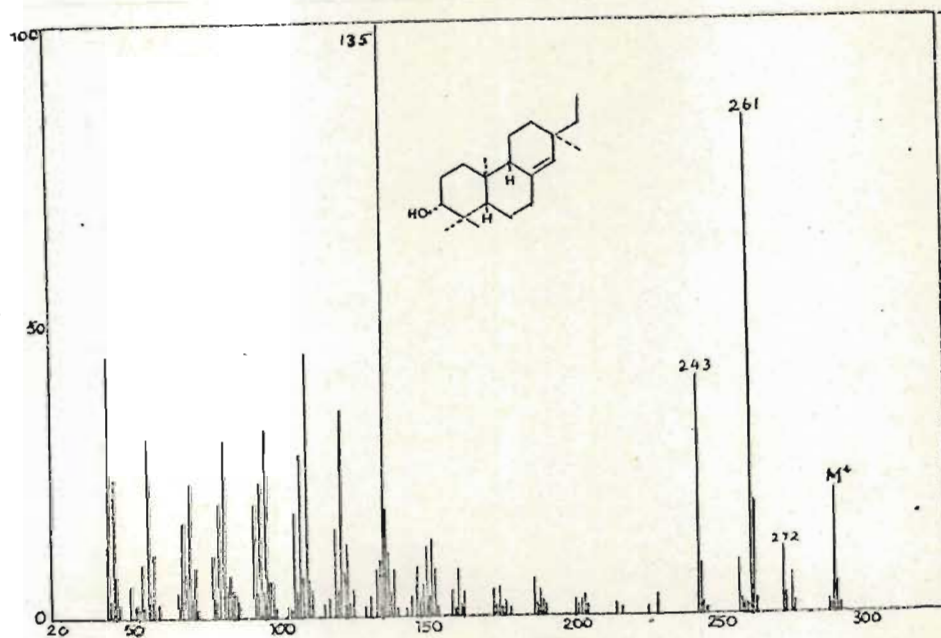
PIMARA-8(14),15-DIENE

FIGURE 11   MASS SPECTRUM OF 3α-HYDROXY-ent-
ISOPIMAR-8(14)-ENE
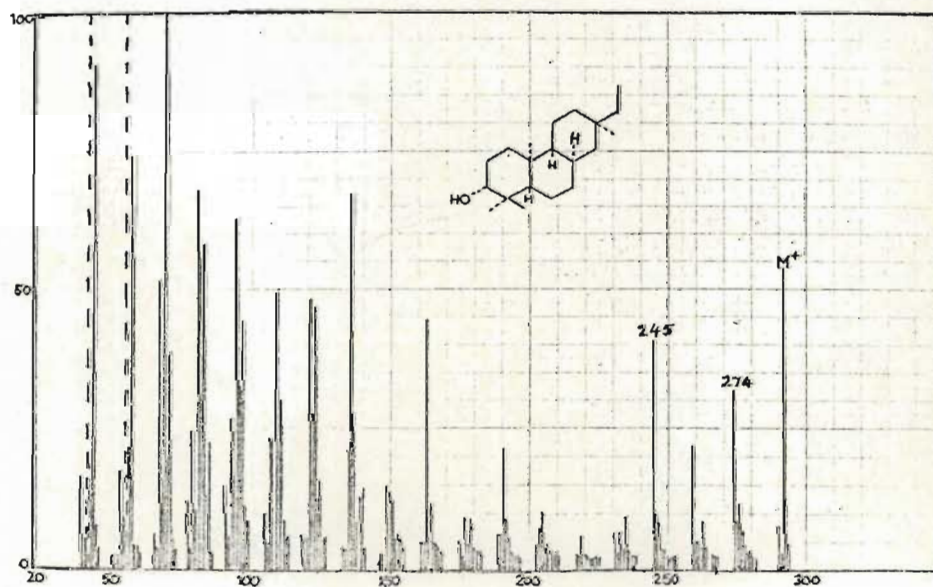
FIGURE 12   MASS SPECTRUM OF 3α-HYDROXY-ent-ISOPIMARANE
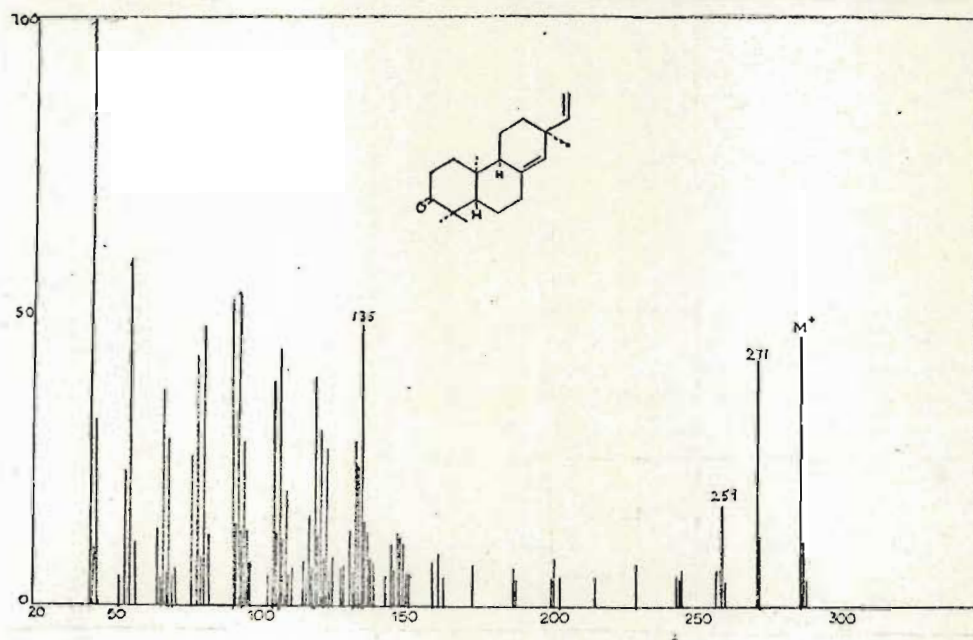
FIGURE 13   MASS SPECTRUM OF <u>ent</u>-ISOPIMARA-8(14),15-DIEN-3-ONE
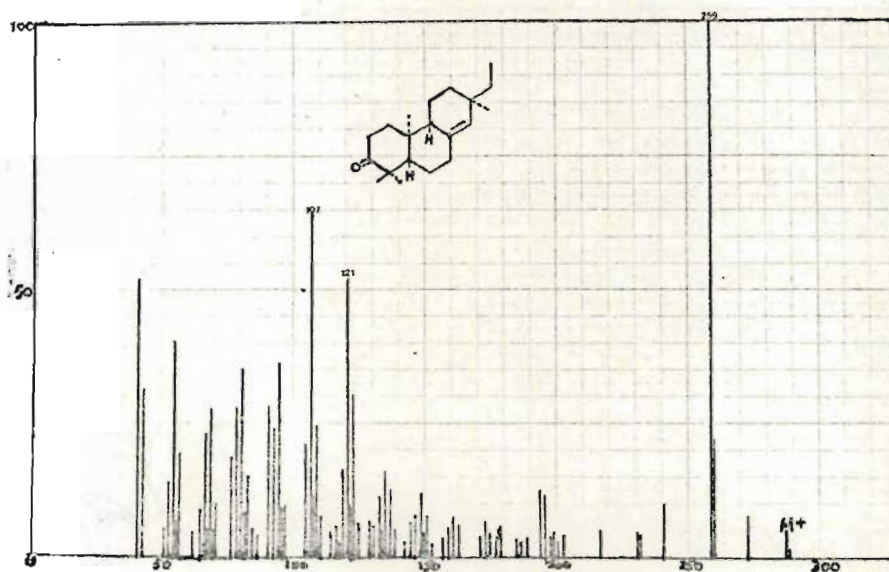
FIGURE 14   MASS SPECTRUM OF <u>ent</u>-ISOPIMAR-8(14)-EN-3-ONE
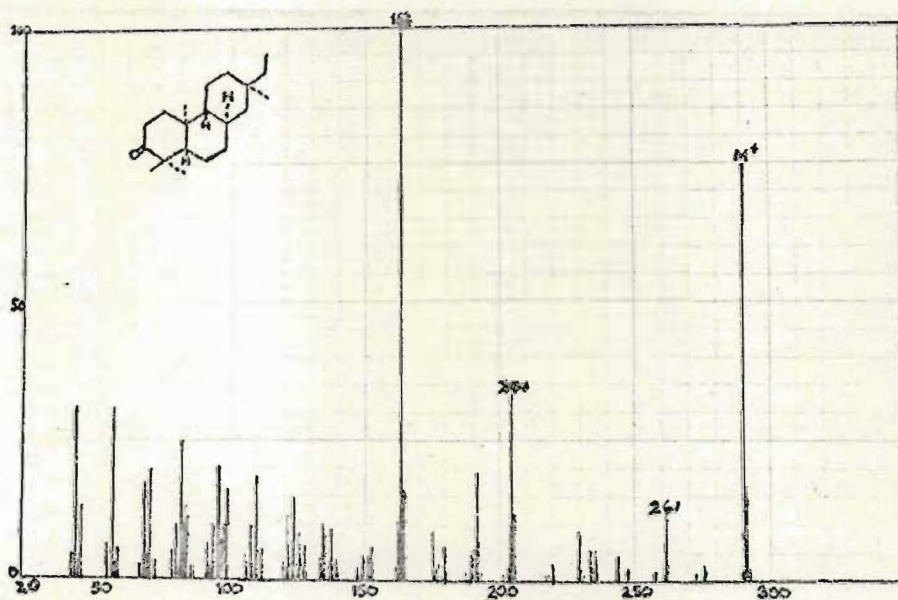
FIGURE 15 MASS SPECTRUM OF ent-ISOPIMARAN-3-ONE
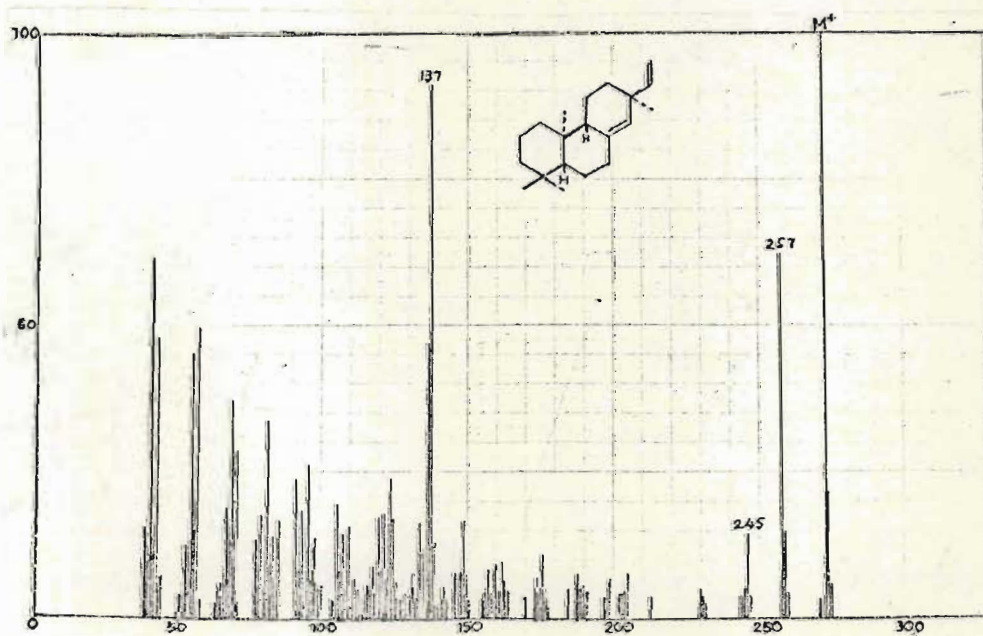
FIGURE 16 MASS SPECTRUM OF ent-ISOPIMARA-8(14),15-DIENE

1.2. 3α-Hydroxy-ent-isopimara-8(14),15-dien-12-one, II.

The second crystalline component obtained in fair yield (0.2% of the heartwood) by means of column chromatography was 3α-hydroxy-ent-isopimara-8(14),15-dien-12-one, II.

As in the case of the alcohol, I, this compound produced no colour with the Liebermann-Burchard reagent or on treatment with neutral methanolic ferric chloride, but a positive tetranitromethane test indicated the presence of un-saturated olefinic linkages.

The Oxygen Functions

In the infrared spectrum of this diterpene, $C_{20}H_{30}O_2$ (Figure 17) bands at 3547 and 1696 cm.$^{-1}$ implied that the molecule possessed both hydroxy and carbonyl substituents. The position of the carbonyl absorptions for six-membered ring ketones is usually in the region between 1705 - 1725 cm.$^{-1}$, and thus the low frequency of this absorption in the spectrum of II was probably due to association in the solid state or the proximity of double bonds to the carbonyl function (112).

The formation of both a monoacetate, $C_{22}H_{32}O_3$, XCI, and a mono-oxime, $C_{20}H_{31}NO_2$, XCII, substantiated the presence of a single hydroxy and a single keto group in this compound. In the infrared spectrum of the ketol acetate the expected carbonyl absorption appeared at 1700 cm.$^{-1}$ together with the additional acetate carbonyl stretching vibration at 1723 cm.$^{-1}$ and the ester C-O stretching vibration at 1243 cm.$^{-1}$, while bands due to the

hydroxy group disappeared. The infrared spectrum of the oxime exhibited the characteristic C=N stretching frequency at 1645 cm.$^{-1}$ as a moderately strong peak while the carbonyl band disappeared. A broad band centred around 3300 cm.$^{-1}$ could be assigned to the C-OH and N-OH stretching vibrations.
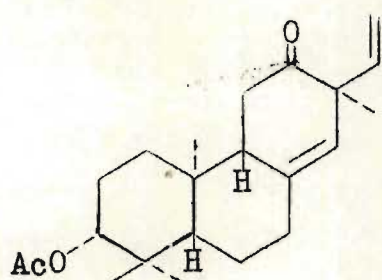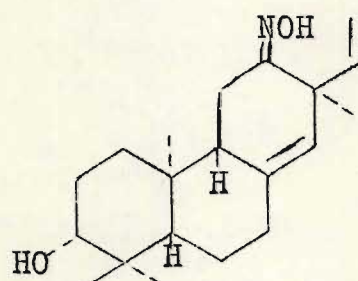
XCI   XCII

However, the failure of the ketol, II, to produce a red colouration with alkaline triphenyltetrazolium chloride (113) revealed that the hydroxy group was not located ∝ to the keto function. This was supported by the fact that the ketol failed to react with sodium metaperiodate solution, and thus the positions of the oxygen functions were deduced chemically and spectroscopically.

The Double Bonds

The infrared spectrum of the ketol, II, also exhibited olefinic absorptions at 3085 and 1628 cm.$^{-1}$ similar to those encountered for the alcohol, I. However, the determination of the number of double bonds present was complicated by the presence of the carbonyl group.

Mild hydrogenation of the ketol employing a palladium on carbon catalyst in ethanolic medium resulted in the rapid formation of the dihydro derivative, $C_{20}H_{32}O_2$, XCIII (a), which on acetylation yielded the acetate, $C_{22}H_{34}O_3$, XCIII (b). Under the more drastic hydrogenating conditions in which Adam's catalyst in glacial acetic acid medium were employed the ketol rapidly absorbed one mole of hydrogen, but this was followed by a slower uptake which continued for two days. At the end of this period the calculated volume of hydrogen absorbed was equivalent to three moles. Thus if it was assumed that one mole of hydrogen had been consumed in the reduction of the carbonyl group, the remaining two moles must have been used to reduce two olefinic linkages. The presence of two double bonds in the ketol, II, was substantiated by performing a Huang-Minlon reduction (99) to remove the carbonyl function. The resulting alcohol was characterised as being identical to I, and hydrogenation under high pressure over Adam's catalyst in glacial acetic acid gave rise to the tetrahydro alcohol, LXXIX. This established unambiguously that the ketol, II, possessed the ent-isopimara-8(14),15-diene skeleton and furthermore, that the hydroxy group was located at C-3. It therefore remained to determine the position of the keto function.

In the infrared spectrum of the dihydro-ketol, XCIII (a) (Figure 18) the free hydroxy vibration appeared at 3542 cm.$^{-1}$ together with a broad band at 3285 cm.$^{-1}$ attributable to some degree of hydrogen bonding. The C-H stretching
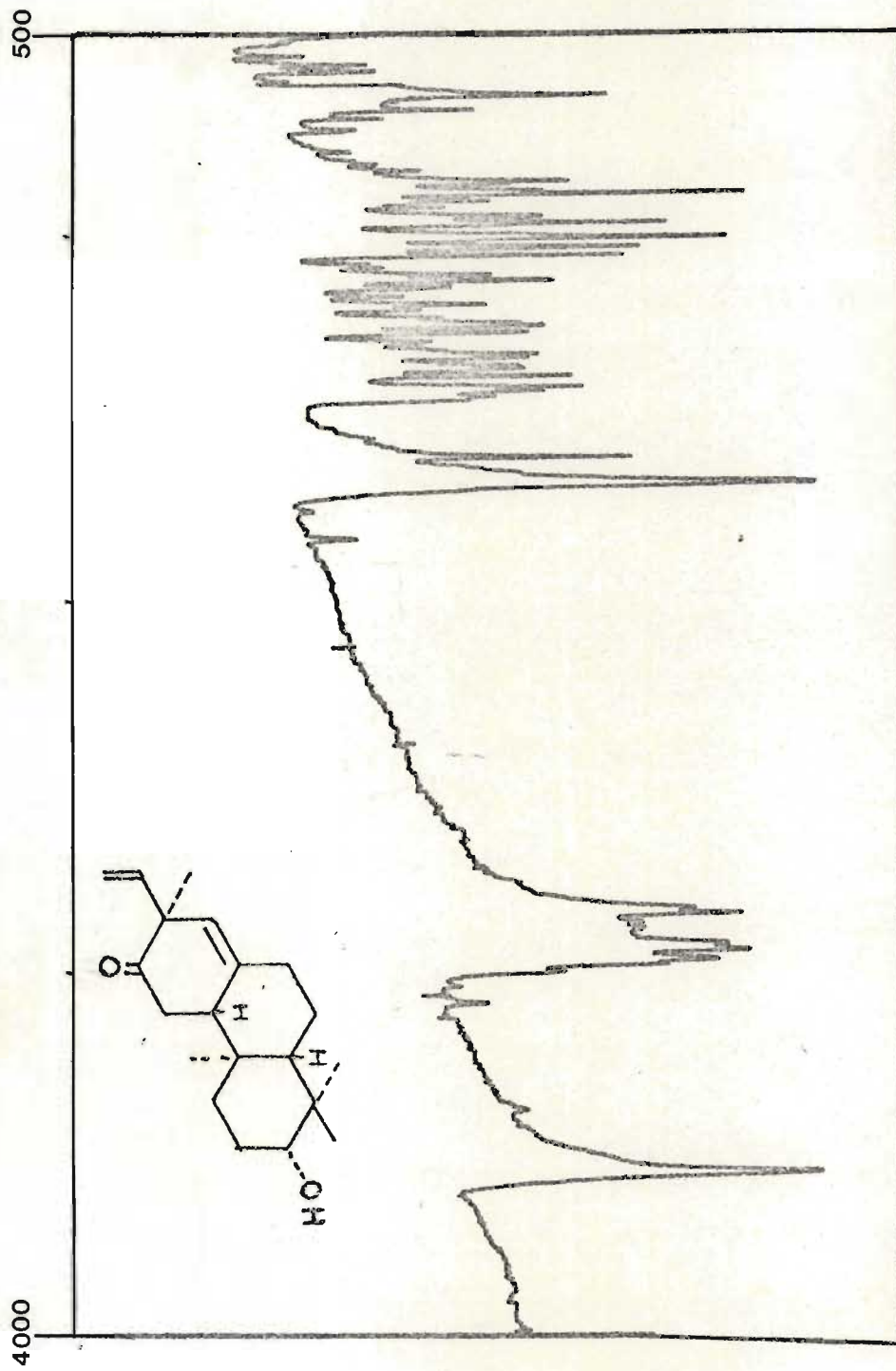
FIGURE 17 IR SPECTRUM OF 3α-HYDROXY-ent-ISOPIMARA-8(14),15-DIEN-12-ONE
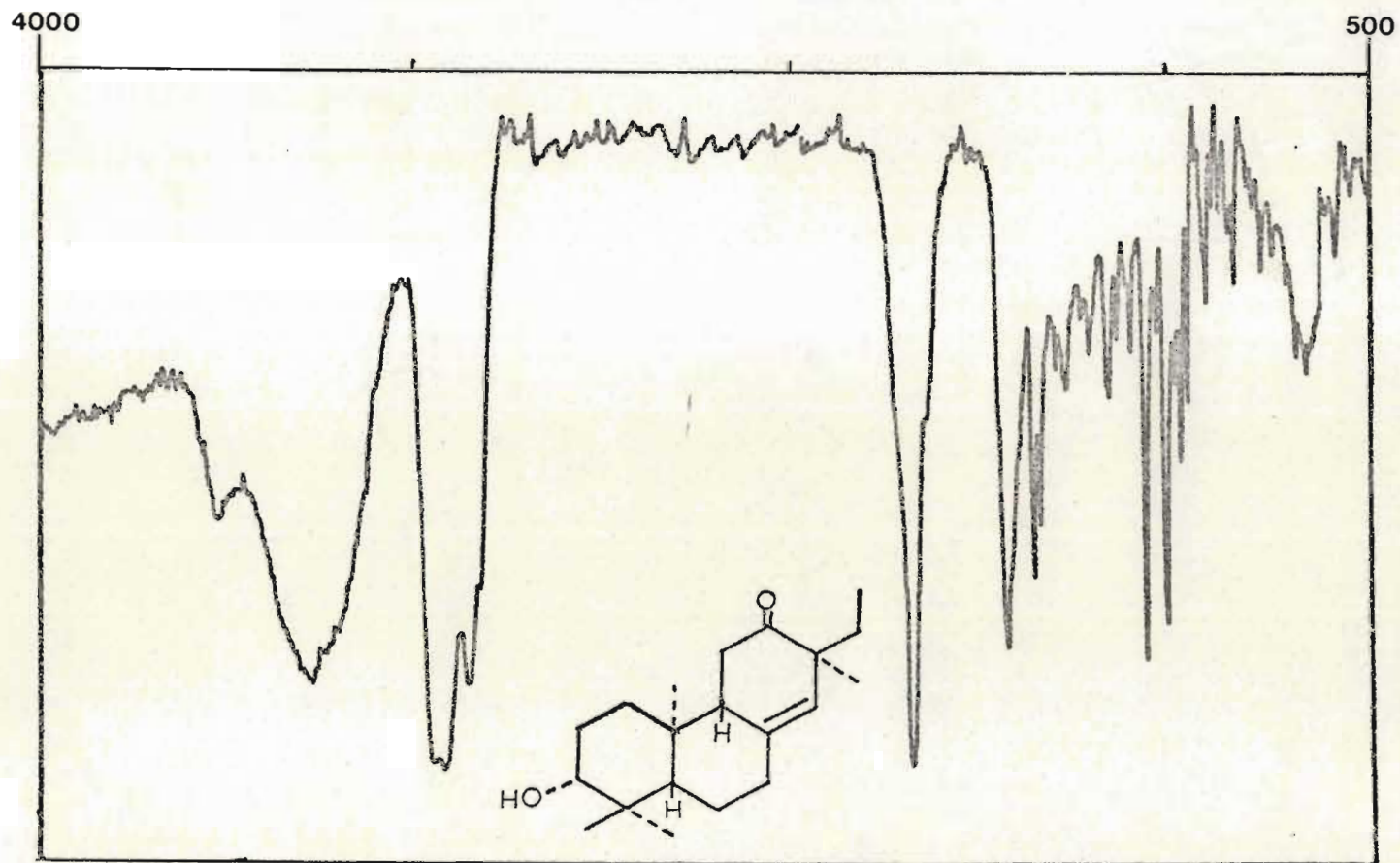
FIGURE 18   IR SPECTRUM OF 3α-HYDROXY-_ent_-ISOPIMAR-8(14)-EN-12-ONE

vibration of the vinyl group was no longer present, while the C=C absorption of the nuclear double bond occurred at 1655 cm.$^{-1}$ although somewhat obscured by the intense carbonyl absorption at 1712 cm.$^{-1}$. The marked increase in frequency of the latter vibration upon hydrogenation of the vinyl group implied that the keto grouping was affected by the C-15, C-16 double bond and thus that the two substituents were probably in close proximity to each other.

(a) R=H
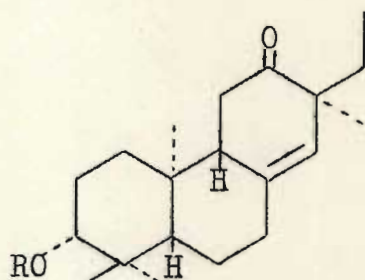
XCIII   (b) R=Ac

The spectrum of the dihydro-acetate, XCIII (b) exhibited no hydroxy absorptions and only a weak band at 1640 cm.$^{-1}$ due to the 8(14)C=C stretching vibration which was almost concealed by the intense band at 1700 cm.$^{-1}$ due to the ketone absorption. An intense acetate carbonyl stretching vibration occurred at 1725 cm.$^{-1}$ accompanied by the characteristic ester C-O vibration at 1242 cm.$^{-1}$.

Location of The Oxygen Functions

Reduction of the ketol, II, with lithium aluminium hydride or sodium in pentyl alcohol resulted in a diol which was chromatographically indistinguishable from the third

unknown compound, III. The facts that both the ketol and the synthetic diol failed to react with glycol splitting reagents and that no ethylidenedioxy derivative could be obtained from the diol implied the absence of a 1,2 or 1,3 oxygenation pattern. Furthermore, the diketone, $C_{20}H_{28}O_2$, XCIV, formed on Jones' oxidation of the ketol, II, did not enolise.

The infrared spectrum of the diketone, XCI, exhibited a double absorption in the carbonyl region at 1695 cm.$^{-1}$, but this did not facilitate the determination of the ketone positions in the molecule. Stretching vibrations due to the terminal methylene group and to the C=C bonds occurred as usual at 3078 and 1625 cm.$^{-1}$ respectively.

However, the ultraviolet absorption spectrum of the diketone, XCIV, in which a maximum appeared at 295 nm. (log ε 2.32) and failed to undergo any shift on the addition of a base, confirmed that the oxygen functions were not present as α- or β- diketo systems (114). Since the hydroxy group in the ketol, II, had been located at C-3, this evidence indicated that the carbonyl function was not situated at C-1 or C-2. In addition, the ultraviolet absorption spectrum of the ketol exhibited a maximum at 300 nm. (log ε 2.28) which is not in the region characteristic of α,β-unsaturated ketones. The position of this absorption in fact suggested that the carbonyl group was in close proximity to a number of alkyl groups or ring junctions, since it has been found that alkyl substitution in cyclohexanone displaces

the n$\rightarrow\pi^*$ band to wavelengths higher than the usual 285 nm.
(115). Due to the absence of α,β unsaturation C-7 could
thus be eliminated as a possible site for the keto group,
leaving C-6, C-11 or C-12 as the remaining positions. Of
these C-6 was considered most unlikely since steric hindrance
of carbonyl groups in this position renders them extremely
resistant to Huang-Minlon reduction (116).

The most satisfactory method of establishing the
position of the keto group was by the formation of an α,β-un-
saturated ketone whose structure could be determined unam-
biguously from its u.v. and n.m.r. spectra. This method has
been employed by Jefferies et al (76) who determined the
position of the C-12 oxygen function in 12β,17-dihydroxy-16α-
(-)-kauran-19-oic acid, LXIX.

