"AN INVESTIGATION OF COMPOUNDS OCCURRING IN *LEONOTIS* SPECIES"

BY

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A Thesis

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Grahamstown,

South Africa.

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To Dr. Kaplan for his encouragement and direction and to Dr. Rivett for his guidance and discussions during the course of study.

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CONTENTS

SUMMARY

Two labdane diterpenoids, 8-hydroxymarrubiin and leonitin were isolated from Leonotis dysophylla *(Benth.) and* Leonotis leonitis *respectively.*

Spectral studies of 8-hydroxymarrubiin, $C_{20}H_{28}O_5$, *showed the presence of a* β*-substituted furan, a* γ*-lactone, three tertiary methyl groups and tertiary hydroxyl group(s). The NMR spectrum of 8-hydroxymarrubiin and marrubiin,* $C_{20}H_{28}O_4$, *were almost identical with the exception of the* C_{17}*-methyl group which appeared as a singlet in 8-hydroxymarrubiin and as a doublet in marrubiin. The extra oxygen atom was therefore assumed to be present as a hydroxyl group substituted in the* C_8*-position. This was further confirmed by the formation of an epoxide and a* $\gamma\delta$*-dilactone.*

The structure of 8-hydroxymarrubiin was confirmed by its correlation with marrubiin via the degradation products marrubenol and the γ*-dilactone.*

Leonitin, $C_{22}H_{30}O_7$, *was shown by spectral and chemical evidence to be a diterpenoid dilactone possessing an ester function and an ether linkage. Comparison of the* ^{1}*NMR spectra of compound X and leonitin suggested that the acetoxy function occurs in the* C_{20}*-position. This was further supported by the formation of a* $\gamma\delta$*-dilactone. The absence of a* β*-furan moiety was apparent from chemical and spectral evidence.*

A structure for leonitin is proposed and aspects of its stereochemistry discussed.

INTRODUCTION

This review is confined to diterpenoids with normal and rearranged labdane skeletons and is an extension of the review by Kaplan[1] on labdane diterpenoids.

The carbon skeletons of diterpenoids in general either conform to one of the eight types[2] shown (Chart 1) or can be derived from them by secondary rearrangements in accordance with the Biogenetic Isoprene rule.

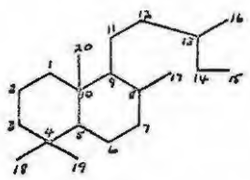
The di-, tri-, tetra- and pentacyclic diterpenoids are all derivable from geranyl-geraniol (1) or geranyl linaloöl (2) via the bicyclic alcohol (3) of the labdane type. It can be noted immediately that in all authenticated cases the relative stereochemistry at carbon atoms 5, 9 and 10 is as shown in (3), in accordance with the results of concerted anti-parellel 1:2 additions and further verified by Scott et al,[3,4] by a combined X-ray and circular dichroism study. The parent skeletons of the eight groups of diterpenoids can then readily be derived from (3).

The skeleton of common labdane diterpenoids can be divided into two partial structures (A ⟶ Y) and a side chain unit which can either contain an asymmetric carbon (a ⟶ g) or have no such symmetry (chart 2).

The labdane diterpenoids reviewed by Kaplan[1] are shown in charts 3, 4, 5. The structural configuration and stereochemistry of some of the diterpenoids in Charts 3, 4, 5 have since been elucidated conclusively and hence have been reviewed again in addition to others in the review.

Chart 1

The carbon atoms are numbered in accordance with the suggestion of M^{c} Crindle and Overton.



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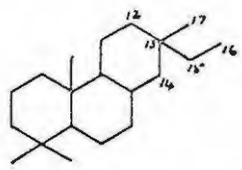

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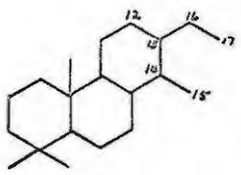

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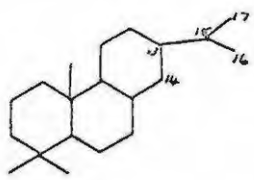

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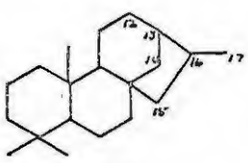

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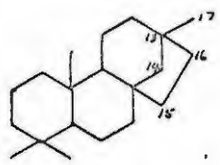

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