In the present investigation the ketol acetate,
XCI, was completely hydrogenated over Adam's catalyst yield-
ing an alcohol, $C_{22}H_{38}O_3$, XCV, from which the carbonyl
group was regenerated by oxidation with Jones' reagent to
give 3α-acetoxy-ent-isopimaran-12-one, $C_{22}H_{36}O_3$, XCVI. The
α-bromoketone, $C_{22}H_{35}BrO_3$, XCVII, was produced upon treat-
ment of the ketone, XCVI, with a solution of bromine in
acetic acid and a trace of saturated hydrogen bromide
solution (76). Dehydrobromination of this compound was
most efficiently effected by refluxing its dimethylformamide
solution with a mixture of lithium bromide and lithium
carbonate (76), to afford the α,β-unsaturated ketone,
$C_{22}H_{34}O_3$, XCVIII.
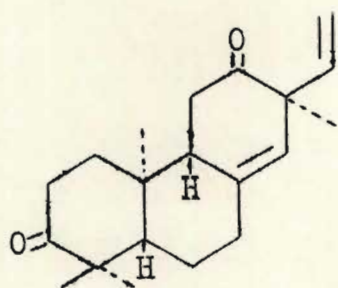
XCIV
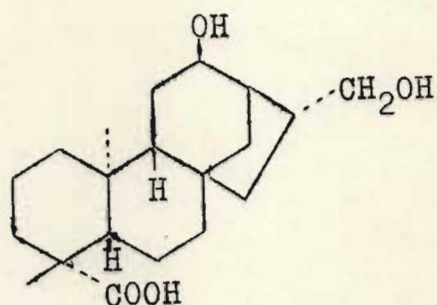
LXIX
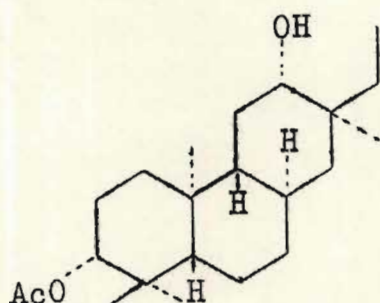
XCV
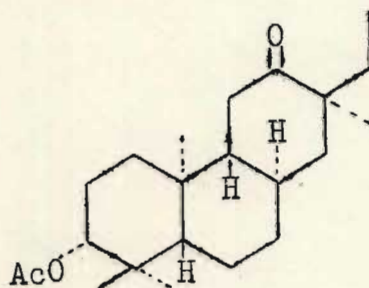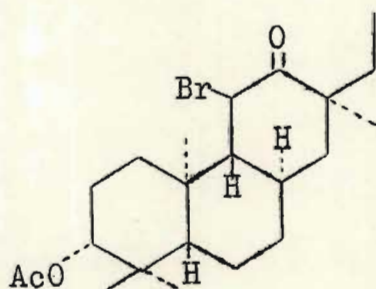
XCVI

XCVII
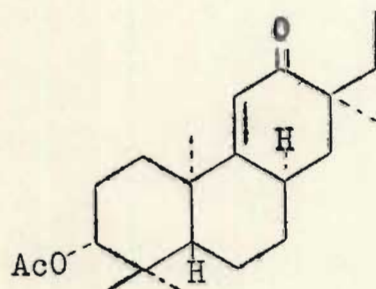
XCVIII

The infrared spectrum arising from the alcohol, XCV, displayed no olefinic absorptions as expected, but a sharp band due to the hydroxy function appeared at 3510 cm.$^{-1}$ together with the acetate carbonyl and ester C-O vibrations at 1718 and 1240 cm.$^{-1}$ respectively.

The disappearance of the hydroxy band and the appearance of a new intense band at 1700 $cm.^{-1}$ in the spectrum of the ketone, XCVI indicated that oxidation of XCV had regenerated the required ketone. α-Bromination produced a broadening of the carbonyl band and a slight bathochromic shift to 1695 $cm.^{-1}$ in the spectrum of the α-bromoketone, XCVII. This effect, due to the strongly electronegative character of the bromine atom, indicated the axial orientation of the bromine (109) but not its location in the molecule.

Its ultraviolet absorption spectrum, however, was most useful in determining the configuration of the bromo- group. Cookson (117) had shown that in conformationally rigid steroid and triterpenoid ketones, a distinction could be made between axial and equatorial bromine on the α-carbon atom. In general an axial substituent caused an average bathochromic shift of 28 nm. while an equatorial substituent produced an average hypsochromic shift of 5 nm. Thus the maximum at 325 nm. (logε 1.89) in the u.v. spectrum of the α-bromoketone compared with the maximum at 300 nm. (logε 1.52) for the parent ketone indicated that the bromine was axially orientated, giving rise to 3α-acetoxy-11β-bromo-ent-isopimaran-12-one, XCVII.

The infrared spectrum of the α,β-unsaturated ketone, XCVIII, exhibited its carbonyl stretching frequency at 1657 $cm.^{-1}$ and new absorptions due to the conjugated double bond at 3048 and 1603 $cm.^{-1}$ in agreement with expectation. Ratios of the C=O band intensity to the C=C band intensity

have been employed in establishing the conformation of α,β-unsaturated ketones (118) and in this case the value of approximately 3 falls within the range of 0.6-3.5 for <u>cisoid</u> ketones. Furthermore, in the ultraviolet absorption spectrum an intense maximum at 241 nm. (logϵ 4.16) was in fair agreement with the value of 215 nm. calculated on the basis of the Woodward rules (119) for a 9(11)-en-12-one.

Nuclear Magnetic Resonance Spectra

A study of the n.m.r. data accumulated for the ketol and its derivatives has confirmed many of the structural assignments made. From the n.m.r. spectrum of the ketol, II, (Figure 19) it was obvious that the olefinic double bond systems were identical to those present in the C-3 alcohol, I. The quartet centred at τ4.25 arose from the resonance of the C-15 vinyl proton which formed the X part of an ABX system (observed coupling constants were J_{trans}18 Hz, J_{cis}10 Hz), while the two non-equivalent C-16 protons constituted the AB part and gave rise to a multiplet at τ4.89 (observed geminal splitting J_{gem}1.5 Hz). However, the C-14 proton resonance at τ4.76 showed no detectable allylic coupling which was perhaps indicative of the location of the carbonyl group in the C-ring causing a conformational deviation which was not conducive to allylic interaction between the protons at C-14 and C-9.

The C-3 proton adjacent to the hydroxy group

resonated as a multiplet at τ6.76 (observed axial-
equatorial splitting approximately 6 Hz) which was con-
sistent with its assigned axial orientation (96). In the
region around τ7.5 a multiplet occurred arising from signals
due to protons adjacent to the carbonyl function and the
allylic C-7 methylene protons, but this splitting pattern
could not be readily analysed.

The signals of the four methyl groups at C-13, C-4
equatorial and axial and C-10 resonated as singlets at τ8.78,
8.87, 9.20 and 9.29. A comparison of these values with
those for the alcohol, I, showed that the introduction of a
keto- group caused a deshielding of the C-13 methyl group
by 0.17 p.p.m. and a shielding of the C-10 methyl group by
0.10 p.p.m. These effects could be explained by the
anisotropy of a carbonyl group at C-12 (76).

In the spectrum of the ketol acetate, XCI, the only
significant differences were those caused by acetylation.
The C-3 proton shifted predictably downfield to τ5.5 and
exhibited axial-equatorial splitting of the order of 6 Hz
which was indicative of the equatorial configuration of the
acetate moiety (96). An intense singlet at τ7.96 was also
attributable to the acetate methyl protons.

The spectrum of the dihydroketol, XCIII (a),
differed from that of the ketol by the significant absence
of signals due to the C-15 and C-16 vinyl protons. This
resulted in a spectrum with an uncomplicated olefinic region

displaying a singlet at τ4.7 due to the C-14 proton. The methyl region presented a somewhat more complex splitting pattern since hydrogenation of the C-15(16) double bond resulted in a primary methyl group at C-16 which resonated as a partially concealed triplet centred at τ9.2.

Acetylation of the dihydroketol gave rise to a spectrum in which the C-3 proton signal shifted downfield to τ5.5 and a new singlet appeared at τ7.98 due to the acetate methyl group.

The saturated nature of the tetrahydro ketone, XCVI, was confirmed by the absence of any signals in the olefinic region of its n.m.r. spectrum. A quartet at τ7.8 could be attributed to the two non-equivalent methylene protons adjacent to the carbonyl group since this resonance disappeared on α-bromination of the ketone. The methyl region of the spectrum was very similar to that discussed in the spectrum of the dihydro compound.

The spectrum of the α-bromoketone, XCVII, (Figure 20) proved to be very important diagnostically. From the study (105) of a large number of α-haloketones an interesting observation was that the chemical shift of hydrogens attached to the halogen bearing carbon atom occurred in the region between τ4.9 - 6.0. Furthermore, the signal for an equatorially orientated hydrogen was found to resonate at higher field than its epimer bearing an axially orientated proton. This reversal of the usual axial-equatorial relationship discussed in the spectra of the alcohols and acetates has

been reported for several pairs of epimeric α-bromocyclo-hexanones (106). Thus in the case of this α-bromoketone, XCVII, the doublet at τ6.0 indicated that the bromine was in the axial configuration, thereby substantiating the evidence provided by the ultraviolet absorption spectrum discussed earlier. The observed splitting of 4 Hz was consistent with the range (1 - 5 Hz) for vicinal axial-equatorial interaction (104). Since splitting was observed a distinction could be made between C-11 and C-12 as possible sites for the carbonyl group if the C-6 position was discounted. Had the keto-function been located at C-11, bromine substitution at C-12 would have resulted in a singlet due to the C-12 proton whilst bromination at C-9 would not have produced a signal in this region. If the C-6 position was considered a likelihood for the keto-function the same resonance would have arisen, but it will be shown at a later stage that this site did not satisfy all the requirements.

From a consideration of molecular models the chair conformation is probably the most stable conformation for the tetrahydroketone, XCVI, to adopt. If the addition of bromine occurs by the usual   mechanism, then the bromine would approach the molecule from the sterically least hindered side. Since the C-11 site is considerably hindered by the C-10 axial (α) methyl group, approach by the bromine will be from the opposite face of the molecule. This requires that bromine approaches from the β side, and this reaction would produce the 11β-bromo compound with ring C

in the boat conformation. This conformation is preferred
to the theoretically more stable situation where the
bromine atom would be in the equatorial configuration and
ring C in the chair conformation (108). The formation of
the latter product is not favoured because of the severe
interaction which would occur between an equatorial C-11
bromine and the equatorial C-1 α-hydrogen. Supporting
evidence of the fact that the bromine is β-orientated was
provided by the shift in the C-10 methyl signal in the
n.m.r. spectrum. If the axial bromine had been in the α-con-
figuration a deshielding of the C-10 methyl group should
have been observed (76, 120), but instead there was a small
degree of shielding from τ9.13 in the tetrahydro ketone,
XCVI, to τ9.22 in the α-bromoketone, XCVII.

The n.m.r. spectrum of the α,β-unsaturated ketone,
XCVIII, exhibited a doublet at τ4.31 due to the vinylic
proton α- to the carbonyl group. This splitting (2 Hz) could
be attributed to allylic coupling between the C-11 and C-8
protons. From a consideration of molecular models the
dihedral angle between these two protons almost certainly
fell within the range of 60 - 110° which would give rise to
the predicted (93, 94) and observed magnitude for such
coupling constants.

A multiplet at τ7.4 was probably due to the C-8
allylic proton while there was no significant change in the
pattern of splitting of the methyl signals.
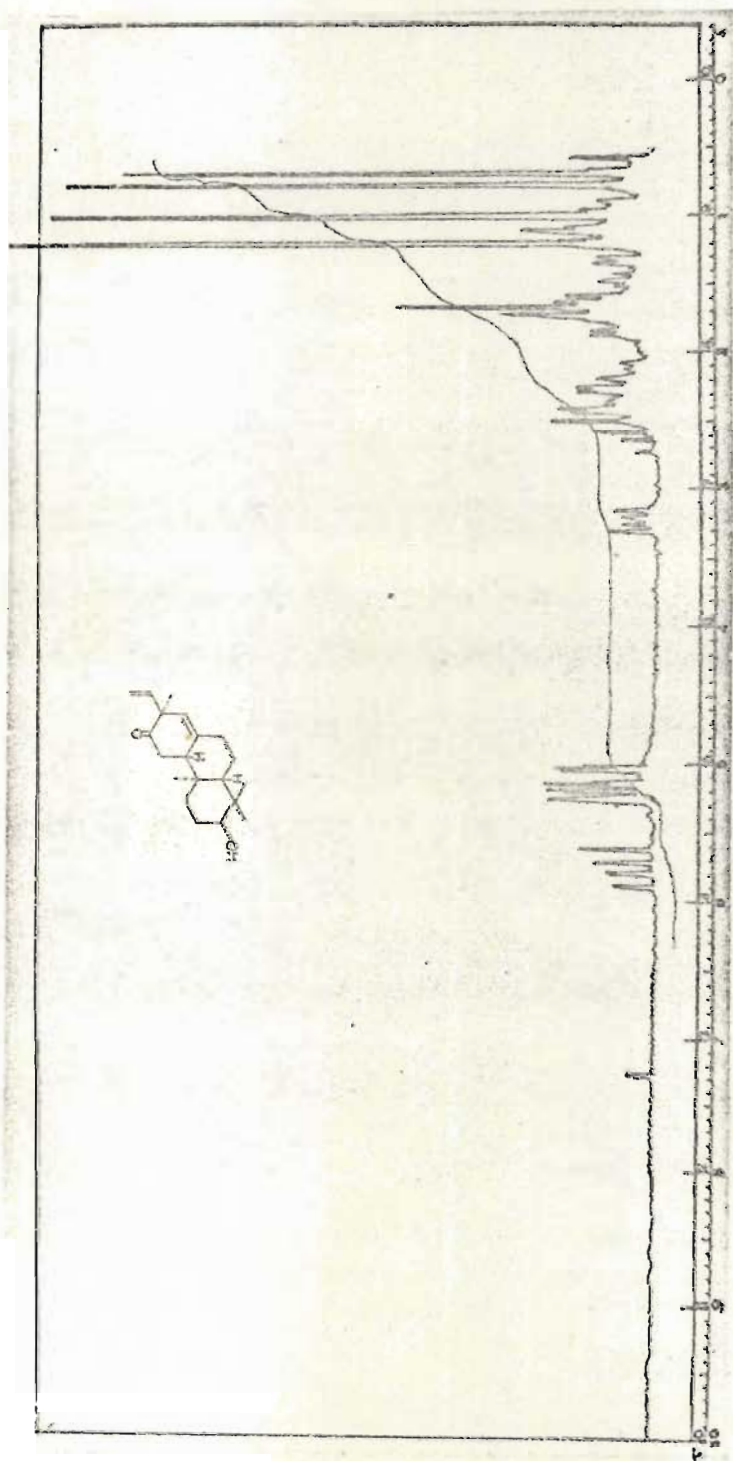
FIGURE 19 NMR SPECTRUM OF 3α-HYDROXY-ent-ISOPIMARA-8(14),15-DIEN-12-ONE
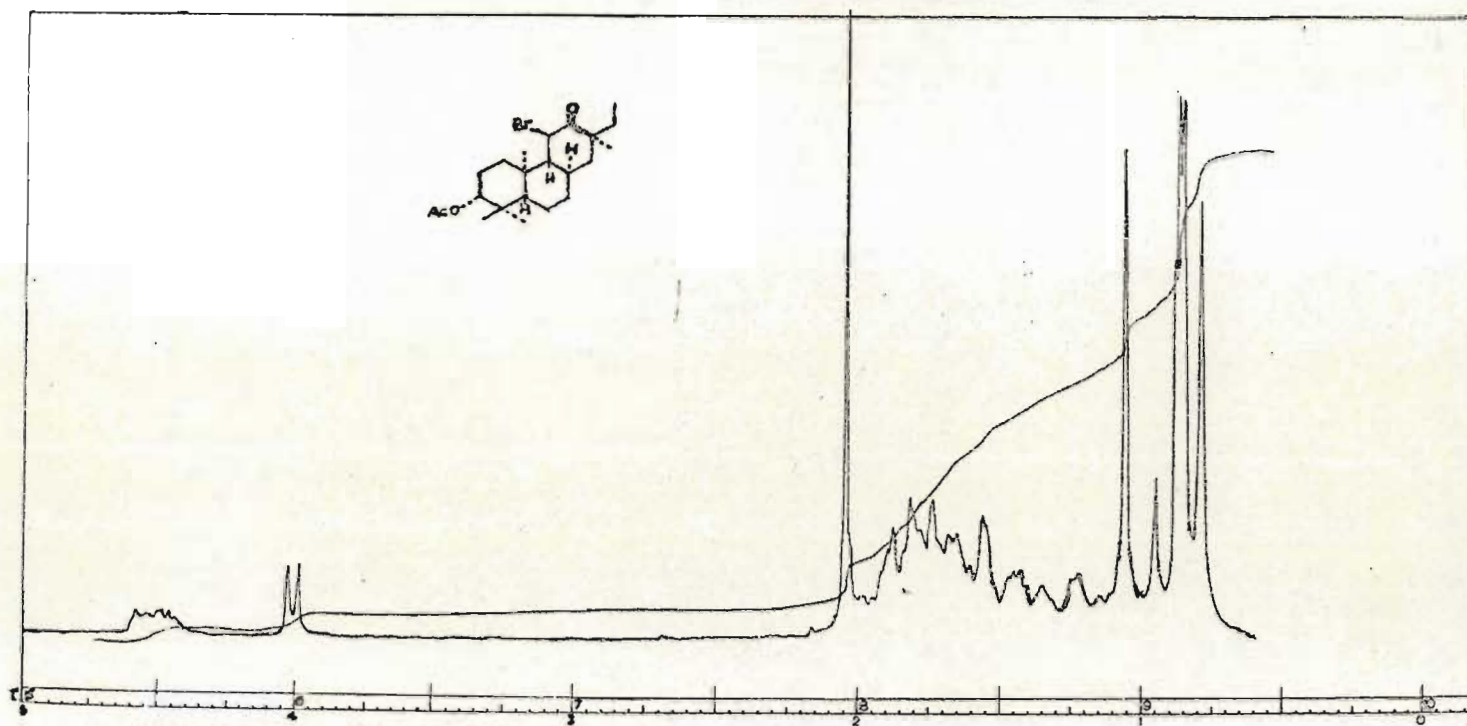
FIGURE 20   NMR SPECTRUM OF 3α-ACETOXY,11β-BROMO-ent-ISOPIMARAN-12-ONE
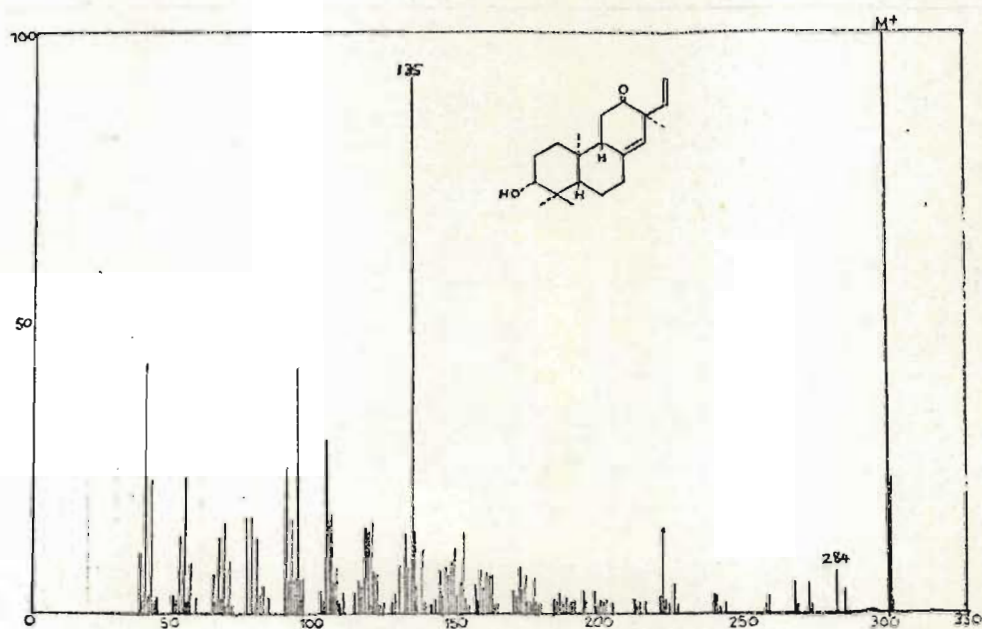
FIGURE 21   MASS SPECTRUM OF 3α-HYDROXY-ent-ISOPIMARA-
8(14),15-DIEN-12-ONE
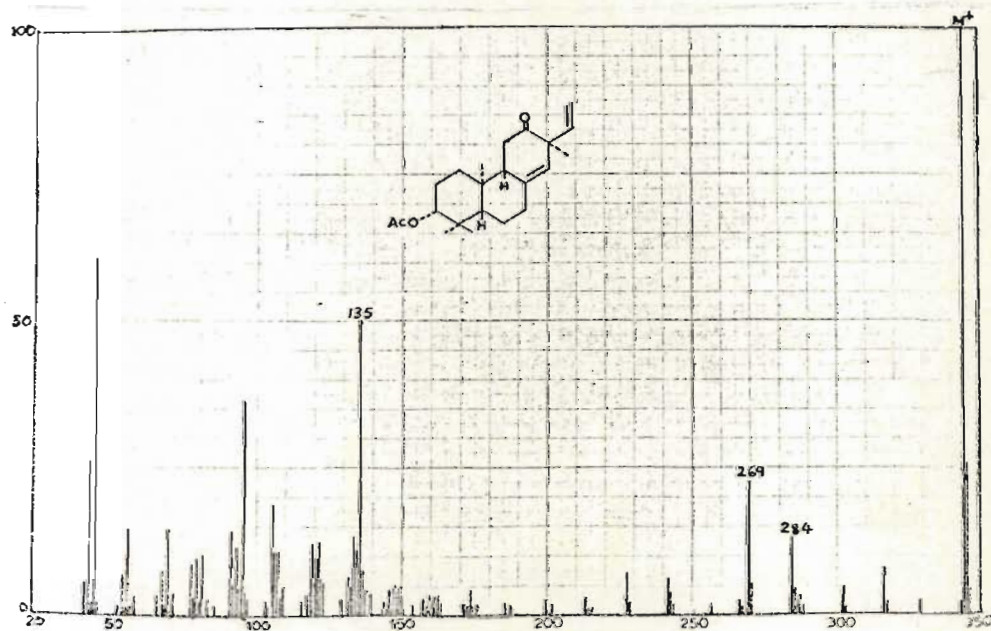
FIGURE 22   MASS SPECTRUM OF 3α-ACETOXY-ent-ISOPIMARA-
8(14),15-DIEN-12-ONE
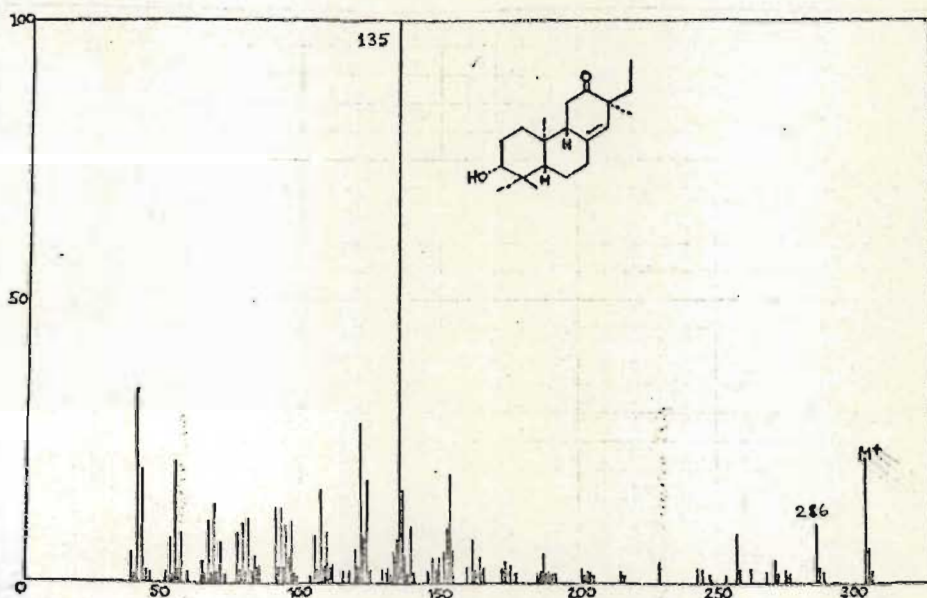
FIGURE 23   MASS SPECTRUM OF 3α-HYDROXY-ent-ISOPIMAR-
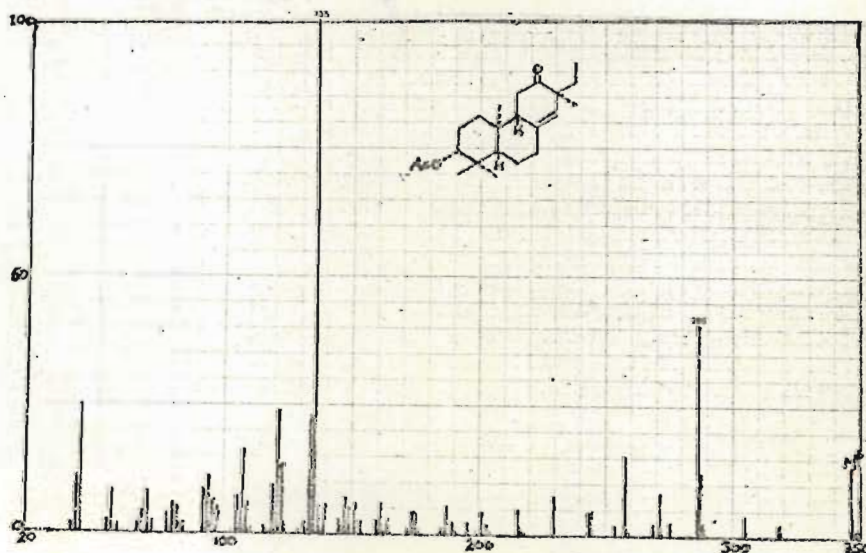
8(14)-EN-12-ONE

FIGURE 24   MASS SPECTRUM OF 3α-ACETOXY-ent-ISOPIMAR-

8(14)-EN-12-ONE

Mass Spectra

The spectra of the ketol, II, and its derivatives displayed fragmentation patterns which were very similar to those of the alcohol, I, and its derivatives discussed in the previous section.

Significant differences were only apparent in the upper half of the spectrum of the ketol (Figure 21) where the molecular ion at m/e 302 formed the base peak and marked decreases in the M-15 (m^* 273, 302 → 287) and M-33 ions were noted. In the spectra of both the ketol and its acetate (Figure 22) there was a complete absence of the M-27 fragment due to the loss of the C-13 vinyl group, whilst in the spectra of their dihydro derivatives (Figures 23 and 24) there was a large decrease in the abundance of the M-29 fragment. The decreased intensities in these ions which are formed by the loss of a C-13 alkyl substituent were not unexpected since the loss of one of these groups would give rise to a positive charge ∝- to the carbonyl group. Due to the electron withdrawing effect of a carbonyl group the positive charge would not be delocalised, and thus the formation of these ions was not a favoured process.

In addition to these changes in intensity three "new" fragments occurred at M-28, M-42 and M-75. The first of these may be explained by the elimination of carbon monoxide by the favoured fission of the bonds adjacent to the carbonyl group (121). This fragmentation was confirmed

by a metastable peak at m/e 249 (302   274).
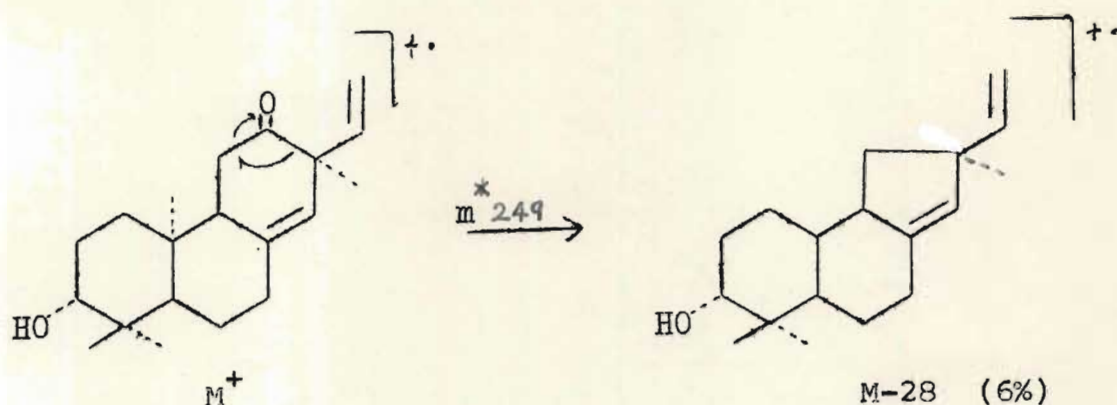
The formation of the M-42 fragment probably arose
from the molecular ion by a Retro-Diels-Alder fragmentation
leading to the elimination of the carbonyl group and the
α- methylene group as ketene. Fission of the bond β- to the
carbonyl group is usually a less favoured process than
fission of the α- bond, and in accordance with this the M-42
fragment was present in slightly lower abundance than the
M-28 ion in the spectrum of the parent ketol, II.
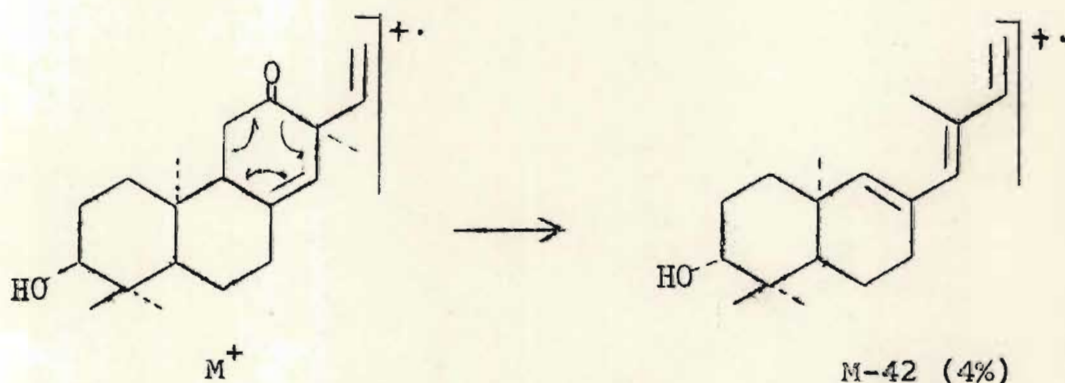
Finally, the M-75 fragment could be explained by
one of two possible fragmentations. The first would involve
the loss of the elements of water and the C-10 methyl group

from the M-42 ion as shown below, while a second process would involve the loss of ketene from the M-18 ion by the mechanism outlined above to give a M-60 ion (not present in the spectrum) which would then rapidly lose the C-10 allylic methyl group to produce the M-75 ion. Of the two possible pathways, the former was preferred.

M-42   M-75 (5.5%)
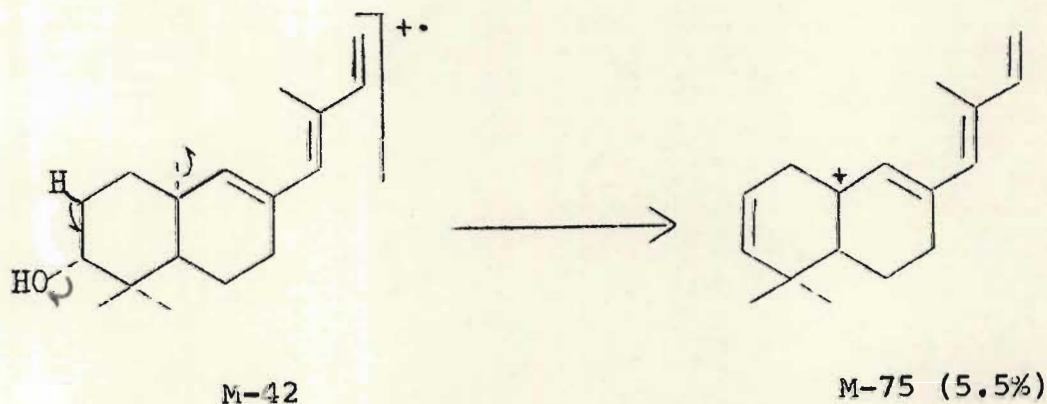
A peak appearing at m/e 148 in the spectrum of the alcohol, I, was absent in the spectrum of the ketol, but replaced by a peak at m/e 133 (M-169) which was derived from the M-28 ion by a process similar to that explained for the formation of the m/e 148 fragment from the alcohol, I , and is illustrated on page 94.

In the spectrum of the ketol acetate, XCI, (Figure 22) the molecular ion which again formed the base peak appeared at m/e 344. The main differences in fragment-ation, however, were the replacement of the M-18 fragment by an M-60 ion and the M-(18+15) ion by the relatively abundant M-(60+15) ion (22.5%).

A scheme of the overall fragmentation of the ketol, II, and its acetate, XCI, is shown in Scheme 5.

M-75 M-42 M-28 M-169

M-168 M-150 M$^+$ M-18

M-167 M-149 M-15 M-33

M-183

SCHEME 5

93

M-169 (13.5%)
m/e 133
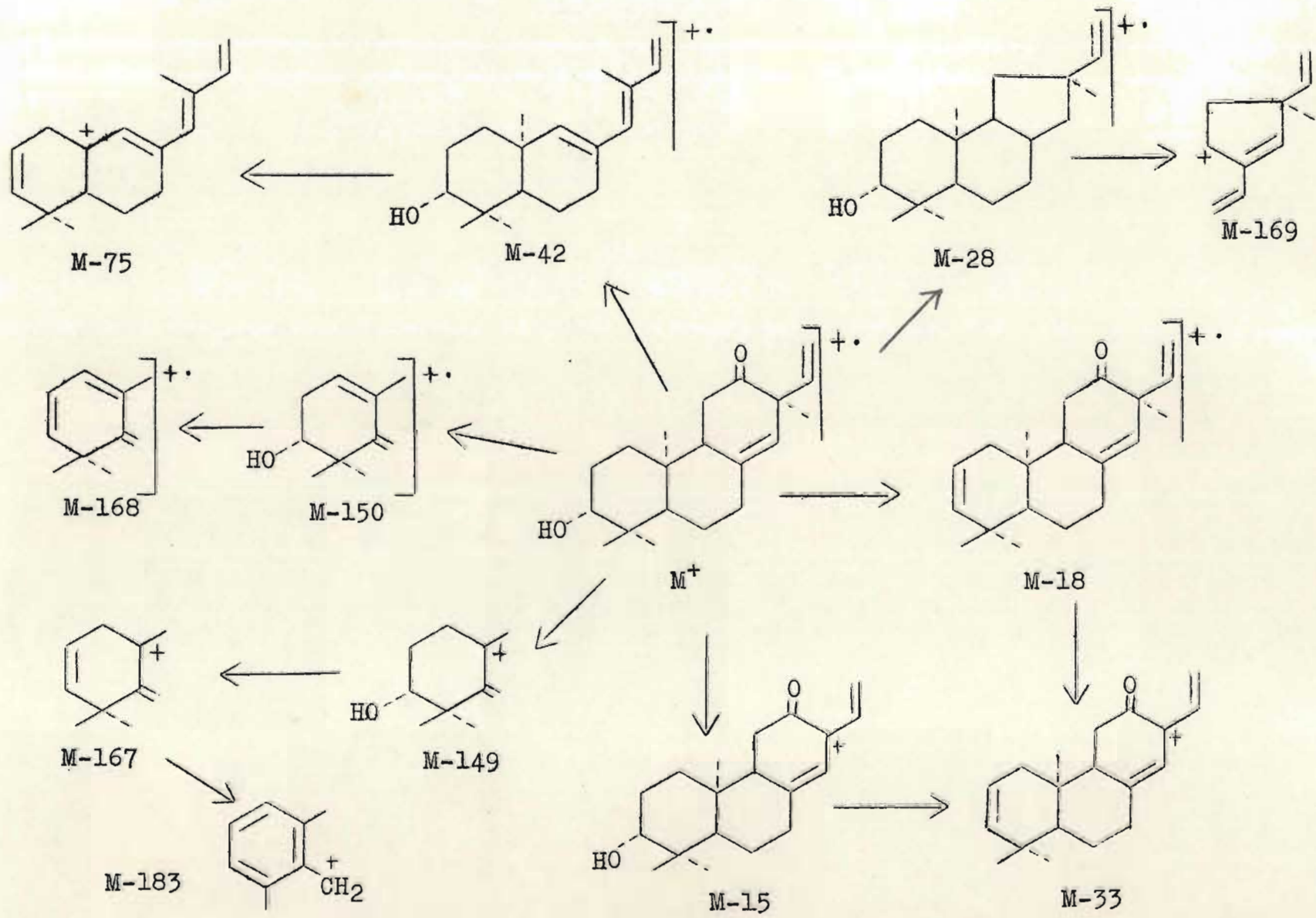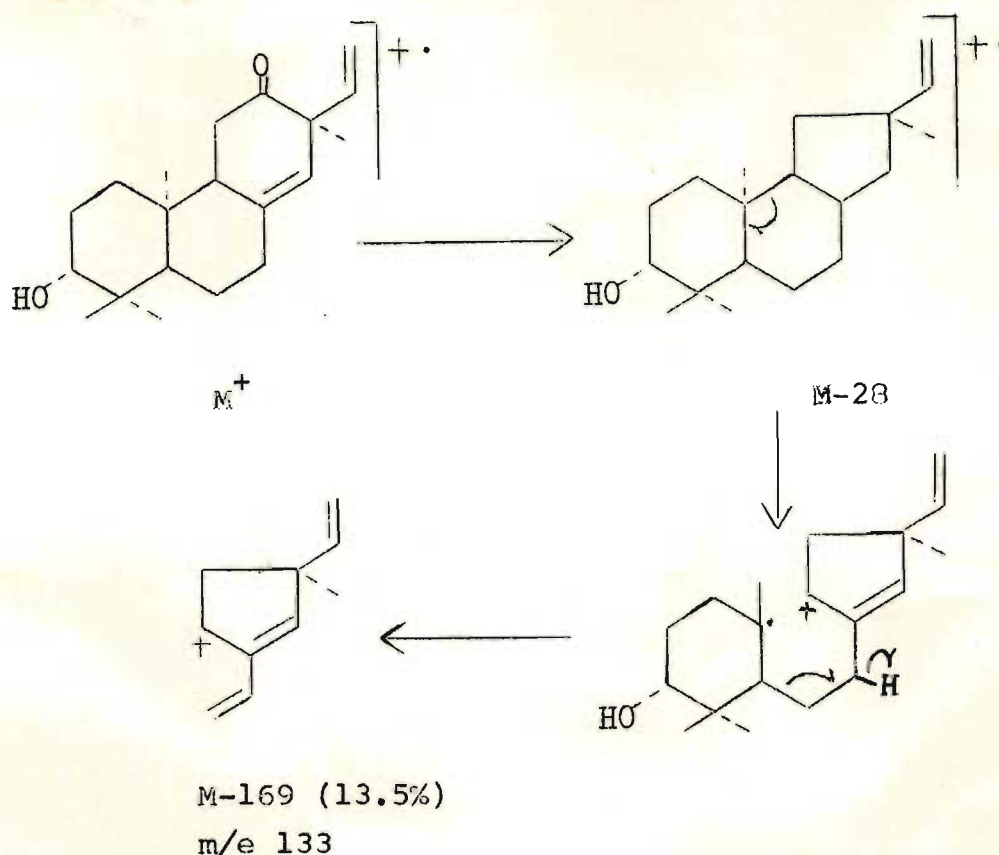
The spectrum of the tetrahydroketol acetate, XCVI, displayed the molecular ion at m/e 348 (66.5%) as well as a number of prominent peaks in the upper half of the spectrum. Since the mechanisms for the formation of these fragments have already been discussed, the proposed structures for the most significant ions are shown in Scheme 6.

In the spectrum of the α-bromoketone, XCVII, there were two molecular ion peaks at m/e 426 and 428 due to the presence in the molecule of two isotopes of bromine (Br^{79} and Br^{81}) in approximately equal abundance. The spectrum was relatively simple and exhibited prominent peaks at M-60 and M-80 arising from the loss of the elements of acetic acid and hydrogen bromide respectively. (page 96)

SCHEME 6

m/e 320, M-28 (100%)

M⁺

m/e 323, M-15 (15%)

m/e 288, M-60 (53.5%)

m/e 273, M-75 (40%)

m/e 260, M-88 (37.5%)

m/e 245, M-103 (32%)

M⁺   M-80 (14%)
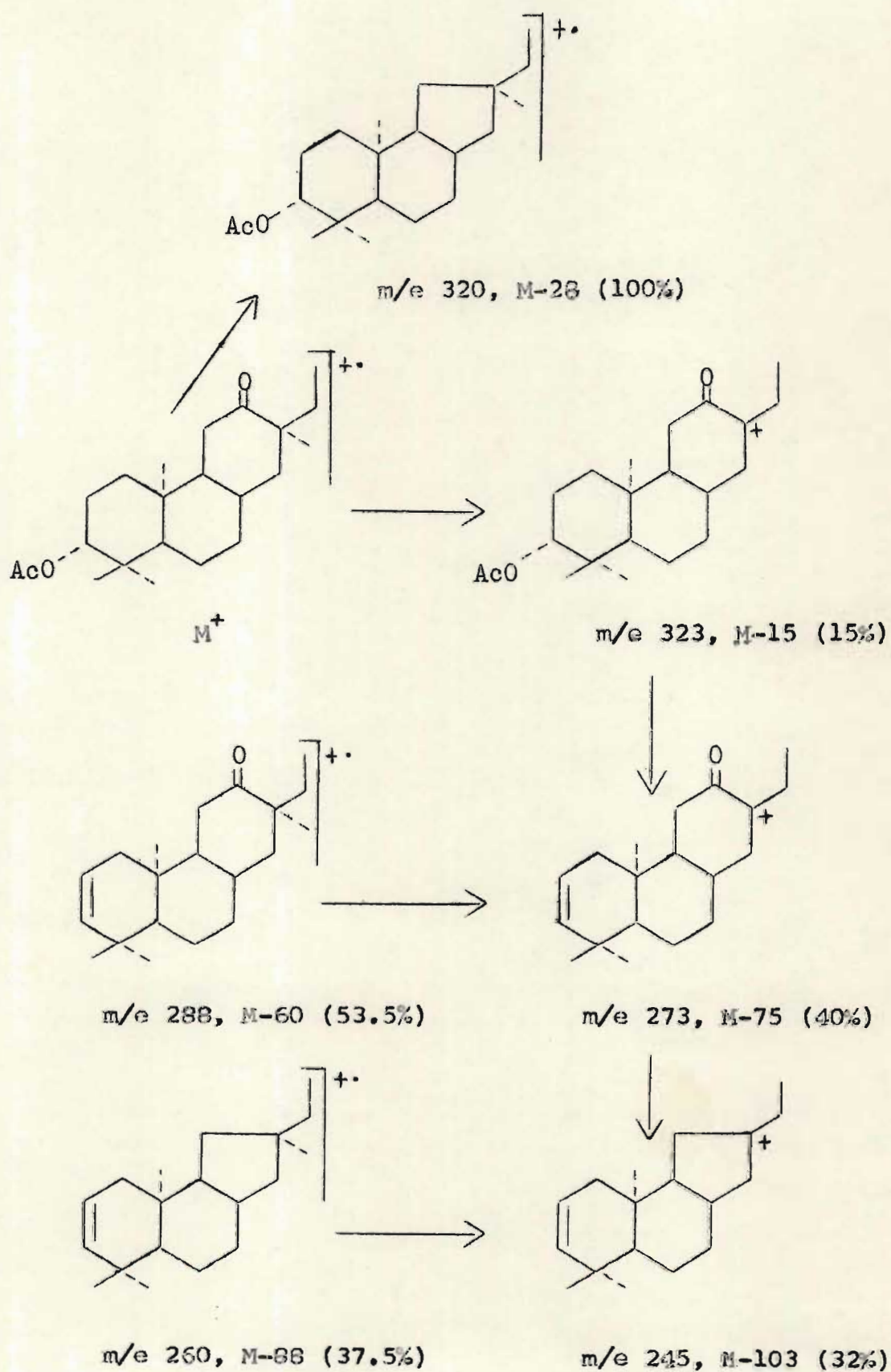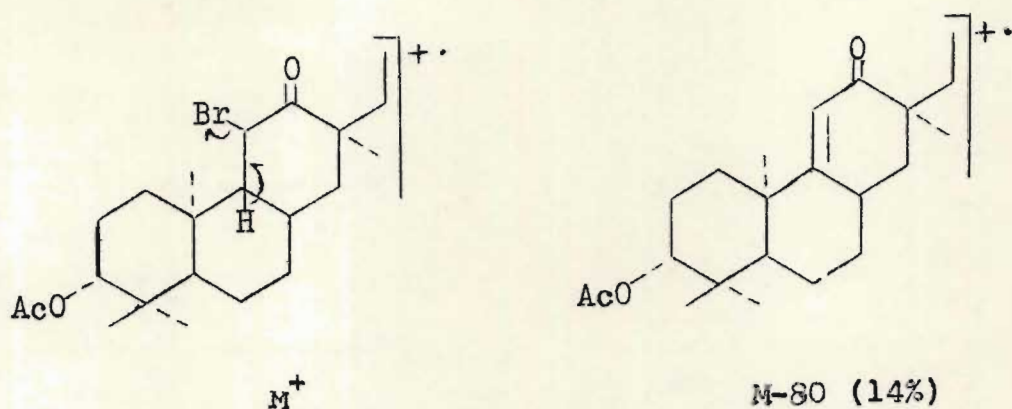
The loss of carbon monoxide from the M-80 ion
would account for a prominent ion at M-108, from which
an allylic methyl group could readily be lost from C-10
or C-13 to produce the ion at M-123.

M-80   M-108 (51%)   M-123 (16%)
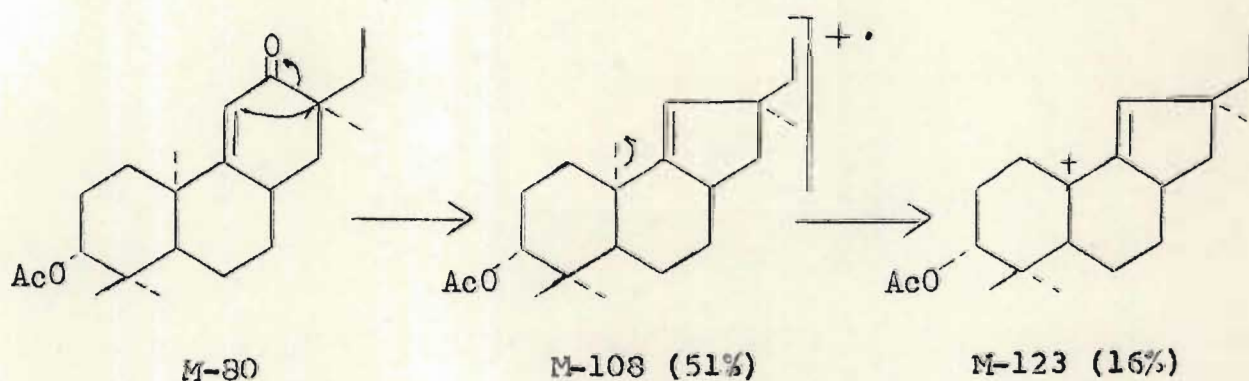
An intense ion at M-141 probably arose from the
M-60 ion by loss of the bromine atom. If this ion then
underwent the loss of a molecule of carbon monoxide then
this would have resulted in the ion at M-169. (page 97)

The mass spectrum of the α,β-unsaturated ketone,
XCVIII, was extremely simple with the molecular ion occurr-
ing at m/e 346. The most significant ions were those which
appeared as M-28 and M-60 fragments. The former ion which

arose by the loss of the elements of carbon monoxide as shown by the metastable peak at m/e 292.6 (346 →318) formed the base peak in the spectrum. Another metastable ion at m/e 289 indicated that a methyl group had been eliminated from the M-28 ion to produce the fragment at m/e 303 (M-43). This process was probably similar to that outlined for the analogous ion in the previous spectrum. As usual the M-60 ion could be explained by the loss of the elements of acetic acid by the McLafferty rearrangement. The remainder of the spectrum was very similar to all the spectra previously discussed, although the ions were present in much lower abundance.

M-60   M-141 (66%)   M-169 (37%)
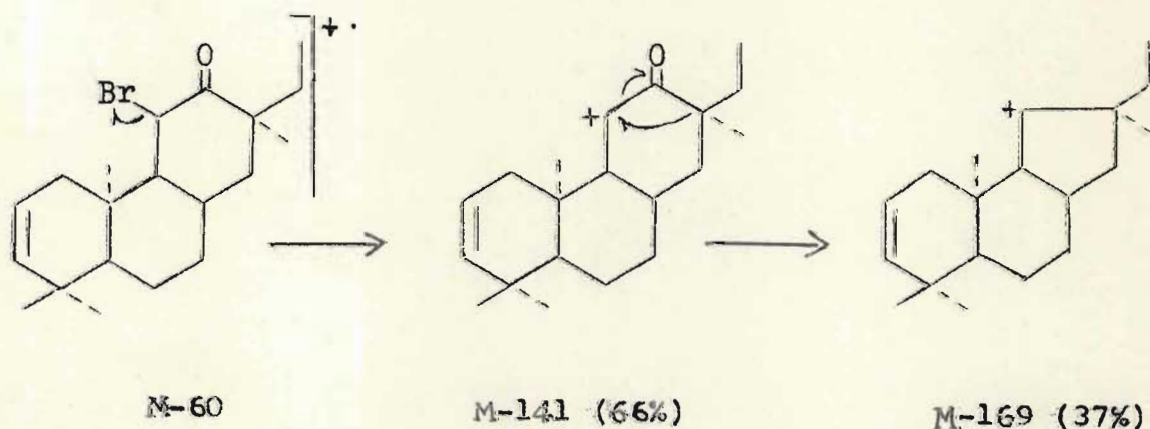
1.3.   3α,12α-Dihydroxy-ent-isopimara-8(14),15-diene, III.

The third compound to be isolated chromatographically in this series was obtained in lower yield than the ketol, II, and comprised approximately 0.1% of the heartwood. It has been identified as 3α,12α-dihydroxy-ent-isopimara-
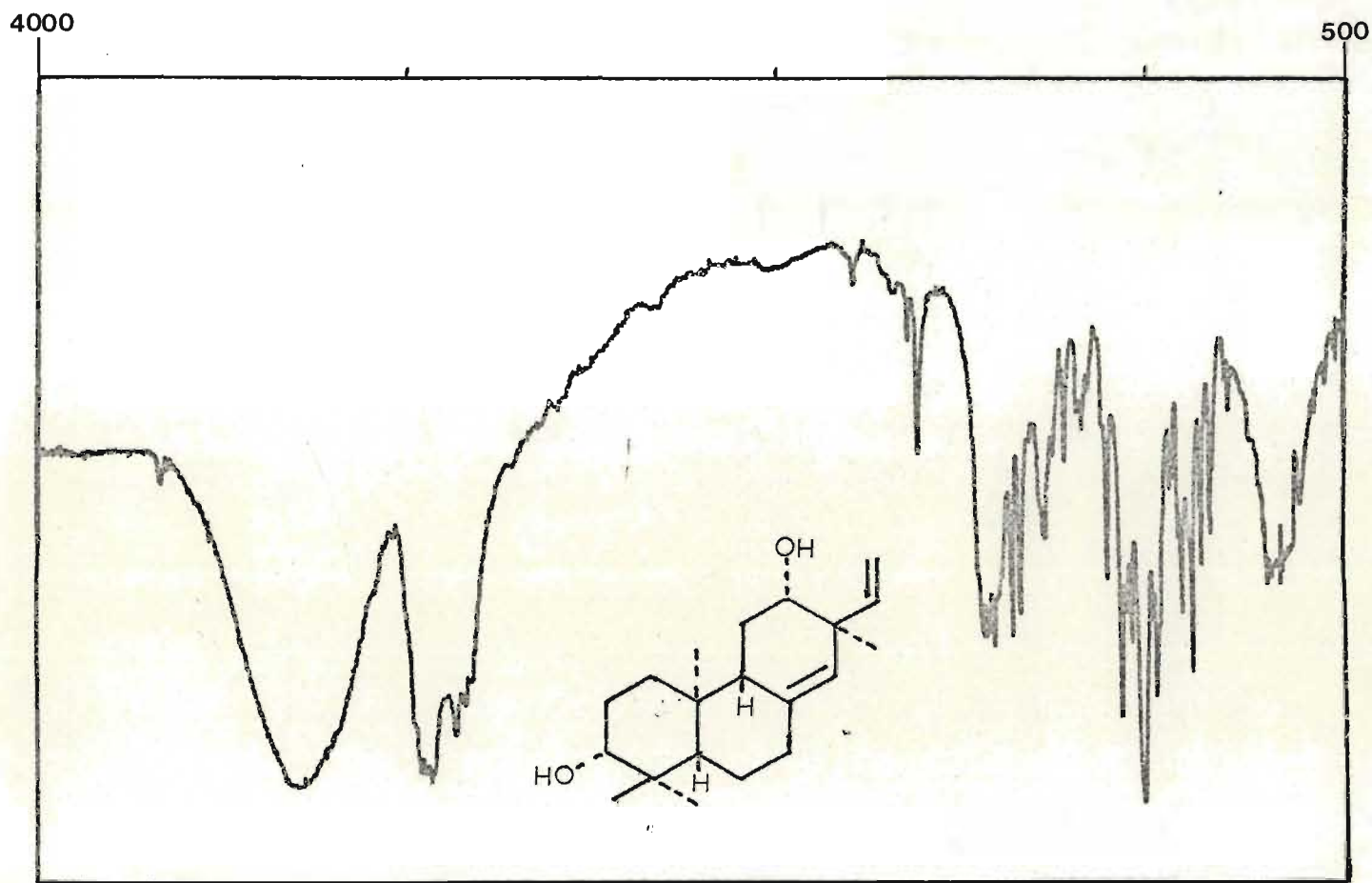
FIGURE 25   IR SPECTRUM OF 3α,12α-DIHYDROXY-<u>ent</u>-ISOPIMARA-8(14),15-DIENE

8(14),15-diene, III, by establishing its relationship with
the ketol and by a study of its spectral data.

Except for the tetranitromethane test the usual
colour reactions failed to provide any indication as to the
nature of the functional groups. However, evidence regarding
the diterpenoid character of the compound was indicated by
its molecular formula of $C_{20}H_{32}O_2$. The infrared spectrum of
this diterpene III, (Figure 25) exhibited no carbonyl absorp-
tion, but a broad hydroxy band was evident at 3290 cm.$^{-1}$.
This suggested that both oxygen functions were present as
hydroxy groups. Confirmation of this was provided by the
conversion of the diol, III, to the diacetate, XCIX.
Furthermore, the failure of the diol to react with periodic
acid showed that the two hydroxy groups were not vicinal.
Jones' oxidation of the diol, III, afforded a diketone which
was found to be identical in all respects to the diketone,
XCIV, obtained by a similar oxidation of the ketol, II.
The formation of the diketone established that both the
basic skeletons and the sites of oxygenation of the ketol
and the diol were identical.

A comparison of the n.m.r. spectra of the ketol
and the diol confirmed the similarity of their skeletons.
The spectrum of the diol (Figure 26) exhibited a quartet at
$\tau 4.22$ and a multiplet centred at $\tau 4.93$ characteristic of the
C-13 vinyl ABX system (observed J_{trans} 18 Hz, J_{cis} 10 Hz, J_{gem}
1.5 Hz). The singlet at $\tau 4.83$ due to the C-14 proton was
partially obscured by the AB multiplet.
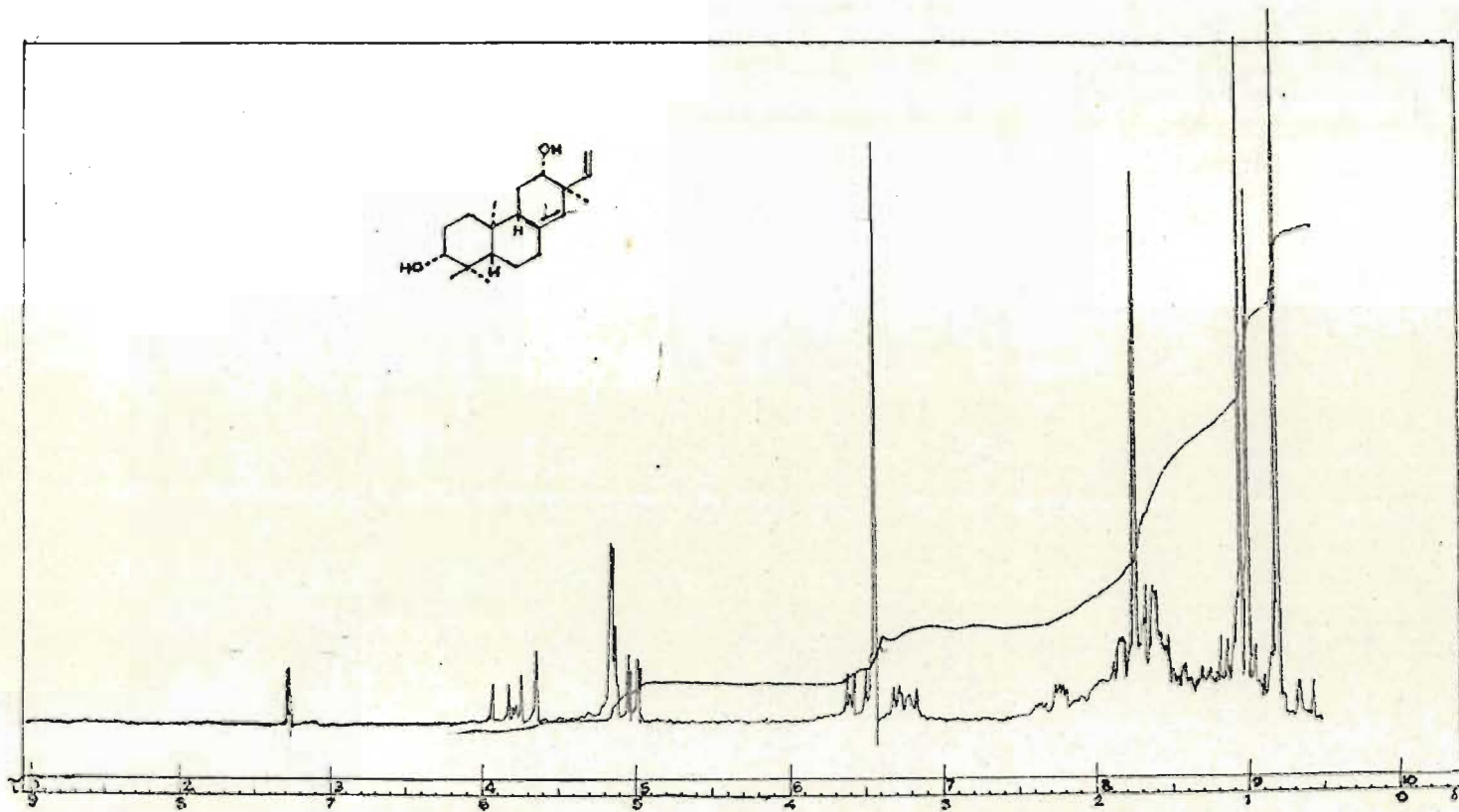
FIGURE 26 NMR SPECTRUM OF 3α,12α-DIHYDROXY-ent-ISOPIMARA-8(14),15-DIENE
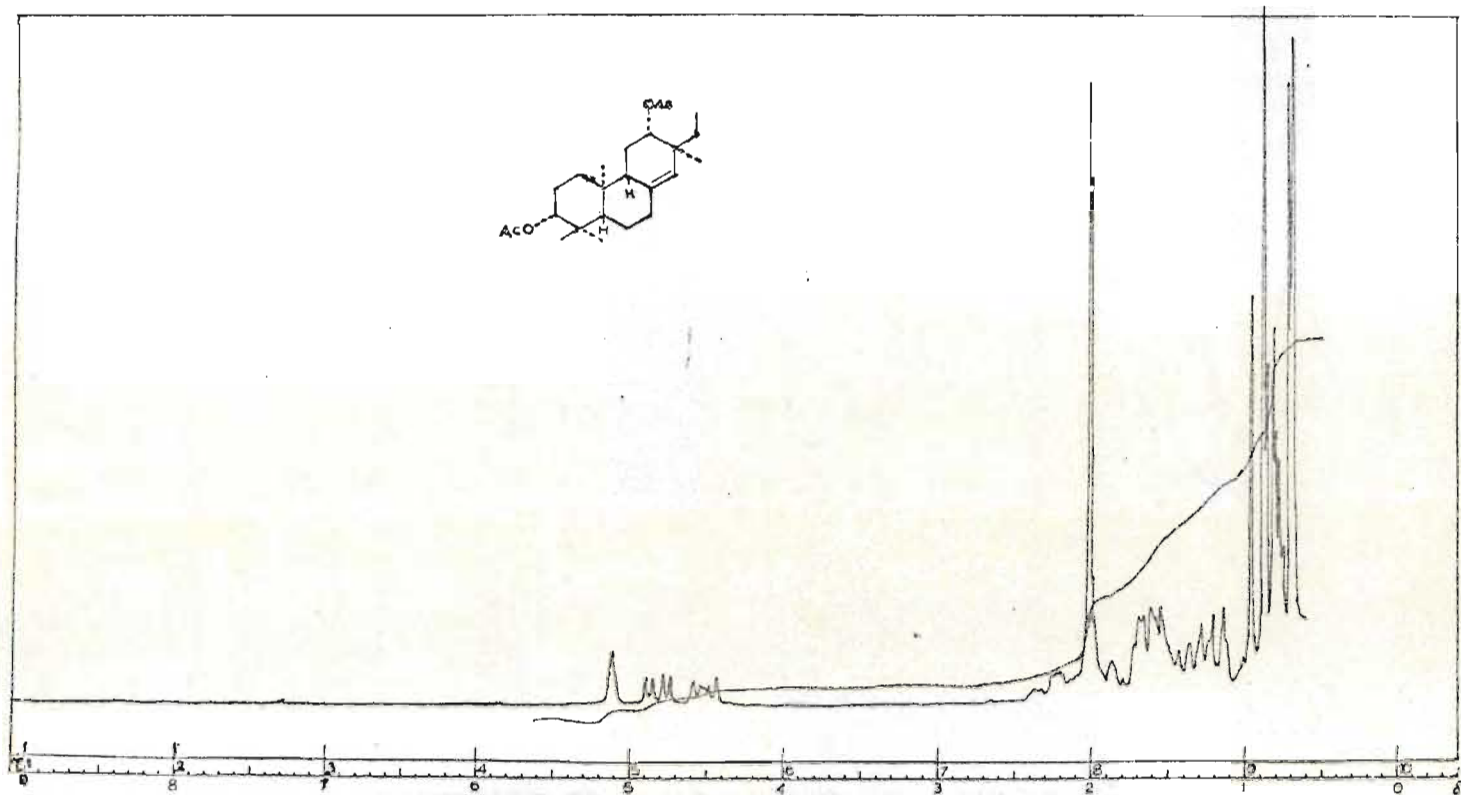
FIGURE 27   NMR SPECTRUM OF 3α,12α-DIACETOXY-ent-ISOPIMAR-8(14)-ENE

Most significant were the signals due to the
protons adjacent to the hydroxy groups. The C-3 axial
proton resonated as usual as a multiplet at τ6.75 which is
in good agreement with the chemical shift for this signal
(τ6.73) in the spectrum of the alcohol, I. A broad multi-
plet at τ6.45 was assigned to the proton adjacent to the
second hydroxy group, but this signal was partially obscured
by a hydroxy proton signal at τ6.54. This signal together
with another at τ8.24 was removed by exchange with deuterium
oxide, thereby simplifying the multiplet at τ6.45.

On acetylation of the diol the C-3 and C-12 proton
signals shifted from τ6.75 and 6.45 to τ5.48 and 5.19 respec-
tively. However, this did not clarify the situation as the
signal at τ5.19 was now complicated by part of the vinyl
multiplet in this region. In order to overcome this problem
the diol was hydrogenated over palladium on carbon in ethanol
or over Adam's catalyst in acetic acid to produce a dihydro-,
C (a), or tetrahydro-derivative, CI, respectively. Acetyl-
ation of the dihydrodiol, C (a), afforded the dihydrodiacet-
ate, C (b), whose n.m.r. spectrum (Figure 27) exhibited a
multiplet at τ5.49 due to the C-3 proton and an ABC quartet
at τ5.19 due to a proton adjacent to two non-equivalent
protons. Since it was established that the positions of
oxygenation in the ketol, II, and the diol, III, were ident-
ical and furthermore, that the carbonyl function in the
ketol was located at probably C-12 or only tentatively at
C-6, it followed that the second hydroxy group in the diol

must also be situated at either of these two positions.
It was obvious from the observed quartet due to the proton
adjacent to this hydroxy group that such a signal could only
have arisen if the oxygen function was located at C-12. Had
the hydroxy function been in the C-6 position a more complex
signal would have been expected due to coupling with the C-5
and C-7 protons. Thus in the diol, III, the functional
groups were located at C-3 and C-12, which therefore con-
firmed that in the ketol, II, the carbonyl group was at C-12.

The half-width and chemical shift of the C-3
proton signal were consistent with the axial orientation
which had been assigned to the previous congeners discussed.
By a similar consideration of the C-12 proton signal it
seemed extremely likely that this hydrogen was probably also
axially orientated. However, in order to obtain a chemical
confirmation of the stereochemistry at C-12 the ketol, II,
was stereospecifically reduced with sodium in pentyl alcohol
(122, 123) to furnish exclusively the equatorial (α) hydroxy
compound, which was identical in all respects to the diol,
III. In contrast a similar reduction of the ketol, II,
using lithium aluminium hydride afforded a mixture of both
equatorial and axial isomers of the diol in the ratio of
approximately 3:2. Thus the diol has been shown to be
3α,12α-dihydroxy-ent-isopimara-8(14),15-diene, III.
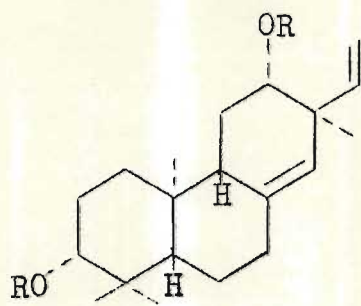
III   R=H
XCIX   R=Ac
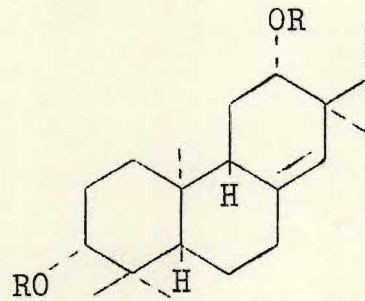
C (a) R=H
   (b) R=Ac
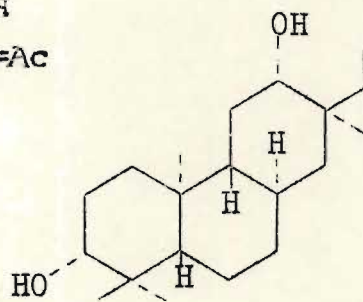
CI

Mass Spectra

The mass spectrum of the diol, III, displayed a fragmentation pattern which was very similar to that of the alcohol, I. Its molecular ion occurred at m/e 304 in agreement with its molecular formula of $C_{20}H_{32}O_2$. The ion at m/e 135 once again formed the base peak in the spectra of the parent diol, III, and its dihydro and acetyl derivatives.

Minor differences which were observed were due to the ions resulting from the elimination of the C-12 hydroxy group. Thus the ion at M-44 for the diol, III, probably arose by a retro Diels-Alder fragmentation of the C ring in the molecular ion and corresponds with the M-42 ion in the spectrum of the ketol, II, (Figure 21). The cleavage mech-
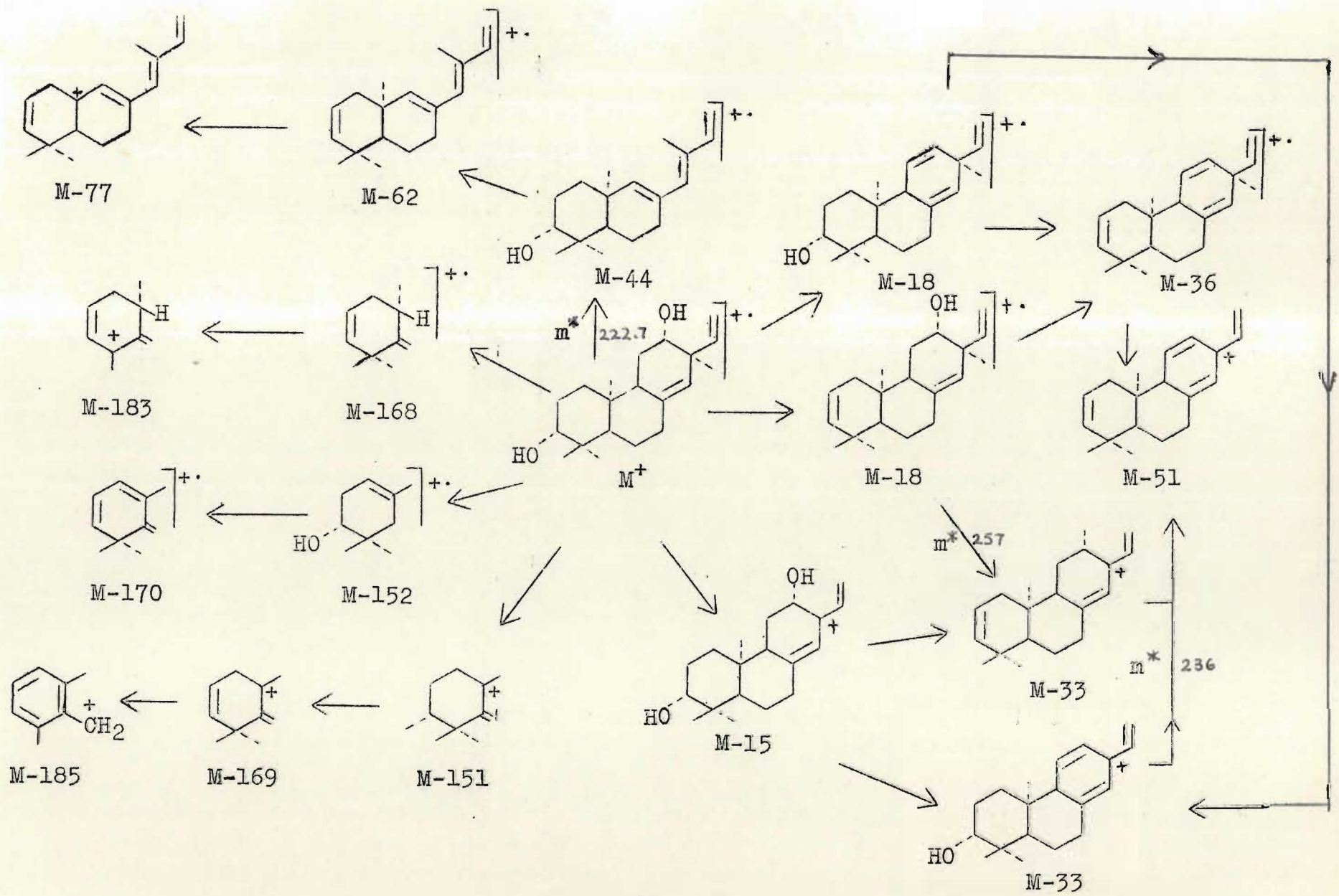
SCHEME 7

anisms giving rise to the other ions have been previously discussed and therefore the overall fragmentation pattern of the diol and its derivatives is illustrated in Scheme 7.

Biogenetic Discussion

The co-occurrence of the diterpenes I, III, II and IV exhibiting an increasing oxidation state of the C ring provides an interesting series for biogenetic speculation.
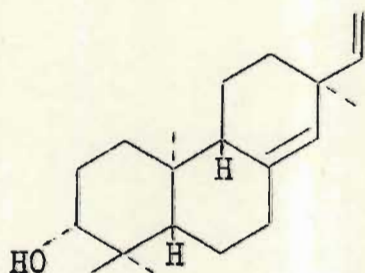
I
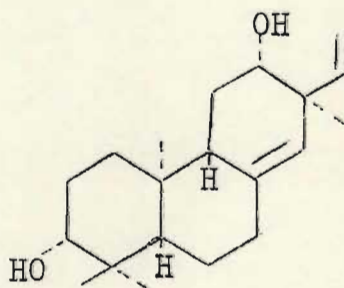
III
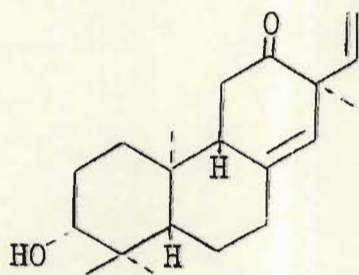
II
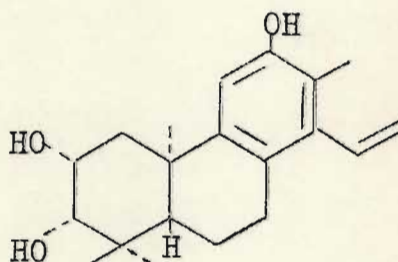
IV

It is suggested that the logical biogenetic pathway involved oxygenation of the alcohol, I, to the diol, III, which in turn may be oxidised to the ketol, II, the precursor of cleistanthol, IV. The aromatisation of ring C of the ketol to give the cleistanth-8,11,13-triene skeleton,

CII, is thought to be initiated by the abstraction of an hydride ion from C-9. Migration of the 8,14-double bond to the 8,9 position together with the transfer of the vinyl group from C-13 and C-14 via a "Claisen" type of intermediate, CIII, and the elimination of a proton from C-14 thus affords the aromatic ring C as outlined below.

CII

I   III   II

IV   CIII
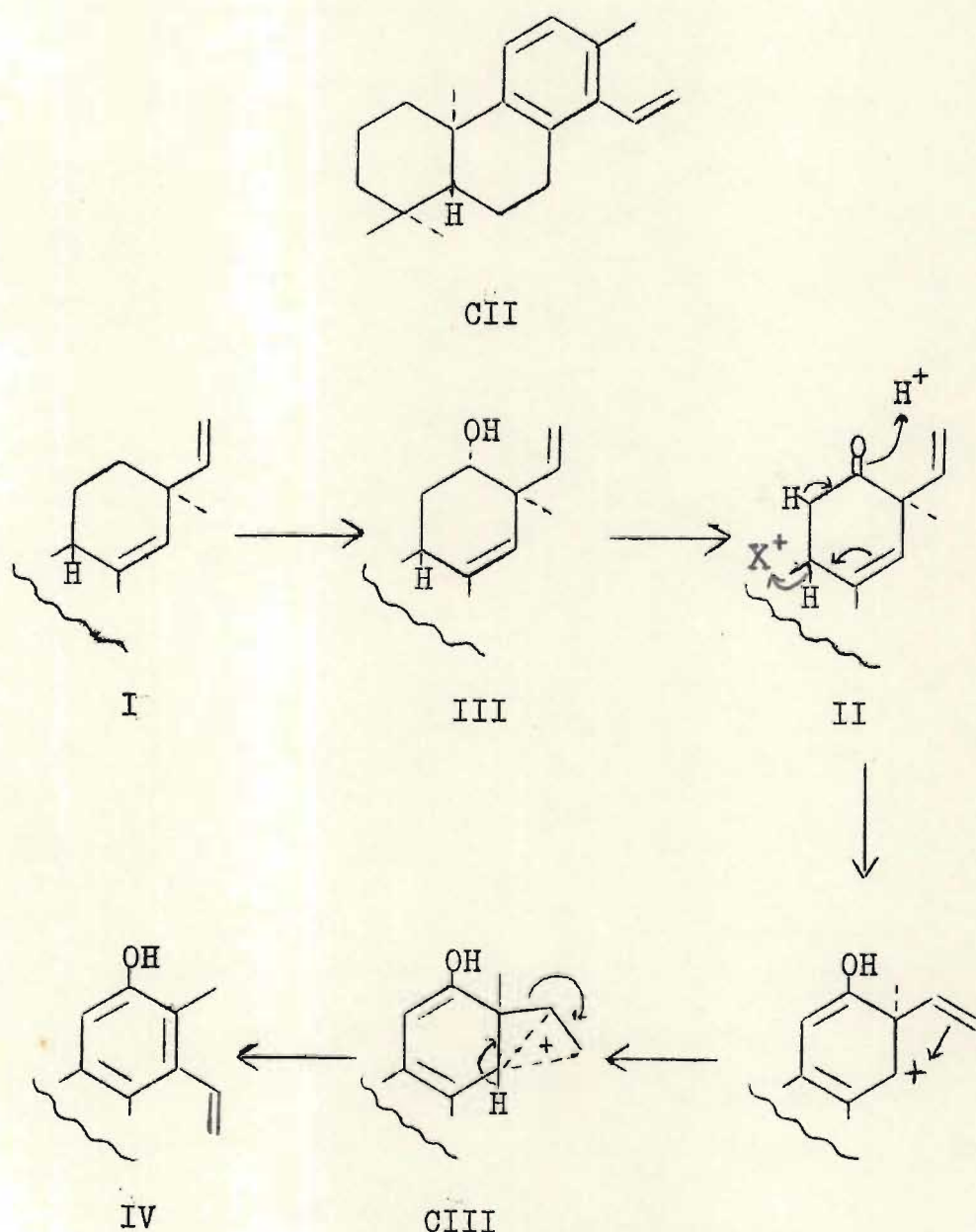
An alternative and tempting sequence for the conversion of the ketol, II, to the phenol, IV, involves the α, β-unsaturated ketone, CIV, as outlined below. Unfortunately, the fact that this compound could not be detected within the extract failed to substantiate this theory.
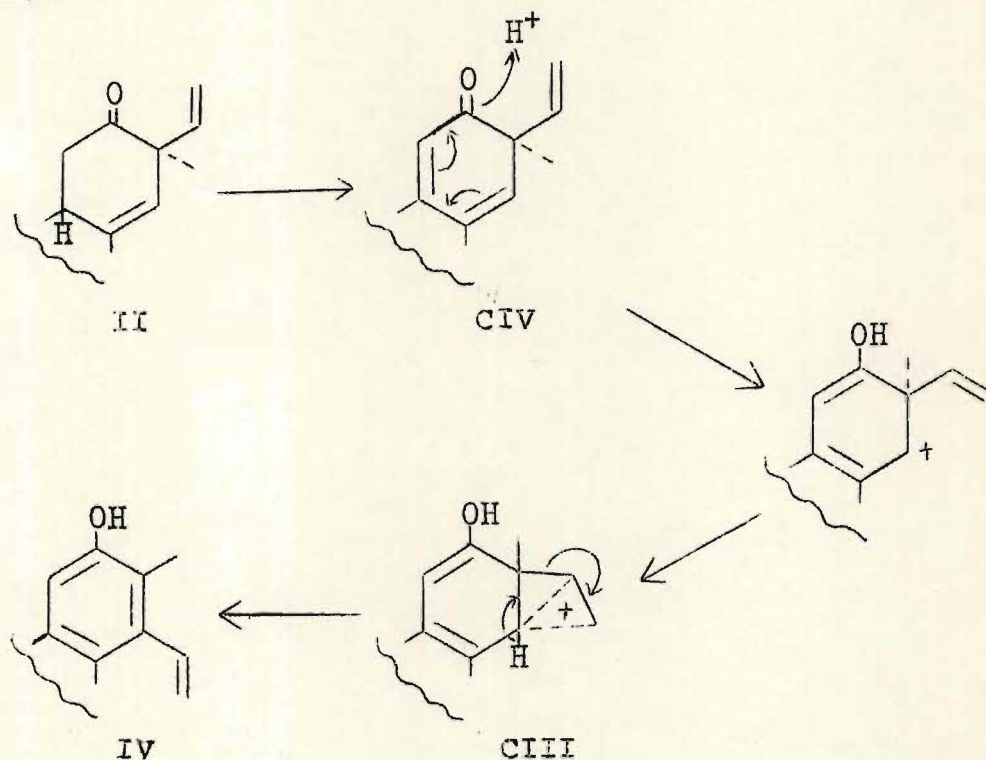
As mentioned in the introduction the pimaradienes are commonly considered (60, 62) to be the biogenetic precursors of the tetracyclic diterpene skeletons. Thus (-)-kaurene, LXX, may be derived from the ent-pimaradiene, CV, as depicted on the next page.

However, due to the epimeric stereochemistry encountered at C-13 in the present series of Cleistanthus compounds isolated, it is considered that the vinyl group

is not favourably orientated for the formation of a tetra-
cyclic skeleton and that migration of this group to C-14
is perhaps a more favoured process. This hypothesis is
supported by the occurrence of the hitherto unknown
cleistanthane skeleton and the apparent absence of tetra-
cyclic diterpenes so frequently encountered in other species
of Euphorbiaceae (e.g. 7,65,68,71,72,74,75).

CV

LXX
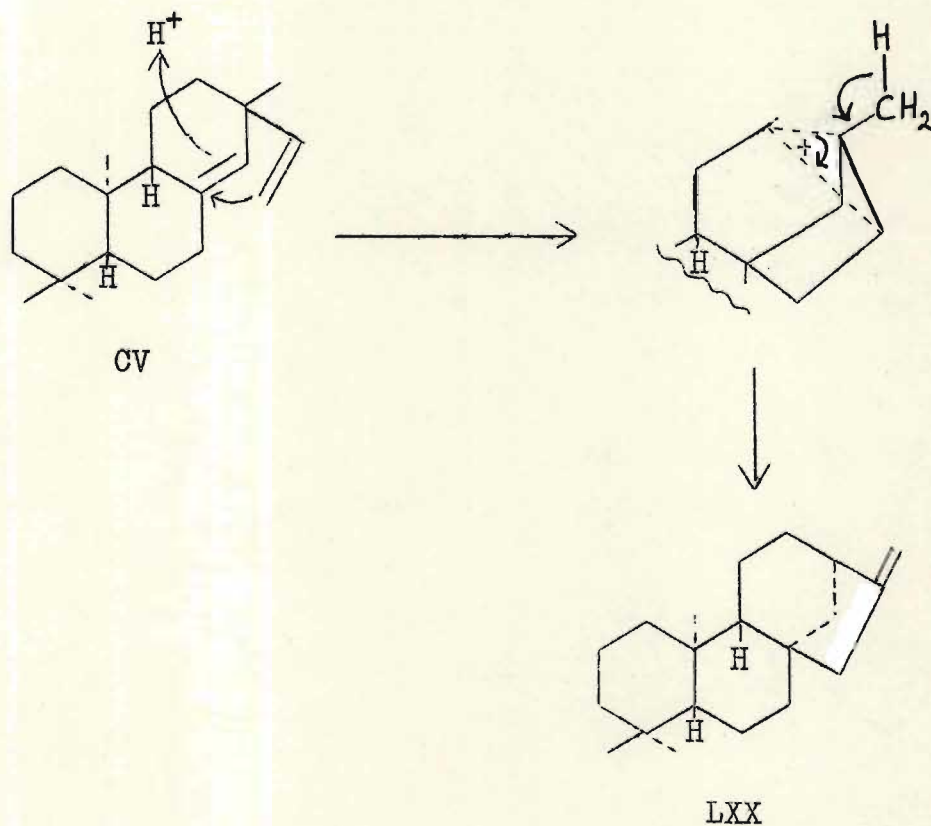
PART 2.   THE DITERPENES OF SPIROSTACHYS AFRICANA

During an earlier investigation of the heartwood
of Spirostachys africana Sond. (syn. Excoecaria africana
Muell. Arg.) the structures of three diterpenoid constituents
were elucidated, but it was reported that there remained an
acidic fraction which was not investigated (7). An attempt
was therefore made to isolate and identify further new com-
pounds from the heartwood in order to compare the species
chemotaxonomically with other Euphorbiaceae.

Spirostachys africana which is commonly known as
Tambootie or African Sandalwood is a deciduous tree that grows
to a height of 20 to 30 feet. It is monotypic and endemic
to Africa, being found mainly in Botswana, the Zululand
forests, throughout the Transvaal and in East Africa (124).
The straight clear trunk which is usually up to 18 ins. in
diameter is covered with a rough, almost black bark, fissured
into characteristic rectangles. Its sapwood is a pale buff
colour while the heavy heartwood is well-defined, rich brown
in colour and possesses a distinctive odour. The sawdust from
the wood, if allowed to enter the eyes, is known to cause
pain and temporary blindness, while the latex is used as a
fish poison by the indigenous tribes and the bark is employed
as an ingredient in herbal medicines.

The milled, air-dried heartwood of Spirostachys
africana was extracted several times with cold light
petroleum, and on concentration this yielded a yellow syrup

in which a crystalline solid precipitated. Extraction of
the sawdust with boiling light petroleum furnished a darker
extract which appeared to be chemically identical with the
cold light petroleum extract as monitored by thin layer
chromatography.

Preliminary fractionation of the crude extract by
means of column chromatography afforded the known ketone, V,
ketol, VI, and diosphenol, VII (7). Since the beyerene and
stachene skeletons are identical these compounds may be named
beyer-15-en-3-one, 3-hydroxybeyer-15-en-2-one and 2-hydroxy-
beyer-1,15-dien-3-one respectively. Although they were
originally reported as stachene derivatives (7), the
beyerene nomenclature will be adhered to throughout this
section for the sake of consistency.

In order to separate the acidic components, the
crude extract was partitioned between light petroleum and
0.1 N aqueous alcoholic potassium hydroxide using the counter-
current technique. This led to the isolation of a new di-
terpene seco-acid to which the name spirostachic acid was
assigned.
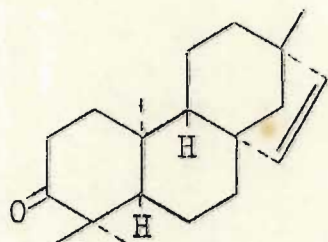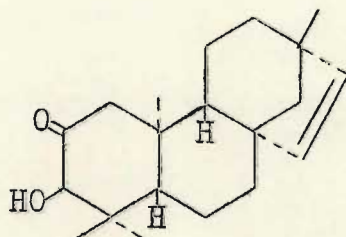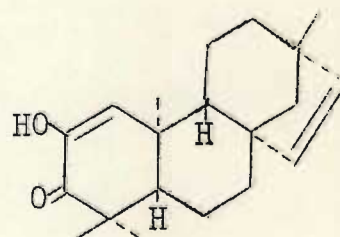
V   VI   VII

2.1. Beyer-15-en-3-one, V, 3-Hydroxybeyer-15-en-2-one, VI and 2-Hydroxybeyer-1,15-dien-3-one, VII.

Repeated column chromatography of the first fraction followed by recrystallisation of the product provided needles of beyer-15-en-3-one, V, which was identified by comparison of its physical constants and spectral data with those of an authentic sample, and the formation of an identical 2,4-dinitrophenylhydrazone.

Colourless needles of the ketol were collected from the mother liquors of the eluates and identified by a positive triphenyltetrazolium chloride test (113). Physical constants and spectral measurements indicated that this component was 3-hydroxybeyer-15-en-2-one, VI.

The third fraction proved to be a complex mixture in which the diosphenol, VII, was the major constituent. Its presence was indicated by the development of an intense brownish-mauve colour on treatment with a neutral ferric chloride solution, and was not further investigated.

2.2. Spirostachic acid or 2,3-Secobeyer-15-en-2,3-dioic acid, VIII.

Repeated crystallisations of the acidic fraction from the counter-current distribution afforded colourless needles shown by various chemical and spectral data to be the seco-diacid, VIII, which has not previously been reported as a natural product.
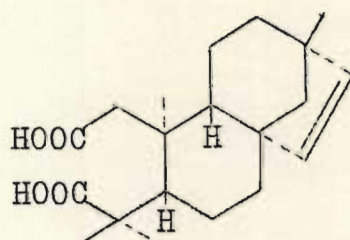
VIII

Elementary analysis established the molecular
formula of $C_{20}H_{30}O_4$, but the nature of the oxygen functions
was not readily determined since colour tests with such re-
agents as neutral ferric chloride, triphenyltetrazolium
chloride and 2,4-dinitrophenylhydrazine proved negative.
Under normal conditions for acetylation no acetate was form-
ed, and hence the oxygens could not have been involved in
hydroxy groupings. The ready solubility of the compound in
alkali and its reprecipitation by acid suggested that the
oxygens were involved in carboxy groups. The number of acid
groupings was determined by a standard titration of the acid,
VIII, with sodium hydroxide, whence it was established that
there were two replaceable hydrogens thereby showing VIII to
be a dibasic acid.

The infrared spectrum of VIII (Figure 28) pro-
vided substantial support for its dicarboxylic nature by the
occurrence in the carbonyl region of two bands at 1718 and
1685 cm.$^{-1}$ (125). Moderately strong absorptions at 2655 and
2560 cm.$^{-1}$ could be ascribed to the O-H stretching vibrations
in dimeric acids which have been well investigated (126).
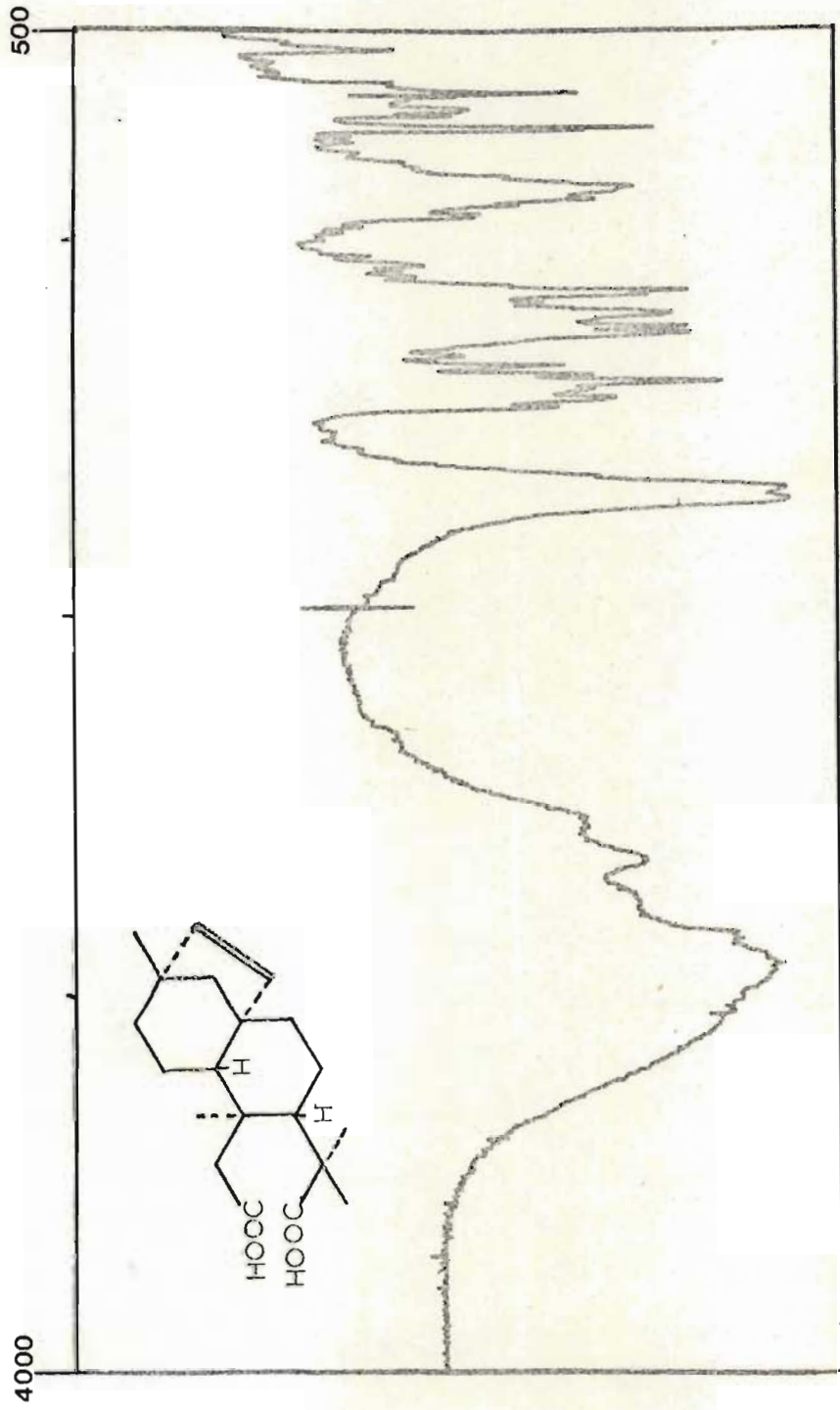
FIGURE 28   IR SPECTRUM OF SPIROSTACHIC ACID

The bands which appeared at 1410, 1280 and 935 cm.$^{-1}$ are characteristic of carboxylic acid dimers and are attributable to the coupled C-O and O-H vibrations and the O-H out-of-plane bending in the dimer (127).

Absorptions occurring at 1385 and 1362 cm.$^{-1}$ signified the presence of the gem-dimethyl groupings (128). The ratio intensity of these bands and the appearance of a further absorption near 1385 cm.$^{-1}$ indicated (129) the presence of an angular methyl group between two six-membered rings i.e. probably at C-10. The similarity of these infrared absorption measurements to the reported (7) values of the compounds previously isolated from Spirostachys implied that the diacid, VIII, was derived from a similar hydrocarbon skeleton.

The tetranitromethane test produced a canary yellow colouration in the acid, thereby indicating some degree of unsaturation, but the ultraviolet absorption spectrum failed to exhibit any characteristic bands above 210 nm., which therefore showed the absence of conjugation in the molecule.

In the infrared spectrum (Figure 28) bands due to the =C-H and C=C stretch vibrations were unfortunately obscured by the intense C-H and C=O absorptions, although an intense band appearing at 746 cm.$^{-1}$ could be attributed to a cis-double bond (7).

The n.m.r. spectrum of the parent acid, VIII, (Figure 29) displayed an ideal AB system consisting of two doublets (130) centred at τ 4.35 and 4.55 (J=8 Hz) comparable with the equivalent cis-arranged C-15 and C-16 protons

in the five-membered rings of a secobeyerene, LXIII, (73) or in the hydrocarbon hibaene, CVI, (131). The broad singlets at τ7.38 and 7.51 each due to two protons probably arose from the C-1 and C-14 methylene resonances, while the poorly defined triplet at τ7.92 due to two protons could possibly be attributed to the quaternary hydrogens at C-5 and C-9 which are in similar environments.

The four tertiary C-methyl signals for hibaene, CVI, are reported to occur at τ9.03, 9.15, 9.19 and 9.27 (131). In the spectrum of the diacid, VIII, these signals appeared at τ8.76, 8.83, 9.01 and 9.15. Assuming that the latter two signals corresponded with the first two mentioned for hibaene, it seems feasible that those signals which underwent a change in chemical shift viz. τ9.19 to 8.76 and τ9.27 to 8.83 were due to the deshielding effects of the two carboxy groups on the gem-dimethyl substituents at C-4. Thus the singlet at τ9.01 was probably due to the C-13 methyl group within the shielding influence of the nuclear double bond, while that at τ9.15 could be attributed to the C-10 angular methyl substituent.
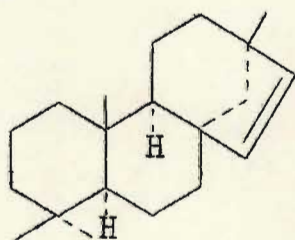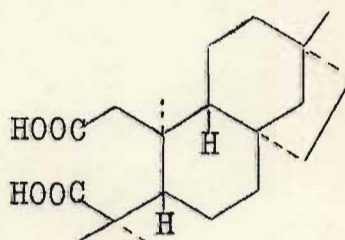
CVI   CVII
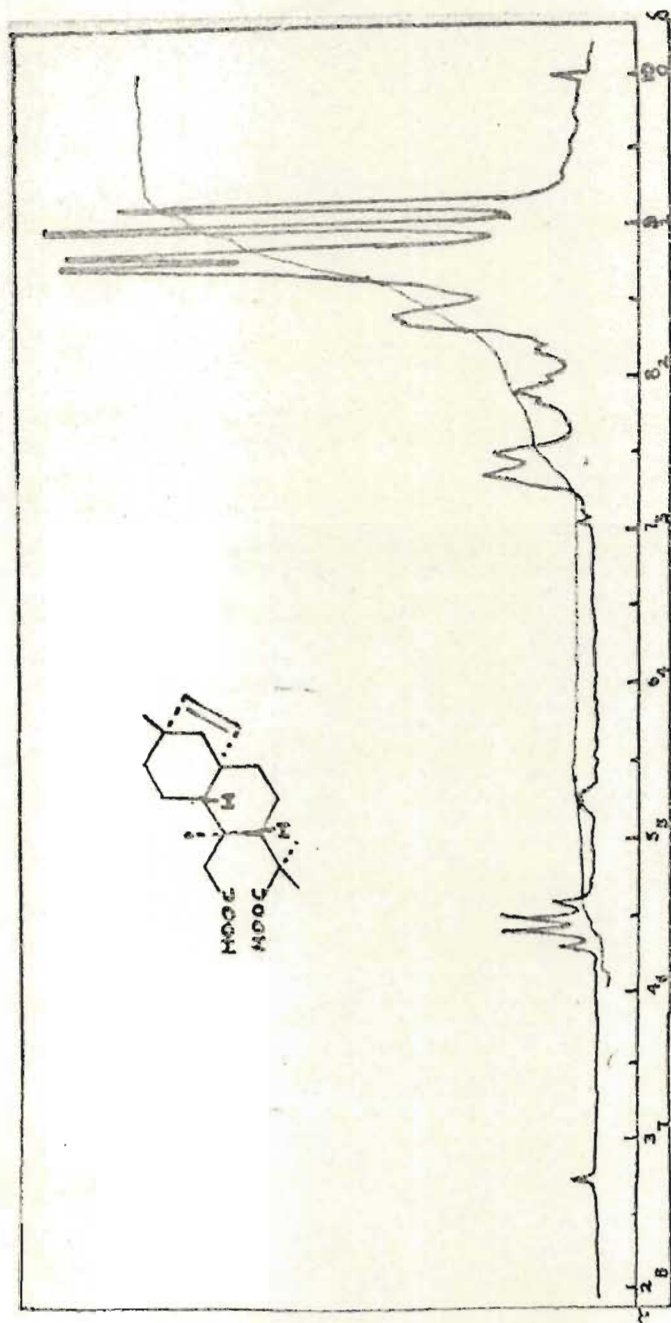
FIGURE 29   NMR SPECTRUM OF SPIROSTACHIC ACID

In order to determine the number of olefinic double
bonds present in the diacid, VIII, a quantitative hydrogen-
ation was performed over Adam's catalyst in acetic acid.
The absorption of hydrogen was completed over a short period
of time during which one mole of hydrogen was absorbed. This
formation of the dihydro-derivative, CVII, confirmed the
presence of only one double bond in the parent acid, VIII.
The infrared spectrum of CVII was very similar to that of
VIII except for the disappearance of the band at 746 cm.$^{-1}$.

Its n.m.r. spectrum clearly showed the compound to
be fully saturated by the absence of any signals in the
olefinic region, whilst the remainder of the spectrum appear-
ed to be less resolved due possibly to the change in con-
formation or ring strain caused by saturation of the D ring.

The Methyl Esters of Spirostachic Acid.
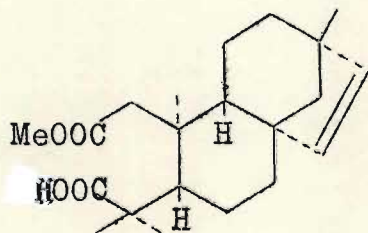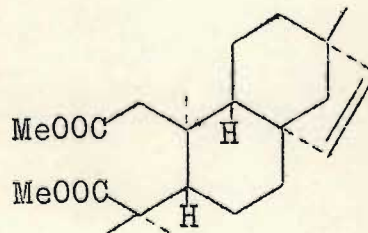
CVIII   CIX

Attempts to methylate the diacid, VIII, by the
Fischer-Speier method for esterification (132) resulted in

the formation of only a monomethyl ester, $C_{21}H_{32}O_4$, CVIII.
Its infrared spectrum exhibited a band at 2653 cm.$^{-1}$ attribu-
table to the O-H stretching vibration in a carboxylic acid
dimer (125), while an observable effect of this methylation
was the separation of the carbonyl stretching absorptions
into two bands at 1740 and 1685 cm.$^{-1}$ due to the ester and
free acid carbonyls respectively. Furthermore, the charac-
teristic short wavelength ester band occurred at 1158 cm.$^{-1}$
accompanied by bands between 1300 - 1000 cm.$^{-1}$.

Many investigators observed that the rate of
formation of the ester, which proceeds by an S_N2 mechanism,
depended on the nature of the substituents in the position
α- to the carboxy group rather than steric effects alone (133).
The proximity of an electron-donating group was found to
inhibit the attack of the reagent in the bond making process,
and thus it was deduced that in the ester, CVIII, esterific-
ation had occurred at the C-2 rather than the C-3 carboxy
group which was adjacent to the electron-donating C-4 gem-
dimethyl substituents. A similar case of monomethylation was
reported (69) for the diacid, LVII (a), isolated from an
Australian Euphorbiaceae. The 17-methyl ester was readily
furnished under Fischer-Speier conditions, while the C-19
carboxy grouping remained unchanged.

The dimethyl ester, $C_{22}H_{34}O_4$, CIX, was the pre-
dominant product on treatment of the diacid, VIII, with
diazomethane. A comparison of the fragments in the mass
spectra of the acid and its esters confirmed the seco-acid

nature of the parent compound, VIII, and furthermore that the acid groups were located at C-2 and C-3.
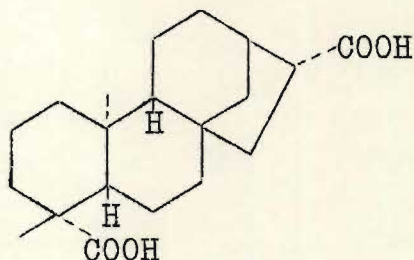
LVII (a)

Other Derivatives of Spirostachic Acid.

Reduction of the diacid, VIII, with lithium aluminium hydride produced a mixture of products, which on purification by means of column chromatography, furnished a major constituent whose identity as the diol, CX, was substantiated by the molecular formula, $C_{20}H_{34}O_2$. Its infrared spectrum showed that the carboxy groups were no longer present since the absorptions around 2650 and 1700 $cm.^{-1}$ disappeared and were replaced instead by a characteristic intense hydroxy hump around 3340 $cm.^{-1}$. In addition to the usual _cis_ double bond peak at 746 $cm.^{-1}$ a weak band was detectable at 3052 $cm.^{-1}$ due to the =C-H stretch vibrations. An intense band at 1013 $cm.^{-1}$ may be tentatively assigned to the C-O stretching vibrations of the hydroxy attachments.

Spirostachic acid, VIII, on treatment with acetic anhydride furnished a crystalline product, $C_{20}H_{28}O_3$, deduced to be an anhydride, CXI, from its spectral data. The infrared

spectrum displayed the anhydride carbonyl absorptions at 1796 and 1758 $cm.^{-1}$ together with the usual _cis_ double bond absorption at 746 $cm.^{-1}$ whilst the n.m.r. spectrum was not significantly changed from that of the free acid.
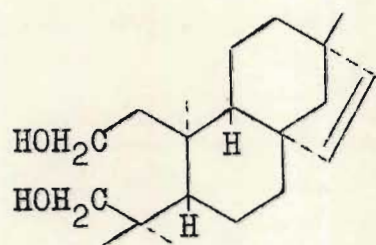
CX
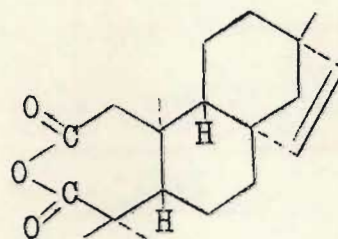
CXI
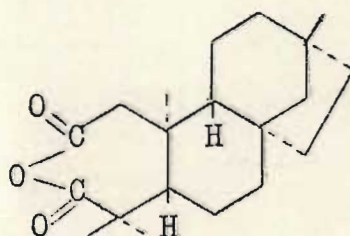
CXII

Similar treatment of dihydrospirostachic acid, CVII, with acetic anhydride afforded a crystalline anhydride, $C_{20}H_{30}O_3$, CXII. The physical characteristics of this anhydride were identical with those reported (7) for the anhydride derived from 2,3-secobeyeran-2,3-dioic acid, CVII, which had been synthesised to facilitate the structural elucidation of the _Spirostachys_ constituents. These results served to confirm that dihydrospirostachic acid and 2,3-secobeyeran-2,3-dioic acid were one and the same compound possessing the structure shown in CVII.

Thus it was deduced that the seco-diacid, VIII, differed from 2,3-secobeyeran-2,3-dioic acid, CVII, by only an olefinic double bond and this was shown by infrared and n.m.r. data to be situated at C-15, C-16.

The Mass Spectra of Spirostachic Acid and its Derivatives

The mass spectra of spirostachic acid and its dihydro, monomethyl and dimethyl derivatives presented a series of fragmentation patterns which proved most valuable in the assignment of the basic 2,3-secobeyerane skeleton to these compounds.

Because of the well-known fact that esters are more volatile than their corresponding acids, studies were concentrated on spectra of both the mono- and dimethyl esters in relation to the parent acid. Comprehensive surveys have been conducted on a large number of spectra of long chain aliphatic esters in particular (134,135), and their basic patterns of cleavage and rearrangement can be applied to alicyclic esters as well.

The spectrum of spirostachic acid, VIII, (Figure 30) displayed a weak molecular ion peak at m/e 334 whilst in the spectrum of its dihydro derivative, CVII, (Figure 31) the molecular ion peak was absent. However, in both spectra the M-18 fragment appeared and this may have arisen by the loss of the elements of water from the molecular ion to give the anhydride ion as shown on the next page.

$$M^+ \qquad\qquad\qquad\qquad M-18$$
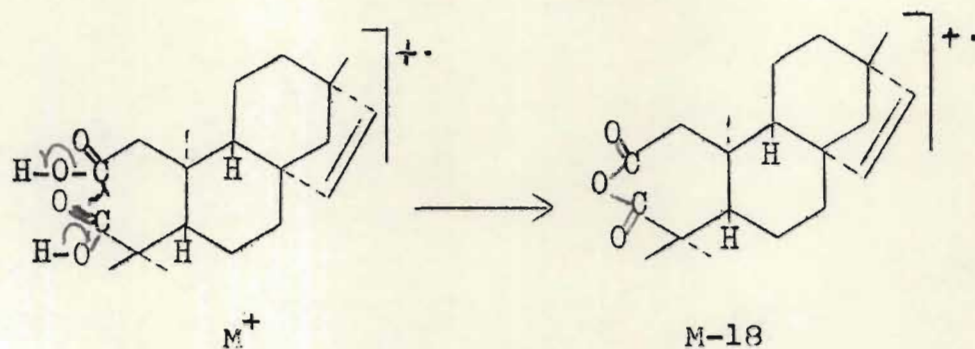
In the spectra of monomethylspirostachate, CVIII, (Figure 32) and dimethylspirostachate, CIX, (Figure 33) the molecular ion peaks appeared at m/e 348 and 362 respectively in low intensities, but M-18 peaks were undetectable. The base peaks in all these spectra occurred at m/e 187 except in the case of dihydrospirostachic acid in which the peak appeared as expected at m/e 189. These fragments probably arose by the mechanisms illustrated in Scheme 8 in which β-cleavage to either of the carboxyl groups is followed by a McLafferty rearrangement (136). Thus the m/e 189 fragment in the spectrum of the dihydro- compound would be the corresponding ion containing a saturated D ring.

SCHEME 8

VIII $R_1 = R_2 = H$

CIX $R_1 = Me,\ R_2 = H$

CX $R_1 = R_2 = Me$

m/e 187
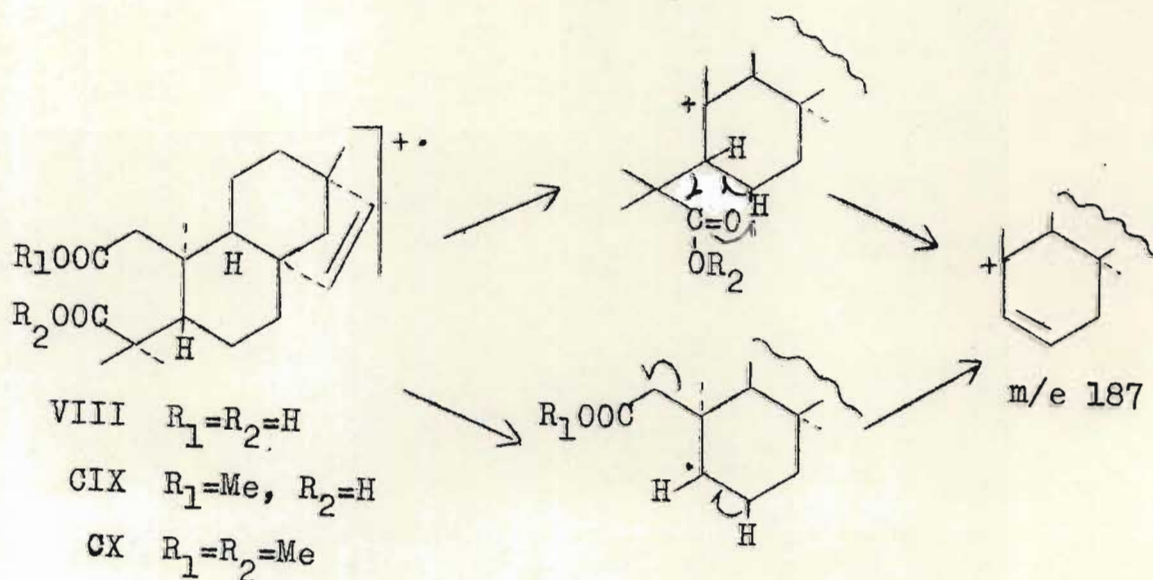
The next peaks of significance for the diacid, VIII, appeared at m/e 275 (42%) and 247 (43%). These ions are shown in Scheme 8 to arise by cleavage of the C-1, C-10 and C-4, C-5 bonds respectively, and have been confirmed by accurate mass measurements which correspond with the formulae $C_{18}H_{27}O_2$ and $C_{16}H_{23}O_2$ for the two respective fragments. The analogous peaks for monomethylspirostachate occurred at m/e 275 (38.5%) and 261 (12%) respectively, which confirmed that under Fischer-Speier conditions the C-2 carboxyl group underwent esterification in preference to the C-3 grouping. In the case of dimethylspirostachate the corresponding fragments appeared at m/e 289 (39.5%) and 261 (12.5%) respectively. An accurate mass measurement of the former ion was consistent with the formula $C_{19}H_{29}O_2$ again due to the loss of the C-1, C-2 moiety ($H_2\dot{C}-CO_2Me$) from the molecular ion. Similarly the formula $C_{17}H_{25}O_2$ was determined for the latter ion, which indicated the loss of the C-3, C-4 moiety ($Me_2\dot{C}-CO_2Me$) from the parent ion.

Prominent peaks observed at m/e 274 (30.5%) and 246 (41%) in the spectrum of the parent acid could again be explained by McLafferty rearrangements.
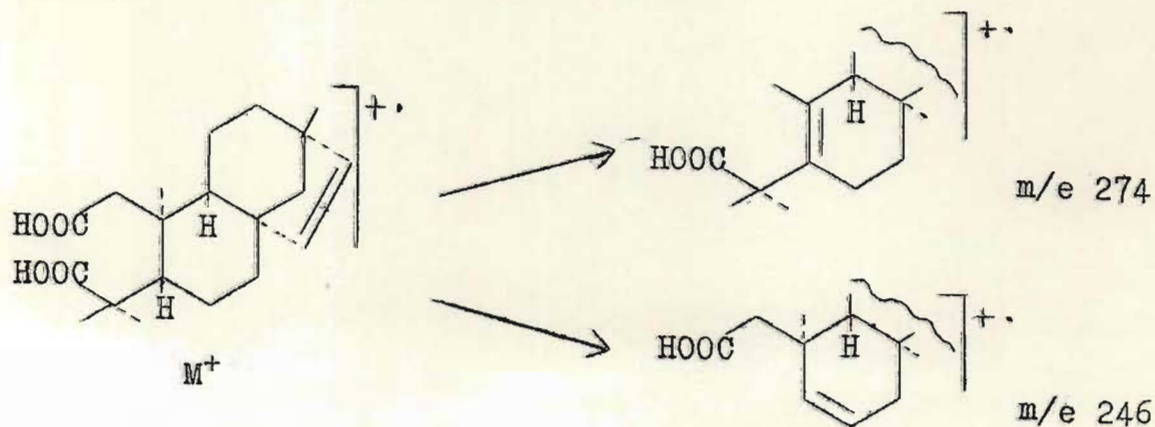
In the spectrum of the monomethyl compound the corresponding
ions were detected at m/e 274 (12%) and m/e 260 (9%), whilst
in the diester spectrum they were present at m/e 288 (7%)
and 260 (7%), but in lower abundance.

A peak at m/e 188 (17%) in the spectra of all the
unsaturated compounds may be explained by the ion arising
from the simultaneous cleavages of the bonds β to the carbon-
yl function as shown below.

$$M^+ \qquad\qquad\qquad m/e\ 188$$
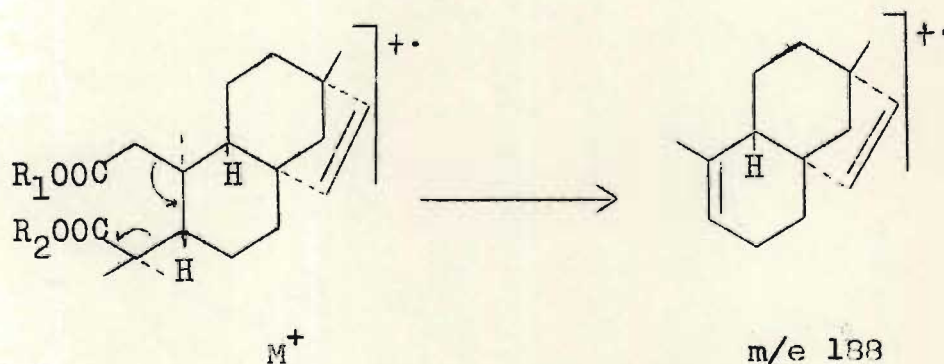
Hydrogenation of the parent acid, VIII, caused an increase
of two a.m.u. to this ion which appeared at m/e 190.

Another significant ion common to the unsaturated
series occurred at m/e 159, but moved to m/e 161 on hydro-
genation, thereby indicating that the fragment contained the
D ring. A metastable peak at m/e 135.5 signified that this
ion arose from the fragment at m/e 187. The mechanism of
this fragmentation probably involves a more complex re-
arrangement than usual and the following pattern is
suggested.

m/e 187   m/e 159
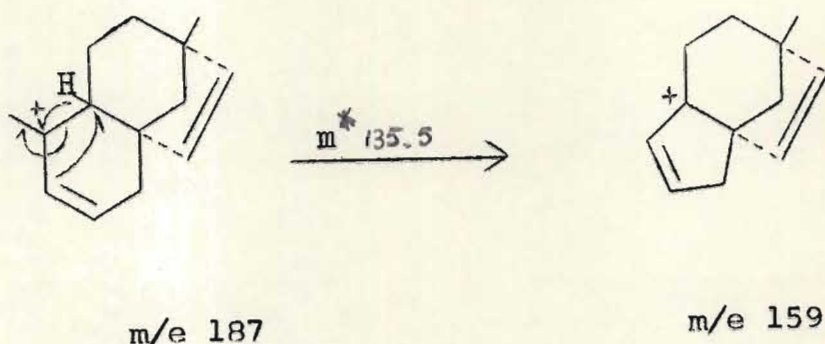
In the upper regions of the spectra less prominent peaks were observed. These were mainly due to various combinations of bond cleavages α- to the carbonyl functions. The spectrum of spirostachic acid, VIII, displayed a M-45 fragment at m/e 289 which resulted from the loss of -COOH from the C-2 position. Thus the corresponding fragment M-(45+14) for the monomethyl ester occurred at m/e 289, and at m/e 303 for the dimethyl ester.

The M-46 fragments found in the spectra of the acids, VIII and CVII, and monomethylspirostachate, CVIII, all probably arose by loss of the elements of formic acid from either of the acid groups, and these occurred at m/e 288, 290 and 302 respectively.

Further fragmentation patterns for some of the less significant peaks are illustrated in Scheme 9, whilst the majority of the low mass ions being "remaining portions" of the major fragments will not be discussed again. The overall picture gained from these fragmentations serves to substantiate the structure assigned to spirostachic acid, VIII.

SCHEME 9

M$^+$ M-58

M$^+$ M-86

M$^+$ M-89

M-87   M-133
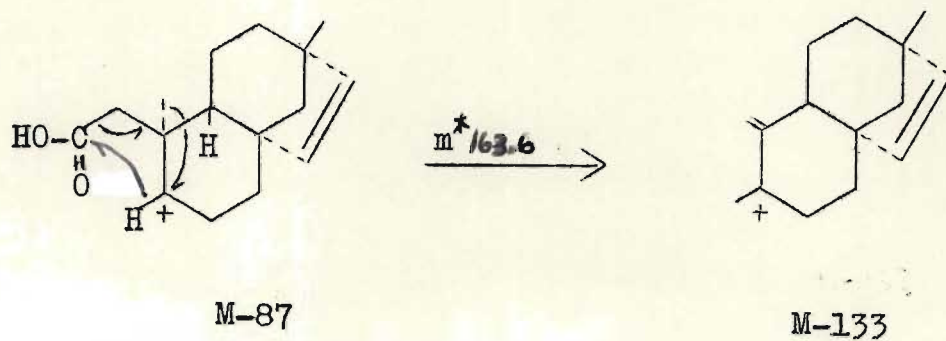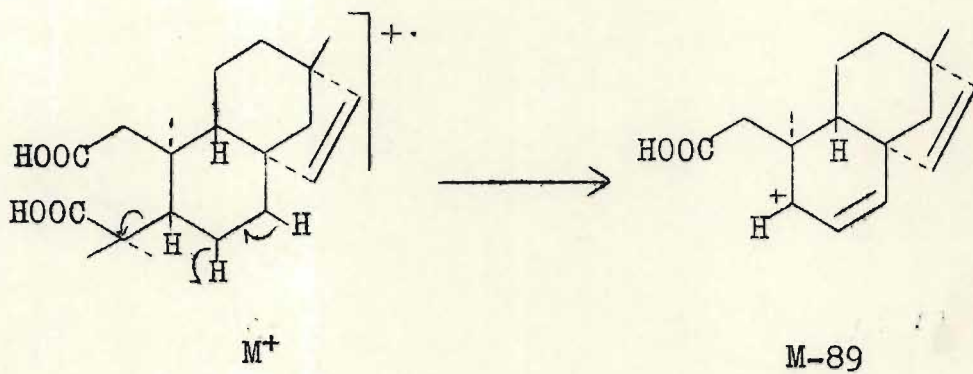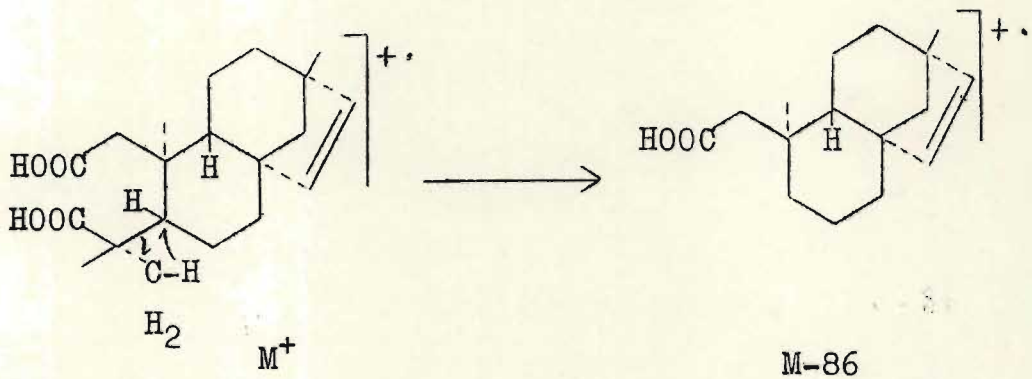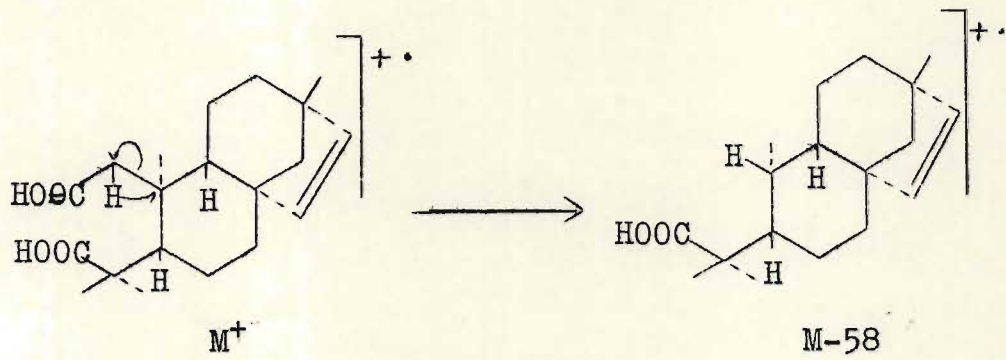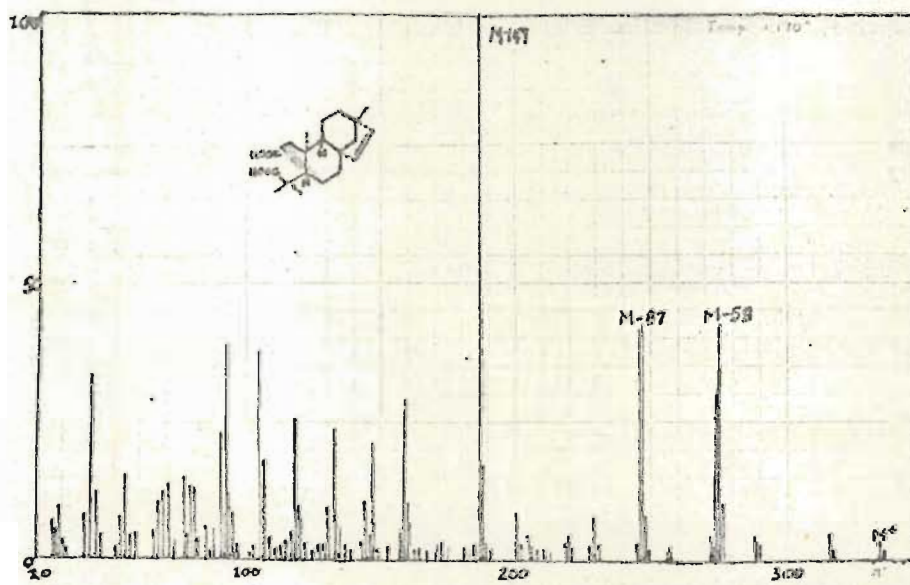
FIGURE 30   MASS SPECTRUM OF SPIROSTACHIC ACID
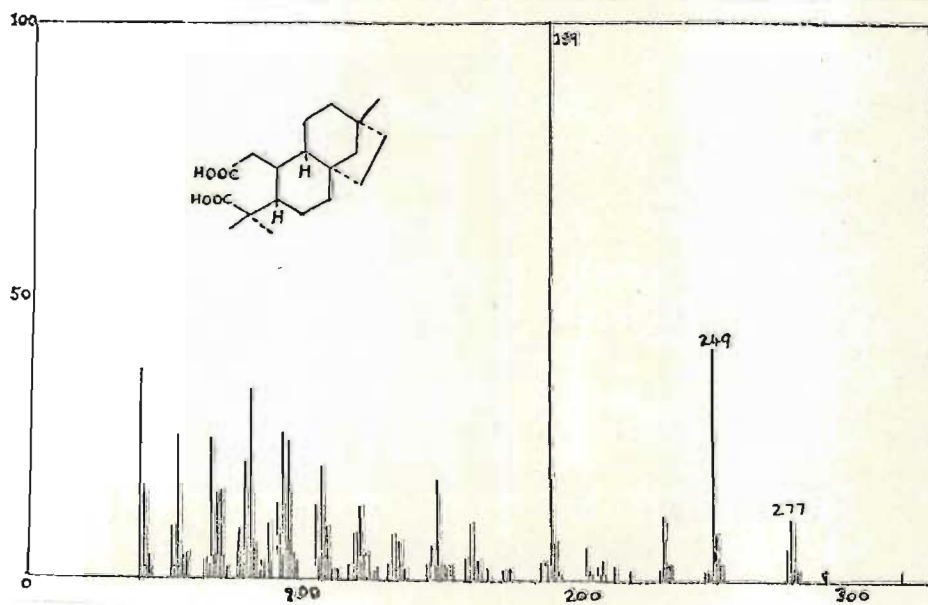
FIGURE 31   MASS SPECTRUM OF DIHYDROSPIROSTACHIC ACID
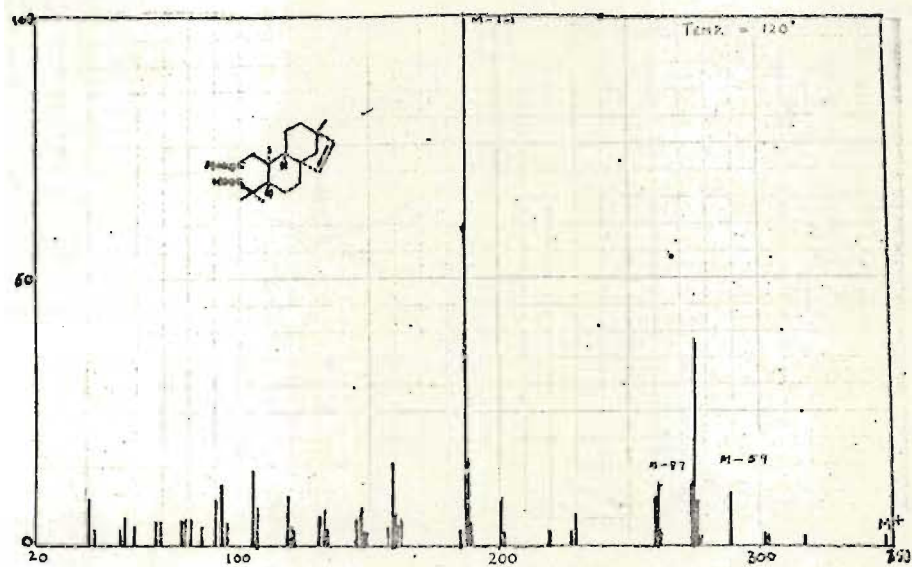
FIGURE 32   MASS SPECTRUM OF MONOMETHYLSPIROSTACHATE
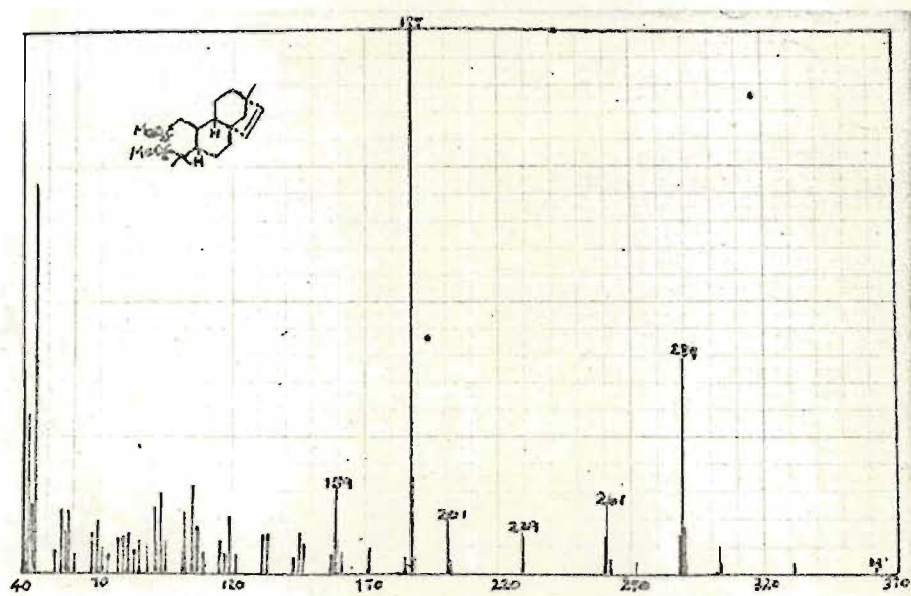
FIGURE 33   MASS SPECTRUM OF DIMETHYLSPIROSTACHATE

The mass spectral fragmentations of the seco-di-
acids and their esters have thus proved to be a powerful
tool in determining the positions of the carboxylic acid
groups. This method may be extended to the determination
of the position of olefinic double bonds or the location of
vicinal hydroxy groups which may be readily converted into
seco-acids. These observations were successfully applied
(1,3) in the structure determination of cleistanthol, IV,
which was converted to the seco-acid, CXIII (a), and its
dimethyl ester, CXIII (b). By the comparison of the mass
spectra of these compounds with those of the spirostachic
acid derivatives it was possible to confirm that alcoholic
hydroxy groups were located at C-2 and C-3.
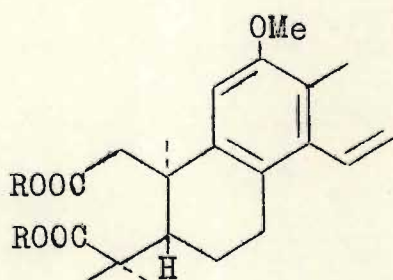
CXIII   (a)   R=H

         (b)   R=Me

Discussion

The co-occurrence of the ketone, V, the ketol, VI,
the diosphenol, VII, and the acid, VIII, in <u>Spirostachys</u>
<u>africana</u> presents another interesting biogenetic sequence
in which the oxidation state of ring A increases from the
ketone, V, through to the seco-diacid, VIII, as shown.
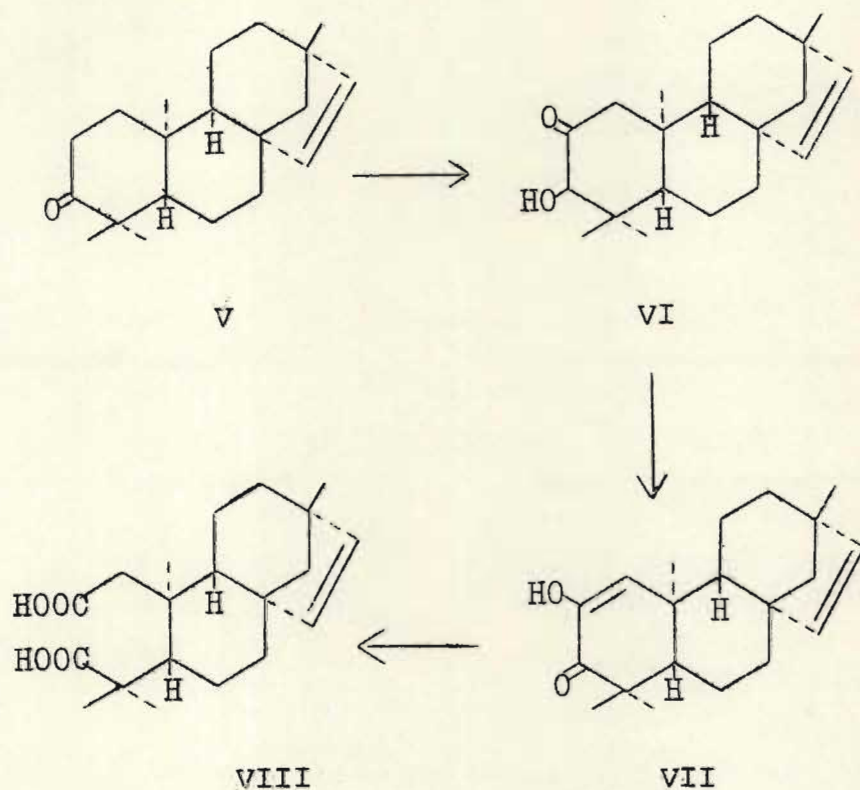
In a number of Western Australian Euphorbiaceae the co-occurrence of diterpenes possessing both kaurene and beyerene (stachene) skeletons has been reported (65,67,73, 74), with the former usually featuring more prominently. In contrast no compounds possessing the kaurene skeleton were identified during the present investigation, although this by no means precludes their presence entirely.

CONCLUSION

From the classical concepts of biosynthesis the
beyerene (stachene) class of tetracyclic diterpenes is con-
sidered to arise by a certain mode of collapse of the carb-
onium ion resulting from the cyclisation of ent-pimaradiene
(Figure 2). As yet no such precursors have been detected in
Spirostachys africana in marked distinction to the presence
of the ent-isopimara-diene precursors in Cleistanthus
schlechteri. Furthermore, from Beyeria brevifolia, a seco-
ent-pimaradiene, XLIV, (58) has been reported to occur to-
gether with three kaurenoid diterpenes (67), thereby probably
implying the former to be the precursor of the latter compounds

A further point of interest worthy of speculation
is the fact that in some species of Euphorbiaceae triter-
penoids have been isolated to the exclusion of any diterpenoid
constituents and vice versa. From the chemotaxonomic point
of view there appears to be no correlation between this
phenomenon and the division into sub-families of the
Euphorbiaceae (137). In fact Croton oblongifolius L (59)
contains pimaradienes, while many Euphorbia (138) which belong
to the same sub-family, Crotonoideae, have yielded mainly
triterpenes. Even greater versatility was shown by the co-
occurrence of diterpenes and triterpenes reported in Beyeria
leschenaultii (68). However, a much more detailed survey is
needed before a reasonable hypothesis for chemotaxonomic
divisions can be proposed.
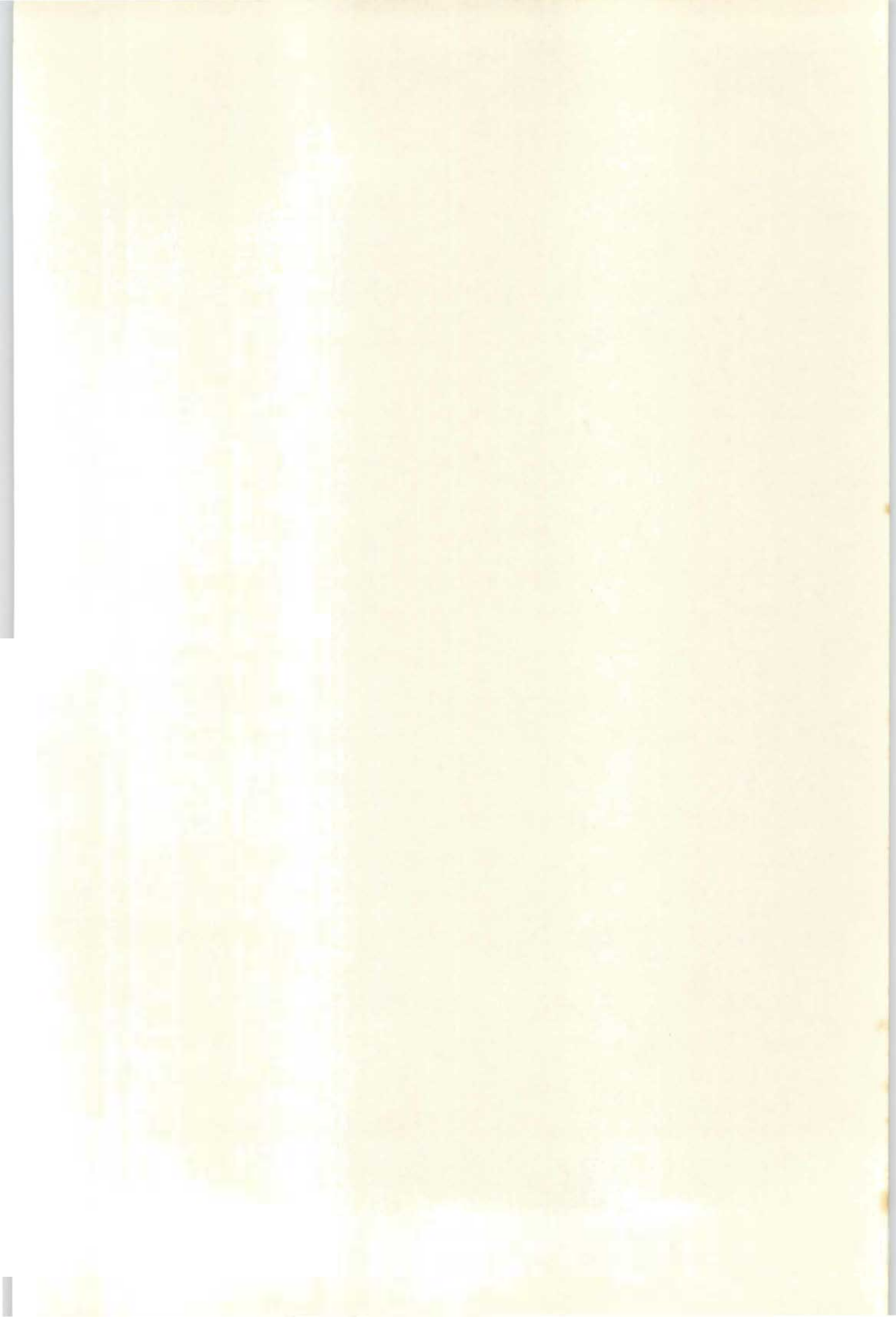
E X P E R I M E N T A L

The following generalisations apply throughout the experimental section, unless otherwise specified. Melting points were determined on a Kofler micro hot-stage and are uncorrected. Optical rotations of solutions in chloroform were measured at room temperature with a Bellingham and Stanley No. 390817 polarimeter. Infrared spectra of samples in potassium bromide discs were recorded on a Perkin-Elmer model 521 spectrophotometer. Ultraviolet absorption spectra of approximately 10^{-3}M solutions in 95% ethanol were recorded with a Beckmann DB UV/visible recording spectrophotometer; logε in parentheses follows $\lambda_{max.}$. Mass spectra were obtained with an A.E.I. MS 9 double focussing mass spectrometer. ^{1}H N.m.r. spectra were measured on Varian A-60 and HA-100 instruments using deuteriochloroform as solvent and tetramethylsilane as internal reference. Optical rotatory dispersion curves of methanolic solutions were recorded on a Jasco ORD/UV-5 instrument. In cases where elementary analyses could not be determined due to lack of material, accurate mass measurements were obtained. The light petroleum used had a boiling range of 56-60°. The solvent employed in crystallisations was aqueous methanol.

Chromatography

Glass plates for thin layer chromatography were coated to a thickness of 250μ with Merck silica gel and activated for thirty minutes prior to use. The solvent systems most commonly used were mixtures of benzene-light petroleum (1:1) and ethanol in varying amounts (0-10%) depending on the polarity of the compounds to be separated.

Two spray reagents were utilised in the detection of the components on thin layer chromatograms. These were 1) a 20% solution of antimony pentachloride in chloroform 2) a 5% solution of p-anisaldehyde in 5% ethanolic sulphuric acid. The plates were sprayed with one of these reagents and heated to 100° for 5-10 minutes to allow the full development of colours.

In routine column chromatography basic aluminium oxide was mainly used. This was found to be more successful in separating the ketol, II, and the diol, III, in better yields, while silica gel of particle size between 0.05 and 0.2 mm. yielded more of the alcohol, I. The solvent systems employed for eluting ranged from light petroleum to mixtures of light petroleum-benzene (1:1) and gradually increasing amounts of ethanol (0-5%). It has been established that with the use of these eluants migration rates of the components are chiefly dependent on the ethanol concentration (139).

Colour Reactions

(a)   Tetranitromethane

The sample (1 mg.) was treated with tetranitro-
methane (1 drop) on a spot plate, and any colour produced
within five minutes was noted. Varying shades of yellow
were produced by compounds containing olefinic double
bonds (81), whilst saturated compounds remained unchanged.

(b)   Zimmermann Test

The sample (100 mg.) was dissolved in 2N potassium
hydroxide in absolute ethanol (1 ml.) and 1% m-dinitro-
benzene in absolute ethanol (1 ml.) and set aside for 10
min. On dilution to 10 ml. with absolute ethanol the fad-
ing of a violet colour indicated that the sample was a C-3
ketone (98).

(c)   Triphenyltetrazolium chloride

The sample (2 mg.) dissolved in absolute ethanol
(1 ml.) was treated with a solution of triphenyltetrazolium
chloride (TTC) in absolute ethanol (1%, 1 ml.), followed by
aqueous sodium hydroxide (10%, 1 drop). The appearance of
a red colour or a red precipitate due to the formation of
the triphenylformazan indicated the presence of an α-ketol
or any other easily oxidised structure (113).

PART 1   CLEISTANTHUS SCHLECHTERI

Source and Extraction of the Heartwood Diterpenes

The heartwood was obtained from three trees of Cleistanthus schlechteri var. schlechteri, Family Euphorbiaceae, collected in the coastal region of Zululand, Natal. Botanical specimens of each were deposited at the Natal Herbarium under the numbers NH 53049, 57826 and 60870. The light petroleum extracts of the heartwood from the three trees were shown to be identical by thin layer chromatography.

The milled, air-dried heartwood (6.68 Kg.) was soaked for 12 hr. at room temperature in light petroleum (8 l.), which on evaporation yielded an orange syrup (102 g.). Further exhaustive extraction of the wood with refluxing light petroleum (48 hr.) caused the separation of crude cleistanthol, IV, (104 g., 1.55%) and a dark orange syrup (95 g.) identical in composition to the cold extract.

Portions of the cold extract (15 g.) dissolved in light petroleum-chloroform (5:1, 75 ml.) were applied to columns of basic aluminium oxide (300 g.). Elution with light petroleum gave a mixture of fatty products (2.1 g.), while further elution with a mixture of benzene-light petroleum (1:1) afforded crystals of 3α-hydroxy-ent-iso-pimara-8(14),15-diene, I, (0.2 g.). Repeated crystallisations gave colourless needles, m.p. 126-127°, $[\alpha]_D$+12.5°(c 1), m/e 288 (M^+).

(Found: C, 83.1; H, 11.3. $C_{20}H_{32}O$ requires C, 83.3; 11.2%)

$\nu_{max.}$ 3325 br (O-H), 3080 (C=CH$_2$) and 1631 (C=C) cm.$^{-1}$

τ 4.19 (1H, q, C-15 H, $J_{obs.}$ 10 and 18 Hz), 4.75 (1H, s, C-14 H), 5.10 (2H, m, 2xC-16 H), 6.73 (1H, m, C-3 H), 8.95, 8.99, 9.17 and 9.19 (4x3H, 4xs, 4xMe).

(Reported for isopimara-8(14),15-dien-3β-ol, LXXXV (c): m.p. 126.5-127.5°, [α]$_D$-19.5°(100); the mass spectrum (103) and i.r. spectrum were identical to those given by I).

The addition of ethanol to the eluting mixture (benzene-light petroleum-ethanol; 1:1:0.01) yielded crystals of 3α-hydroxy-ent-isopimara-8(14),15-dien-12-one, II, (1.8 g.). Recrystallisation from light petroleum-ethanol gave large colourless prisms, m.p. 157-158°, [α]$_D$+290°(c 1), m/e 302 (M$^+$).

(Found: C, 79.3; H, 10.0. $C_{20}H_{30}O_2$ requires C, 79.4; H, 10.0%)

$\nu_{max.}$ 3547 s (O-H), 3085 (=CH$_2$), 1696 (C=O) and 1628 (C=C) cm.$^{-1}$

$\lambda_{max.}$ 300 (2.28) nm.

τ 4.25 (1H, q, C-15 H, $J_{obs.}$ 10 and 18 Hz), 4.76 (1H, s, C-14 H), 4.89 (2H, m, 2xC-16 H), 6.76 (1H, m, C-3 H), 8.78, 8.87, 9.20 and 9.29 (4x3H, 4xs, 4xMe).

A further increase in the ethanol content of the eluant above (1:1:0.04) furnished crystals of 3α,12α-dihydroxy-ent-isopimara-8(14),15-diene, III, (0.6 g.). On recrystallisation colourless, long spars were obtained, m.p. 161-162°, $[α]_D + 28°$ (c 1), m/e 304 (M^+).

(Found: C, 78.7; H, 10.8. $C_{20}H_{32}O_2$ requires C, 78.9; H, 10.6%)

$ν_{max.}$ 3290 br (O-H) and 1635 (C=C) cm.$^{-1}$

$τ$ 4.22 (1H, q, C-15 H, $J_{obs.}$ 10 and 18 Hz), 4.93 (3H, m, C-14 H and 2xC-15 H), 6.45 (1H, m, C-12 H), 6.75 (1H, m, C-3 H), 8.93, 8.98, 9.16 and 9.16 (4x3H, 4xs, 4xMe).

Continued elution with a more polar solvent system (5-10% ethanol) produced a brown gum (6 g.) containing traces of cleistanthol, IV, amongst a mixture of many other constituents.

Acetylation Products

3α-Hydroxy-ent-isopimara-8(14),15-diene, I, (0.1 g.) dissolved in a mixture of dry pyridine (6 ml.) and acetic anhydride (0.5 ml.) was set aside overnight, then poured into ice-water with stirring. The crude product was filtered and dried, and on crystallisation yielded colourless needles of 3α-acetoxy-ent-isopimara-8(14),15-diene, LXXVII, (0.1 g.), m.p. 104-105°, $[α]_D - 7°$ (c 1), m/e 330 (M^+).

(Found: C, 79.9; H, 10.5. $C_{22}H_{34}O_2$ requires C, 80.0, H, 10.4%)

$\nu_{max.}$ 3080 s (C=CH$_2$), 1712 (acetate C=O), 1630 (C=C) and 1240 (ester C-O) cm.$^{-1}$

(Reported for 3β-acetoxyisopimara-8(14),15-diene, LXXXV (d): m.p. 102-104.5°, $[\alpha]_D$+4° (100)).

3α-Hydroxy-ent-isopimara-8(14),15-dien-12-one, II, (0.2 g.) was acetylated by the method described above to give, on crystallisation, colourless prisms of 3α-acetoxy-ent-isopimara-8(14),15-dien-12-one, XCI, (0.19 g.), m.p. 127-128°, $[\alpha]_D$+210.5° (c 1), m/e 344 (M$^+$).

(Found: C, 76.5; H, 9.4. $C_{22}H_{32}O_3$ requires C, 76.7; H, 9.4%)

$\nu_{max.}$ 3078 (C=CH$_2$), 1723 (acetate C=O), 1700 (C-12 carbonyl) and 1243 (ester C-O) cm.$^{-1}$

τ 4.25 (1H, q, C-15 H, $J_{obs.}$ 10 and 18 Hz), 4.74 (1H, s, C-14 H), 4.89 (2H, m, C-16 H), 5.50 (1H, m, C-3 H), 7.96 (3H, s, CH$_3$C=O), 8.78, 9.10, 9.14 and 9.28 (4x3H, 4xs, 4xMe).

3α,12α-Dihydroxy-ent-isopimara-8(14),15-diene, III, (0.1 g.) was acetylated as described above and yielded, after crystallisation, colourless needles of 3α,12α-di-acetoxy-ent-isopimara-8(14),15-diene, XCIX, (0.1 g.), m.p.

117°, $[\alpha]_D$+33° (c 1), m/e 388 (M$^+$).

(Found: C, 73.9; H, 9.4. $C_{24}H_{36}O_4$ requires C, 74.2;
H, 9.3%)

$\nu_{max.}$ 3095 (C=CH$_2$), 1733 and 1721 (C-12 and C-3 acetate
C=O respectively) and 1235 (ester C-O) cm.$^{-1}$

τ 4.39 (1H, q, C-15 H, $J_{obs.}$ 10 and 18 Hz), 4.85 (1H,
s, C-14 H), 5.05 (2H, m, 2xC-16 H), 5.19 (1H, over-
lapped, C-12 H) and 5.48 (1H, m, C-3 H).

Oxime of the Ketol, II

3α-Hydroxy-ent-isopimara-8(14),15-dien-12-one, II,
(0.1 g.) and hydroxylamine hydrochloride (0.2 g.) were
dissolved in a mixture of pyridine (10 ml.) and ethanol
(10 ml.), then set aside for 24 hr. Dilution with ice-
water caused the precipitation of the oxime which was
crystallised to give colourless cubes, m.p. 167-168°,
$[\alpha]_D$+163° (c 1), m/e 317 (M$^+$).

(Found: M$^+$, m/e 317.235202. $C_{20}H_{31}NO_2$ requires M$^+$, m/e
317.235466).

Catalytic Reductions

3α-Hydroxy-ent-isopimara-8(14),15-diene, I, (0.1 g.)
dissolved in ethanol (10 ml.) was stirred with hydrogen in
the presence of palladium-charcoal catalyst (30 mg., 30%)
until the uptake of hydrogen ceased (30 min.). During this

period 8 ml. of hydrogen was absorbed at N.T.P. (theoretical
for 1 mole, 7.6 ml.). Crystallisation of the product
yielded colourless, silky needles of 3α-hydroxy-ent-isopimar-
8(14)-ene, LXXVIII (a), (95 mg.), m.p. $132-133^O$, $[\alpha]_D-23.2^O$
(c 2), m/e 290 (M^+).

(Found: C, 82.7; H, 11.8. $C_{20}H_{34}O$ requires C, 82.7;
H, 11.8%)

$\nu_{max.}$ 3320 (O-H) and 1631 w (C=C) $cm.^{-1}$
(Reported for isopimar-8(14)-en-3β-ol, LXXXVI: m.p. 132-
132.5^O, $[\alpha]_D+25.5^O$, (100)).

3α-Hydroxy-ent-isopimara-8(14),15-diene, I, (0.2 g.)
in glacial acetic acid (20 ml.) was hydrogenated under
high pressure (1400 lb./sq. in.) in the presence of platinum
oxide catalyst (50 mg.) for 24 hr. (fresh catalyst being
added every 8 hr.). Hydrogenation was shown to be complete
by a negative tetranitromethane test. Crystallisation of
the crude product gave colourless needles of 3α-hydroxy-ent-
isopimarane, LXXIX, (0.12 g.), m.p. 135°, $[\alpha]_D-46.8^O$ (c 1),
m/e 292 (M^+).

(Found: C, 82.3; H, 12.5. $C_{20}H_{36}O$ requires C, 82.1;
H, 12.4%)
(Reported for isopimaran-3β-ol, LXXXVII: m.p. $134-135.5^O$,
$[\alpha]_D+21.8^O$, (100)).

3α-Acetoxy-ent-isopimara-8(14),15-diene, LXXVII,

(0.1 g.) was hydrogenated over palladium-carbon catalyst in ethanol (10 ml.) as described above to afford colourless needles of 3α-acetoxy-ent-isopimar-8(14)-ene, LXXVIII (b), (90 mg.), m.p. 117-118°, $[\alpha]_D$-47° (c 1), m/e 332 (M^+).

(Found: C, 79.5; H, 11.0. $C_{22}H_{36}O_2$ requires C, 79.5; H, 10.9%).

3α-Hydroxy-ent-isopimara-8(14),15-dien-12-one, II, (0.2 g.) in ethanol (20 ml.) over palladium-carbon (50 mg., 30%) absorbed 14.1 ml. of hydrogen at N.T.P. (theoretical for 1 mole, 12.8 ml.). Crystallisation of the product gave colourless needles of 3α-hydroxy-ent-isopimar-8(14)-en-12-one, XCIII (a), (0.18 g.), m.p. 114-115°, $[\alpha]_D$+76° (c 1), m/e 304 (M^+).

(Found: C, 78.6; H, 10.6. $C_{20}H_{32}O_2$ requires C, 78.9; H, 10.6%)

$\nu_{max.}$ 3542 (O-H), 1695 (C=O) and 1655 (C=C) $cm.^{-1}$

$\lambda_{max.}$ 292 (1.75) nm.

τ 4.70 (1H, s, C-14 H) and 6.72 (1H, m, C-3 H).

3α-Acetoxy-ent-isopimara-8(14),15-dien-12-one, XCI, was hydrogenated in ethanol over palladium-carbon as described above to give colourless needles of 3α-acetoxy-ent-isopimar-8(14)-en-12-one, XCIII (b), m.p. 108-109°, $[\alpha]_D$+52° (c 1), m/e 346 (M^+).

(Found: C, 76.2; H, 9.9. $C_{22}H_{34}O_3$ requires C, 76.3; H, 9.9%)

$\nu_{max.}$ 1725 (acetate C=O), 1700 (C-12 C=O) and 1242 (ester C-O) cm.$^{-1}$

τ 4.76 (1H, s, C-14 H) and 5.50 (1H, m, C-3 H).

This compound was also obtained by acetylation of 3α-hydroxy-ent-isopimar-8(14)-en-12-one, XCIII (a) as previously described.

3α-Acetoxy-ent-isopimara-8(14),15-dien-12-one, XCI, on hydrogenation over a platinum oxide catalyst in glacial acetic acid for 24 hr. (with the addition of fresh catalyst every 8 hr.) yielded crude 3α-acetoxy-ent-isopimaran-12-ol, XCV. Crystallisation of the product resulted in colourless needles, m.p. 133-134°, m/e 350 (M$^+$).

(Found: C, 75.3; H, 10.9. $C_{22}H_{38}O_3$ requires C, 75.4; H, 10.9%).

3α,12α-Dihydroxy-ent-isopimara-8(14),15-diene, III, (0.1 g.) was hydrogenated over palladium-carbon in ethanol as described above to give, on crystallisation, colourless needles of 3α,12α-dihydroxy-ent-isopimar-8(14)-ene, C (a), (80 mg.), m.p. 172-173°, $[\alpha]_D$-31° (c 1), m/e 306 (M$^+$).

(Found: C, 74.6; H, 11.6. $C_{20}H_{34}O_2 \cdot CH_3OH$ requires C, 74.5; H, 11.6%).

$\nu_{max.}$ 3300 br (O-H) $cm.^{-1}$

τ 4.88 (1H, s, C-14 H) and 6.53 (2H, m, C-3 and C-12 H).

3 α,12α-Diacetoxy-<u>ent</u>-isopimara-8(14),15-diene, XCIX, (0.1 g.) when hydrogenated over palladium-carbon in ethanol yielded colourless, long needles of 3 α,12 α-diacetoxy-<u>ent</u>-isopimar-8(14)-ene, C (b), (90 mg.), m.p. 132-133°, [α] $_D$ -5.8° (c 1), m/e 390 (M^+).

(Found: C, 73.9; H, 9.9. $C_{24}H_{38}O_4$ requires C, 73.8; H, 9.8%)

$\nu_{max.}$ 1725 (acetate C=O) and 1238 (ester C-O) $cm.^{-1}$

τ 4.89 (1H, s, C-14 H), 5.19 (1H, q, C-12 H, $J_{obs.}$ 5 and 11 Hz) and 5.49 (1H, m, C-3 H).

This product was also obtained by acetylation of 3α,12α-dihydroxy-<u>ent</u>-isopimar-8(14)-ene, C (a), by the method previously described.

3 α,12α-Dihydroxy-<u>ent</u>-isopimara-8(14),15-diene, III, (0.1 g.) was hydrogenated over platinum oxide catalyst in glacial acetic acid for 24 hr. (fresh catalyst being added every 8 hr.) to give, on crystallisation, colourless

needles of $3\alpha,12\alpha$-dihydroxy-ent-isopimarane, CI, (90 mg.), m.p. 164-165°, $[\alpha]_D$-6° (c 1), m/e 308 (M$^+$).

(Found: C, 74.1; H, 11.6. $C_{20}H_{36}O_2 \cdot CH_3OH$ requires C, 74.1; H, 11.8%)

$\nu_{max.}$ 3380 br (O-H) cm.$^{-1}$

τ 6.73 (2H, m, C-3 and C-12 H).

Oxidation Products

3α-Hydroxy-ent-isopimara-8(14),15-diene, I, (0.1 g.) dissolved in acetone (5 ml.), freshly-distilled over potassium permanganate, was treated dropwise with Jones reagent (97) until the reaction mixture remained orange-brown. The mixture was set aside at 5° for 1 hr. after which the addition of ice-water induced the deposition of crystals of ent-isopimara-8(14),15-dien-3-one, LXXXI, (80 mg.). Recrystallisation yielded colourless, long rods, m.p. 60°, $[\alpha]_D$+45° (c 2), m/e 286 (M$^+$).

(Found: C, 83.6; H, 10.5. $C_{20}H_{30}O$ requires C, 83.9; H, 10.6%)

$\nu_{max.}$ (CHCl$_3$) 3078 (C=CH$_2$), 1697 (C=O) and 1630 (C=C) cm.$^{-1}$

[ϕ] +2810° (max., 315 nm.) and 0° (min., 280 nm.),

τ 4.25 (1H, q, C-15 H, $J_{obs.}$ 10 and 18 Hz), 4.74 (1H, s, C-14 H), 5.12 (2H, m, 2xC-16 H), 8.93, 8.98, 8.98

and 9.02 (4x3H, 4xs, 1xMe).

The ketone, LXXXI, gave a 2,4-dinitrophenyl-hydrazone, m.p. 177-178°.

(Found: C, 66.8; H, 7.6. $C_{26}H_{34}N_4O_4$ requires C, 66.9; H, 7.4%).

(Reported for isopimara-8(14),15-dien-3-one, LXXXV (b):

m.p. 59-60°, $[\alpha]_D$-56°; 2,4-dinitrophenylhydrazone, m.p. 176.5-177° (100)).

3α-Hydroxy-ent-isopimara-8(14),15-dien-12-one, II, (0.1 g.) when oxidised with Jones reagent, as described above, yielded ent-isopimara-8(14),15-dien-3,12-dione, XCIV, (70 mg.) which crystallised as colourless, long spars, m.p. 84-85°, $[\alpha]_D$+312° (c 1), m/e 300 (M^+).

(Found: C, 79.8; H, 9.6. $C_{20}H_{28}O_2$ requires C, 80.0; H, 9.4%)

$\nu_{max.}$ 3078 (C=CH$_2$), 1695 s (C=O) and 1625 (C=C) cm.$^{-1}$

$\lambda_{max.}$ 236 sh (2.48) and 295 nm. (2.32), unchanged on addition of base.

3α,12α-Dihydroxy-ent-isopimara-8(14),15-diene, III, on oxidation with Jones reagent, yielded a diketone identical to ent-isopimara-8(14),15-dien-3,12-dione, XCIV.

3α-Hydroxy-ent-isopimar-8(14)-ene, LXXVIII (a),

on oxidation with Jones' reagent followed by crystallisation of the product, gave colourless needles of ent-isopimar-8(14)-en-3-one, LXXXII, m.p. 59-60°, $[\alpha]_D$+12.5^{O}, m/e 288 (M^{+}).

$\nu_{max.}$ 1695 (C=O) cm.$^{-1}$

3α-Hydroxy-ent-isopimarane, LXXIX, was oxidised with Jones reagent as described above to give colourless needles of ent-isopimaran-3-one, LXXIII, m.p. 70-71°, $[\alpha]_D$-51.5^{O} (c 1), m/e 290 (M^{+}).

(Found: M^{+}, m/e 290.259496. C$_{20}$H$_{34}$O requires M^{+}, m/e 290.260952)

$\nu_{max.}$ 1695 (C=O) cm.$^{-1}$

$\lambda_{max.}$ 291 (1.32) nm.

3α-Acetoxy-ent-isopimaran-12-ol, XCV, on oxidation with Jones reagent and crystallisation yielded colourless needles of 3α-acetoxy-ent-isopimaran-12-one, XCVI, m.p. 105°, $[\alpha]_D$-61.3^{O} (c 2), m/e 348 (M^{+}).

(Found: C, 75.9; H, 10.3. C$_{22}$H$_{36}$O$_3$ requires C, 75.8; H, 10.4%)

$\nu_{max.}$ 1718 (acetate C=O), 1700 (C-12 C=O) and 1240 (ester C-O) cm.$^{-1}$

τ 5.51 (1H, m, C-3 H) and 7.80 (2H, q, 2xC-11 H).

Huang-Minlon (99) Reduction Products

3α-Hydroxy-ent-isopimara-8(14),15-dien-3-one,
II, (0.5 g.) and hydrazine hydrate (1.5 ml.) dissolved in
a mixture of ethanol (1 ml.) and diethylene glycol (12 ml.)
was refluxed for 30 min. before the addition of a concentrat-
ed solution of potassium hydroxide (3 g.) in water (3 ml.).
The reaction mixture was refluxed for a further 90 min. after
which the condenser was removed to allow the evaporation
of water vapour. Heating was continued until the temperature
reached 200° when refluxing was continued for 2 hr. The
cooled mixture, on dilution with water, produced a flocculent
precipitate which on repeated recrystallisation gave
colourless needles of 3α-hydroxy-ent-isopimara-8(14),15-diene,
I, (0.35 g.), m.p. 128-129°, [α]$_D$+13.5° (c 2). The infrared
and mass spectra were identical to those obtained for the
alcohol, I, isolated from the heartwood extract.

ent-Isopimara-8(14),15-dien-3-one, LXXXI, or
ent-isopimara-8(14),15-dien-3,12-dione, XCIV, was reduced
as described above to produce crude ent-isopimara-8(14),15-
diene, LXXXIV. Crystallisation from light petroleum and
chloroform yielded colourless crystals, m.p. 38-39°,
[α]$_D$+7° (c 1), m/e 272 (M$^+$).

(Found: M$^+$, m/e 272.249103. $C_{20}H_{32}$ requires M$^+$, m/e

272.250389).

(Reported for isopimara-8(14),15-diene, LXXXV (a): m.p. 40.5-41°, $[\alpha]_D$-12° (100)).

Reductions of the Ketol, II, to the Diol, III

(a) With LiAlH$_4$

3α-Hydroxy-ent-isopimara-8(14),15-dien-12-one, II, (0.5 g.) dissolved in ether (10 ml.) was added dropwise to a suspension of lithium aluminium hydride (1.5 g.) in dry ether (20 ml.). The mixture was heated under reflux for 20 hr., after which the excess reagent was decomposed by the addition of dilute sulphuric acid and heating at 100°. Separation and evaporation of the ether layer produced a colourless solid which crystallised as long rods of 3α,12α-dihydroxy-ent-isopimara-8(14),15-diene, III, (0.3 g.), m.p. 161°, $[\alpha]_D$+24° (c 1), m/e 304 (M$^+$).

(Found: C, 79.1; H, 10.6. $C_{20}H_{32}O_2$ requires C, 78.9; H, 10.6%).

(b) With Sodium in Pentyl Alcohol (7)

Small portions of sodium (1.75 g.) were added to a refluxing solution of 3α-hydroxy-ent-isopimara-8(14),15-dien-12-one, II, (0.5 g.) in pentyl alcohol (40 ml.). After all the metal had dissolved the mixture was steam-distilled to remove all the alcohol. From the aqueous suspension a flocculent precipitate was filtered off to yield, on

crystallisation, colourless rods of $3\alpha,12\alpha$-dihydroxy-ent-isopimara-8(14),15-diene, III, (0.4 g.).

The infrared and mass spectra of both reduction products were superimposable on those of the diol, III, obtained from the heartwood extract, while mixed melting points of both with an authentic sample of III were undepressed.

Bromination Products

ent-Isopimaran-3-one, LXXXIII, (0.1 g.) dissolved in dry benzene (10 ml.) was treated dropwise with a solution of bromine (50 mg.) in benzene (3 ml.). The initial slow uptake of bromine was followed by a more rapid absorption. The reaction appeared to be complete after 30 min. after which the benzene was evaporated to give a gum. Crystallisation afforded colourless needles of $1\alpha,2\beta$-dibromo-ent-isopimaran-3-one, LXXXXIX, (95 mg.), m.p. 115-116°, $[\alpha]_D$-10° (c 1), m/e 446, 448, 450 (M$^+$).

(Found: C, 53.5; H, 7.2. $C_{20}H_{32}OBr_2$ requires C, 53.6; H, 7.1%)

$\nu_{max.}$ 1720 (C=O) cm.$^{-1}$

τ 6.25 and 6.92 (2H, 2xd, C-1 and C-2 H, $J_{obs.}$ 16 Hz).

ent-Isopimaran-3-one, LXXXIII, (0.1 g.) dissolved in glacial acetic acid (10 ml.) was treated dropwise with

a solution of bromine (50 mg.) in glacial acetic acid
containing one drop of acetic acid saturated with HBr.
After the colour was discharged (10 min.) dilution with
ice-water induced the precipitation of pale yellow crystals
which were filtered and washed with dilute aqueous sodium
thiosulphate. The crystals were shown by t.l.c. to be a
mixture of the mono- and dibromo compounds. These components
were separated by means of preparative t.l.c. Recrystallis-
ation furnished colourless needles of 2β-bromo-ent-isopimaran-
3-one, LXXXVIII, (80 mg.), m.p. 101-102°, m/e 368, 370 (M^+).

(Found: M^+, m/e 368.170117. $C_{20}H_{33}OBr$ requires M^+,
m/e 368.171342)

$\nu_{max.}$ 1720 (C=O) cm.$^{-1}$

τ 4.99 (1H, m, C-2 H).

3α-Acetoxy-ent-isopimaran-12-one, XCVI, (120 mg.)
dissolved in glacial acetic acid (15 ml.) was treated drop-
wise with a solution of bromine (50 mg.) in acetic acid
(3 ml.). The mixture was set aside for 5 days at room
temperature, after which dilution with ice-water yielded
a pale yellow precipitate which was filtered and washed with
dilute aqueous sodium thiosulphate. Crystallisation of the
precipitate (100 mg.) afforded colourless flakes of 3α-
acetoxy-11β-bromo-ent-isopimaran-12-one, XCVII, m.p. 149-150°,
$[\alpha]_D$-34° (c 1), m/e 426, 428 (M^+).

(Found: C, 61.6; H, 8.4. $C_{22}H_{35}O_3Br$ requires C, 61.8; H, 8.2%)

$\nu_{max.}$ 1720 (acetate C=O), 1695 (C-12 C=O) and 1245 (ester C-O) cm.$^{-1}$

$\lambda_{max.}$ 325 nm. (1.89)

τ 5.49 (1H, m, C-3 H), 6.00 (1H, d, C-11 H, $J_{obs.}$ 4 Hz), 7.97 (3H, s, $C\underline{H}_3C=O$), 8.95, 9.15, 9.16 and 9.22 (4x3H, 4xs, 4xMe).

Dehydrobromination of the bromoketone, XCVII

A mixture of 3α-acetoxy-11β-bromo-ent-isopimaran-12-one, XCVII, (0.2 g.), lithium carbonate (0.2 g.) and lithium bromide (0.25 g.) in dimethylformamide (8 ml.) was heated under reflux for 90 min. under a stream of nitrogen. Dilution of the cooled reaction mixture with water yielded colourless crystals of the α,β-unsaturated ketone (0.1 g.). Recrystallisation gave small plates of 3α-acetoxy-ent-isopimar-9(11)-en-12-one, m.p. 143-145°, $[\alpha]_D$ -38° (c 1), m/e 346 (M^+).

(Found: C, 76.3; H, 9.9. $C_{22}H_{34}O_3$ requires C, 76.3; H, 9.9%)

$\nu_{max.}$ 3048 (C=C-H), 1729 (acetate C=O), 1657 (C-12 C=O), 1603 (C=C) and 1235 (ester C-O) cm.$^{-1}$

$\lambda_{max.}$ 239 (4.16) nm.

τ 4.31 (1H, d, C-11 H, $J_{11,8}$ 2 Hz), 5.52 (1H, m, C-3 H),
7.40 (1H, m, C-8 H), 7.97 (3H, s, C$\underline{H}_3$C=O), 8.87, 9.03,
9.07 and 9.13 (4x3H, 4xs, 4xMe).

Attempted Preparation of the Isopropylidenedioxy derivative from the Diol, III

3α,12α-Dihydroxy-ent-isopimara-8(14),15-diene,
III, (50 mg.) dissolved in acetone (5 ml.) and conc.
hydrochloric acid (1 drop) was set aside overnight at 0°.
On dilution with water the product was recovered unchanged.

Isopimara-8(14),15-dien-3-one

A sample of this compound was obtained for purposes
of comparison with the enantiomer, LXXXI. The compound had
m.p. 59-60°, [φ] -2460° (max., 315 nm.) and 0° (min., 280 nm.),
and its infrared and mass psectra were superimposable on
those of LXXXI.

Preparation of Jones' Reagent (97)

A cold solution of chromic oxide (26.7 g.) in conc.
sulphuric acid (23 ml.) and distilled water (40 ml.) was made
up to 100 ml. with water. This solution is 8 N with respect
to oxygen.

PART 2 SPIROSTACHYS AFRICANA

Source and Extraction of the Heartwood Diterpenes

The heartwood was obtained from two trees of
Spirostachys africana Sond. (syn. Excoecaria africana Muell.
Arg.), Family Euphorbiaceae, which had been collected near
Hluhluwe and Inanda, Natal. Botanical specimens of each
were deposited at the Natal Herbarium, Durban, under the
numbers NH52617 A and 52617 B. The light petroleum extracts
from both trees were shown to be identical by comparative
thin layer chromatography.

The milled, air-dried heartwood (641 g.) was
placed in a soxhlet extractor and washed through with three
portions (2 l. each) of cold light petroleum. These
washings were combined and, on evaporation, produced a
yellow syrup (98 g.). Exhaustive extraction of the wood
with refluxing light petroleum (24 hr.) followed by
evaporation of the solvent resulted in a dark orange syrup
(10 g.) which was similar in composition to the cold extract.

Portions of the cold light petroleum extract (10 g.)
dissolved in light petroleum-benzene (4:1, 40 ml.) were
chromatographed on columns of silica gel (200 g., 0.05-0.2 mm.)
to effect a preliminary separation of the components.
Elution with light petroleum yielded a wax-like substance
thought to be a long-chain fatty acid. The addition of
benzene (10%) to the eluant produced a waxy solid found to
be a mixture of two compounds. Further elution with a

mixture of benzene-light petroleum (1:1) produced a solid
ascertained to be a mixture of three components, whilst
increasingly polar solvents failed to effect a separation
of the remaining constituents.

Beyer-15-en-3-one, V

The waxy solid (0.5 g.) obtained from the prelim-
inary separation was rechromatographed on silica gel (25 g.,
0.08 mm.) using light petroleum as eluant. The first 4
fractions collected furnished crystals of beyer-15-en-3-one,
V, (0.1 g.) which, on recrystallisation from light petroleum,
gave colourless needles, m.p. 40-41°, $[\alpha]_D$+27° (c 1), m/e
286 (M$^+$).

(Found: C, 84.1; H, 10.8. $C_{20}H_{30}O$ requires C, 83.9;
H, 10.6%)

$\nu_{max.}$ 3045 (H-C=C-H), 1702 (C=O), 748 (C=C) cm.$^{-1}$

$\lambda_{max.}$ 288 (1.62) nm.

The 2,4-dinitrophenylhydrazone was recrystallised
from ethanol to give orange needles, m.p. 194-196°.
(Reported for beyer-15-en-3-one, V, (7): m.p. 35-36.5^O,
$[\alpha]_D$+22°, 2,4-dinitrophenylhydrazone m.p. 194-196°).

Hydrogenation of beyer-15-en-3-one, V

Beyer-15-en-3-one, V, (5 mg.) was hydrogenated
over palladium-carbon catalyst (2 mg., 30%) in ethanol
(2 ml.) until no further absorption was observed. One mole

of H_2 was absorbed to give beyeran-3-one which crystallised from light petroleum as colourless needles, m.p. 63-64° and failed to react with tetranitromethane.

3-Hydroxybeyer-15-en-2-one, VI

The next 3 fractions eluted produced a colourless compound on evaporation. Crystallisation from light petroleum afforded needles of 3-hydroxybeyer-15-en-2-one, VI, (50 mg.), m.p. 128-129°, $[\alpha]_D$+34° (c 1), m/e 302 (M$^+$).

$\nu_{max.}$ 3460 (O-H), 1712 (C=O) and 745 (C=C) cm.$^{-1}$

$\lambda_{max.}$ 286 (1.5) nm.

2-Hydroxybeyera-1,15-dien-3-one, VII

The mixture originally obtained from the preliminary separation of the crude extract was dissolved in light petroleum (50 ml.) and extracted with a solution of potassium hydroxide (0.2 g.) in aqueous ethanol (2:3, 60 ml.). The aqueous layers were in turn extracted with light petroleum (30 ml.), and the combined organic layers, on concentration and cooling, yielded crude 2-hydroxybeyera-1,15-dien-3-one (10 mg.), m.p. 129-131°.

$\lambda_{max.}$ 271 (3.96) nm.

EtOH+KOH

$\lambda_{max.}$ 316 (3.7) nm.

On treatment with neutral ferric chloride a

mauve-brown colour was observed indicative of a diosphenol.

2,3-Secobeyer-15-en-2,3-dioic acid, VIII

Portions (10 g.) of light petroleum extract dissolved in light petroleum (50 ml.) were subjected to partition by means of counter-current extraction along 12 tubes in which the phases were light petroleum (2 l.) and potassium hydroxide (10 g.) in aqueous ethanol (1:1, 2 l.), which had been previously shaken and set aside for 2 hr. to allow mutual saturation of the two phases. The aqueous layers from the first four tubes were collected and acidified with conc. hydrochloric acid. This resulted in the deposition of colourless crystals which were filtered and recrystallised from dilute acetic acid to give needles of 2,3-secobeyer-15-en-2,3-dioic acid, VIII, (2 g.), m.p. 217-218°, $[\alpha]_D$-29° (c 2), m/e 334 (M$^+$).

(Found: C, 71.8; H, 8.8. $C_{20}H_{30}O_4$ requires C, 71.8; H, 9.0%)

$\nu_{max.}$ 2653 (COOH), 1718 and 1685 (C=O) and 746 (C=C) cm.$^{-1}$

τ 4.35 and 4.55 (2H, 2xd, C-15 and C-16 H, $J_{obs.}$ 6 Hz), 8.76, 8.83, 9.01 and 9.15 (4x3H, 4xs, 4xMe).

Accurate mass measurements of major fragments in the mass spectrum substantiated the structure assigned to the diacid, VIII.

Found: m/e. 187.147869 (M-147). $C_{14}H_{19}$ requires m/e, 187.148668.

Found: m/e, 247.170782 (M-87). $C_{16}H_{23}O_2$ requires m/e, 247.169795.

Found: m/e, 275.198895 (M-59). $C_{18}H_{27}O_2$ requires m/e, 275.201094.

Hydrogenation of 2,3-Secobeyer-15-en-2,3-dioic acid, VIII

2,3-Secobeyer-15-en-2,3-dioic acid, VIII, (5.4 mg.) was hydrogenated over platinum oxide catalyst (3 mg.) in glacial acetic acid (2 ml.) and after the absorption of 0.43 ml. H_2, further uptake ceased (theoretical for 1 mole, 0.38 ml.). Crystallisation of the product yielded colourless needles of 2,3-secobeyeran-2,3-dioic acid, CVII, m.p. 215°, $[\alpha]_D$-20° (c 1), m/e 336 (M$^+$).

(Found: C, 71.5; H, 9.6. $C_{20}H_{32}O_4$ requires C, 71.4; H, 9.6%),

$\nu_{max.}$ 2657 (COOH), 1710 and 1688 (C=O) cm^{-1}.

τ 8.68, 8.80, 8.92 and 9.04 (4x3H, 4xs, 4xMe).

Hydrogenation in ethanolic medium proceeded less rapidly than in the above-mentioned case, but gave rise to the same product.

Methylation of 2,3-Secobeyer-15-en-2,3-dioic acid, VIII

(a)   Fischer-Speier Method (132)

2,3-Secobeyer-15-en-2,3-dioic acid, VIII, (0.1 g.) dissolved in dry methanol (10 ml.) and conc. sulphuric acid (3 drops) was refluxed for 15 hr. Dilution of the cooled

mixture with ice-water produced a white precipitate which,
after repeated crystallisations, afforded colourless
needles of the monomethyl ester, CVIII, (90 mg.), m.p. 120°,
$[\alpha]_D$-10° (c 1), m/e 348 (M$^+$).

(Found: C, 72.7; H, 9.2. $C_{21}H_{32}O_4$ requires C, 72.9;
H, 9.4%)

$\nu_{max.}$ 2653 (COOH), 1740 (ester C=O), 1685 (acid C=O) and
748 (C=C) cm.$^{-1}$

(b)   With Diazomethane

2,3-Secobeyer-15-en-2,3-dioic acid, VIII, (0.1 g.)
dissolved in ether (10 ml.) at 5° was treated dropwise with
cold ethereal diazomethane until the evolution of gas ceased
and a yellow colour persisted. The mixture was set aside
for 2 hr. after which the ether was evaporated under
reduced pressure. The resultant colourless gum could not
be induced to crystallise, but was determined to be the
dimethyl ester, CIX, by the presence of the molecular ion
peak at m/e 362 (M$^+$ for $C_{22}H_{34}O_4$). Its intensity (1%) was
unfortunately too low to allow an accurate mass measurement,
but measurements of the major fragments substantiated the
structural assignment. Found: m/e, 261.184590 (M-101).
$C_{17}H_{25}O_2$ requires m/e, 261.185445. Found: m/e, 289.217930
(M-73). $C_{19}H_{29}O_2$ requires m/e, 289.216743.

Reduction of 2,3-Secobeyer-15-en-2,3-dioic acid, VIII

2,3-Secobeyer-15-en-2,3-dioic acid, VIII, (1 g.) dissolved in dry ether (25 ml.) was gradually added to a suspension of lithium aluminium hydride (3 g.) in dry ether (30 ml.) and refluxed for 12 hr. Excess of $LiAlH_4$ was then destroyed by the addition of dilute sulphuric acid and gentle warming. The ethereal layer was separated and evaporated to a gum which, on filtration through a short silica gel column, yielded colourless crystals of 2,3-secobeyer-15-en-2,3-diol, CX. Recrystallisation from light petroleum-ethanol (9:1) furnished needles, (0.2 g.), m.p. 129-130°, m/e 306 (M^+).

(Found: C, 78.1; H, 11.2. $C_{20}H_{34}O_2$ requires C, 78.4; H, 11.2%)

$\nu_{max.}$ 3338 (O-H) and 748 (C=C) cm.$^{-1}$

Anhydride Formation

2,3-Secobeyer-15-en-2,3-dioic acid, VIII, (0.1 g.) was refluxed with acetic anhydride (5 ml.) for 1 hr. Evaporation of the solvent under reduced pressure resulted in a gum which crystallised from light petroleum as colourless rods of the anhydride, CXI, (50 mg.), m.p. 129-130°, m/e 316 (M^+).

(Found: C, 75.8; H, 8.9. $C_{20}H_{28}O_3$ requires C, 75.9; H, 8.9%)

$\nu_{max.}$ 1796 and 1758 (anhydride C=O) and 746 (C=C) cm.$^{-1}$

τ 4.35 and 4.52 (2H, 2xd, C-15 and C-16 H), 8.67, 8.75, 8.98 and 9.08 (4x3H, 4xs, 4xMe).

2,3-Secobeyeran-2,3-dioic acid, CVII, (50 mg.) was refluxed with acetic anhydride (3 ml.) for 1 hr. The solvent was removed under reduced pressure to give a yellow gum which, on repeated crystallisations from light petroleum, gave colourless needles of the anhydride, CXII, (20 mg.), m.p. 122-123°, m/e 318 (M^{+}).

(Found: C, 75.2; H, 9.6. C$_{20}$H$_{30}$O$_{3}$ requires C, 75.4; H, 9.5%)

$\nu_{max.}$ 1792 and 1750 (anhydride C=O) cm.$^{-1}$

τ 8.67, 8.76, 8.93 and 9.03 (4x3H, 4xs, 4xMe).

Preparation of Ethereal Diazomethane (140)

An aqueous solution of 40% potassium hydroxide (1.5 ml.) was added to ether (5 ml.) and the mixture cooled in ice. To this was added portions of finely powdered nitrosomethylurea (0.5 g.) with continued cooling and shaking. The deep yellow ethereal layer containing diazomethane (0.14 g.) was used for methylating the seco-diacid, VIII.

Determination of the Number of Carboxyl Groups in 2,3-secobeyer-15-en-2,3-dioic acid, VIII

Normality of NaOH standardised with benzoic acid

$$=0.0091 \text{ N}$$

Molarity of 10 ml. aliquots of VIII in methanol

$$=0.011 \text{ M}$$

	1st titration	2nd titration
Volume NaOH	22.6 ml.	22.8 ml.

Average titration value   =22.7 ml. using phenolphthalein as indicator.

$\therefore$ $0.011 \text{ M} = \dfrac{22.7}{10} \times 0.0091$ equivalents

$$= 0.0206 \text{ N}$$

$\therefore$ 2,3-secobeyer-15-en-2,3-dioic acid, VIII, contains two carboxyl groups per molecule.

REFERENCE ABBREVIATIONS

Acta Chem. Scand.	Acta Chemica Scandinavica
Anal. Chem.	Analytical Chemistry
Arch. Pharm.	Archiv der Pharmazie
Austral. J. Chem.	Australian Journal of Chemistry
Berichte	Berichte der deutschen chemischen Gesellschaft.
Bull. Imp. Inst.	Bulletin of the Imperial Institute
Bull. Inst. Chem. Research	Bulletin of the Institute for Chemical Research
Bull. Soc. Chim. France	Bulletin de la Societe chimique de France
Canad. J. Chem.	Canadian Journal of Chemistry
Chem. Comm.	Chemical Communications
Chem. and Ind.	Chemistry and Industry
Coll. Czech. Chem. Comm.	Collection of Czechoslovak Chemical Communications
East African Agric. J.	East African Agricultural Journal
Helv. Chim. Acta	Helvetica Chimica Acta
Ind. J. Chem.	Indian Journal of Chemistry
J. Amer. Chem. Soc.	Journal of the American Chemical Society
J. Chem. Phys.	Journal of Chemical Physics
J. Chem. Soc.	Journal of the Chemical Society

J. Chem. Soc. (B)   Journal of the Chemical Society,
                            Section B, Physical Organic
                            Chemistry
J. Chem. Soc. (C)   Journal of the Chemical Society,
                            Section C, Organic Chemistry
J. Chromatog.   Journal of Chromatography
J. Org. Chem.   Journal of Organic Chemistry
Proc. Chem. Soc.   Proceedings of the Chemical
                            Society
Proc. Roy. Soc.   Proceedings of the Royal Society
S.A. Ind. Chem.   South African Industrial Chemist
Spectrochim. Acta   Spectrochimica Acta
Tet. Letters   Tetrahedron Letters

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Pimarane Diterpenes from *Cleistanthus schlechteri*

By H. A. Candy, Joan M. Pakshong, and K. H. Pegel,* Chemistry Department, Natal University, Durban, South Africa

Source of Heartwood.—Three trees of *Cleistanthus schlechteri* var. Schlechteri, Family Euphorbiaceae, were collected in the coastal region of Zululand, Natal, and botanical specimens of each were deposited in the Natal Herbarium, Durban, under the numbers 53049, 57826, and 60870. The light petroleum extracts of the milled heartwood from the three trees were shown to be identical by t.l.c.

Extraction of the Three Pimarane Diterpenes (II), (V), *and* (VI).—The milled heartwood (6·68 kg.) was soaked for 12 hr. at room temperature in light petroleum (8 l.) which was then evaporated under reduced pressure to yield an orange syrup (102 g.). Further exhaustive extraction of the wood with hot, light petroleum produced crude cleistanthol (I) (104 g., 1·55%) and a dark orange syrup (95 g.) identical in composition to the cold extract. The cold extract (15 g.) dissolved in light petroleum was applied to a basic aluminium oxide column; elution by the same solvent gave a fatty product (2·1 g.), and further elution with a mixture of benzene–light petroleum (1 : 1) afforded crystals of ent-*isopimara*-8(14),15-*dien*-3β-*ol* (II) (0·2 g.), which on repeated crystallisation gave colourless needles, m.p. 126—127°, $[\alpha]_D$ +12·5° (c 1) (Found: C, 83·1; H, 11·3. $C_{20}H_{32}O$ requires C, 83·3; H, 11·2%), m/e 288 (M^+); $\nu_{max.}$ 3325br, 3080, and 1631 cm.$^{-1}$; τ 4·19 (1H, q, 15-H, J 10 and 18 Hz), 4·75 (1H, s, 14-H), 5·10 (2H, m, 16-H), 6·73 (1H, m, 3-H), 8·95, 8·99, 9·17, and 9·19 (4 × 3H, 4 × s, 4 × Me) {reported for isopimara-8(14),15-dien-3β-ol: m.p. 126·5—127·5°, $[\alpha]_D$ −19·5°,[2] and the mass spectrum[3] and i.r. spectrum are identical to those given by (II)}. Addition of ethanol to the eluant (benzene–light petroleum–ethanol, 1 : 1 : 0·01) yielded crystals of ent-3β-*hydroxyisopimara*-8(14),15-*dien*-12-*one* (VI) (1·8 g.), which on recrystallisation from light petroleum–ethanol gave colourless prisms, m.p. 157—158°, $[\alpha]_D$ +290° (c 1) (Found: C, 79·3; H, 10·0. $C_{20}H_{30}O_2$ requires C, 79·4; H, 10·0%). m/e 302 (M^+); $\nu_{max.}$ 3547 (sharp), 3085, 1696, and 1628 cm.$^{-1}$; $\lambda_{max.}$ 300 (2·28) nm.; τ 4·25 (1H, q, 15-H, J_{obs} 10 and 18 Hz), 4·76 (1H, s, 14-H), 4·89 (2H, m, 16-H), 6·76 (1H, m, 3-H), 8·78, 8·87, 9·20, and 9·29 (4 × 3H, 4 × s, 4 × Me). On increasing the ethanol content of the above eluant (1 : 1 : 0·04) ent-*isopimara*-8(14),15-*dien*-3β,12β-*diol* (V) (0·6 g.) was obtained, which crystallised as colourless, long spars, m.p. 161—162°, $[\alpha]_D$ +28° (c 1) (Found: C, 78·7; H, 10·8. $C_{20}H_{32}O_2$ requires C, 78·9; H, 10·6%), m/e 304 (M^+); $\nu_{max.}$ 3290br and 1635 cm.$^{-1}$; τ 4·22 (1H, q, 15-H, J_{obs} 10 and 18 Hz), 4·93 (3H, m, 14-H and 2 × 16-H), 6·45 (1H, m, 12-H), 6·75 (1H, m, 3-H), 8·93, 8·98, 9·16, and 9·16 (4 × 3H, 4 × s, 4 × Me). Further elution furnished a brown gum (6 g.) containing many constituents including cleistanthol.

Acetylation Products.—ent-Isopimara-8(14),15-dien-3β-ol (II) (0·1 g.) dissolved in a mixture of dry pyridine (6 ml.) and acetic anhydride (0·5 ml.) was set aside overnight and then poured into ice–water. The crude product gave on crystallisation needles of ent-3β-*acetoxyisopimara*-8(14),15-*diene* (0·1 g.), m.p. 104—105°, $[\alpha]_D$ −7° (c 1) (Found: C, 79·9; H, 10·5. $C_{22}H_{34}O_2$ requires C, 80·0; H, 10·4%), m/e 330 (M^+) {reported for 3β-acetoxyisopimara-8(14),15-diene: m.p. 102—104·5°, $[\alpha]_D$ +4°}.[2]

ent-3β-Hydroxyisopimara-8(14),15-dien-12-one (VI) (0·2 g.) acetylated as described above, yielded on crystallisation colourless prisms of ent-3β-*acetoxyisopimara*-8(14),15-*dien*-12-*one* (0·19 g.), m.p. 127—128°, $[\alpha]_D$ +210·5° (c 1) (Found:

C, 76·5; H, 9·4. $C_{22}H_{32}O_3$ requires C, 76·7; H, 9·4%), m/e 344 (M^+), $\nu_{max.}$ 3078, 1723, 1700, and 1243 cm.$^{-1}$, τ 4·25 (1H, q, 15-H, J_{obs} 10 and 18 Hz), 4·74 (1H, s, 14-H), 4·89 (2H, m, 16-H), 5·50 (1H, m, 3-H), 7·96 (3H, s, CH_3COO), 8·78, 9·10, 9·14, and 9·28 (4 × 3H, 4 × s, 4 × Me).

ent-*Isopimara*-8(14),15-dien-3β,12β-diol (V) (0·1 g.) acetylated as described above yielded, after crystallisation, needles of ent-3β,12β-*diacetoxyisopimara*-8(14),15-*diene* (0·1 g.), m.p. 117°, $[\alpha]_D$ +33° (c 1) (Found: C, 73·9; H, 9·4. $C_{24}H_{36}O_4$ requires C, 74·2; H, 9·3%), m/e 388 (M^+), $\nu_{max.}$ 3095, 1733, 1721, and 1235 cm.$^{-1}$; τ 4·39 (1H, q, 15-H, J_{obs} 10 and 18 Hz), 4·85 (1H, s, 14-H), 5·05 (2H, m, 16-H), 5·19 (1H, overlapped, 12-H), and 5·48 (1H, m, 3-H).

Oxime of Ketol (VI).—ent-3β-Hydroxyisopimara-8(14),15-dien-12-one (VI) (0·1 g.) and hydroxylamine hydrochloride (0·2 g.) dissolved in a mixture of pyridine (10 ml.) and ethanol (10 ml.) were set aside for 24 hr. On dilution with water the oxime separated out. Crystallisation afforded cubes, m.p. 167—168°, $[\alpha]_D$ +163° (c 1) (Found: M^+, m/e 317·235202. $C_{20}H_{31}NO_2$ requires M^+, m/e 317·235466).

Oxidation Reactions.—ent-Isopimara-8(14),15-dien-3β-ol (II) (0·1 g.) dissolved in acetone (5 ml.) was treated dropwise with Jones reagent (8N-chromic acid) until the solution remained orange-brown. The mixture was set aside at 5° for 1 hr. after which addition of ice water induced the deposition of crystals of ent-*isopimara*-8(14),15-*dien*-3-*one* (III) (80 mg.); recrystallisation gave long rods, m.p. 60°, $[\alpha]_D$ +45° (c 2) (Found: C, 83·6; H, 10·5. $C_{20}H_{30}O$ requires C, 83·9; H, 10·6%), m/e 286 (M^+), $\nu_{max.}$ (CHCl$_3$) 3078, 1697, and 1630 cm.$^{-1}$; $[\phi]$ +2810° (peak, 315 nm.) and 0° (trough, 280 nm.). The compound gave a 2,4-dinitrophenylhydrazone, m.p. 177—178° (Found: C, 66·8; H, 7·6. $C_{26}H_{34}N_4O_4$ requires C, 66·9; H, 7·4%) (reported for isopimara-8(14),15-dien-3-one: m.p. 59—60°, $[\alpha]_D$ −56°; 2,4-dinitrophenylhydrazone, m.p. 176·5—177°).[2]

ent-3β-Hydroxyisopimara-8(14),15-dien-12-one (VI) (0·1 g.) oxidised as described above, yielded ent-*isopimara*-8(14),15-*dien*-3,12-*dione* (70 mg.) which crystallised as long spars, m.p. 84—85°, $[\alpha]_D$ +312° (c 1) (Found: C, 79·8; H, 9·6. $C_{20}H_{28}O_2$ requires C, 80·0; H, 9·4%), m/e 300 (M^+), $\nu_{max.}$ 3078, 1695s, and 1625 cm.$^{-1}$; $\lambda_{max.}$ 236sh (2·48) and 295 nm. (2·32) with no change in basic solution.

ent-Isopimara-8(14),15-dien-3β,12β-diol (V) oxidised as described above yielded the same 3,12-diketone, m.p. 84—85°.

Catalytic Reductions.—ent-3β-Hydroxyisopimara-8(14),15-dien-12-one (VI) (0·2 g.) in ethanol (20 ml.) over palladium–charcoal (50 mg., 30%) absorbed 14·1 ml. of hydrogen at N.T.P. (theor. for 1 mol. 12·8 ml.). Crystallisation of the product gave needles of ent-3β-*hydroxyisopimar*-8(14)-*en*-12-one, m.p. 114—115°, $[\alpha]_D$ +76° (c 1) (Found: C, 78·6; H, 10·6. $C_{20}H_{32}O_2$ requires C, 78·9; H, 10·6%), m/e 304 (M^+), $\nu_{max.}$ 3542, 1712, 1695, and 1655 cm.$^{-1}$, $\lambda_{max.}$ 292 nm. (1·75); τ 4·70 (1H, s, 14-H) and 6·72 (1H, m, 3-H). Acetylation as described above yielded on crystallisation needles of ent-3β-*acetoxyisopimar*-8(14)-*en*-12-*one*, m.p. 108—109°, $[\alpha]_D$ +52° (c 1) (Found: C, 76·2; H, 9·9. $C_{22}H_{34}O_3$ requires C, 76·3; H, 9·9%), m/e 346 (M^+); $\nu_{max.}$ 1725, 1700, and 1242 cm.$^{-1}$, τ 4·76 (1H, s, 14-H) and 5·50 (1H, m, 3-H).

ent-Isopimara-8(14),15-dien-3β,12β-diol (V) was hydrogenated over palladium–charcoal as described above. Crystallisation of the product gave needles of ent-*isopimar*-8(14)-*en*-3β,12β-*diol*, m.p. 172—173°, $[\alpha]_D$ −31° (c 1) (Found: C, 74·6; H, 11·6. $C_{20}H_{34}O_2$.CH_3OH requires C, 74·5; H, 11·3%), m/e 306 (M^+), $\nu_{max.}$ 3300br cm.$^{-1}$; τ 4·88 (1H, s,

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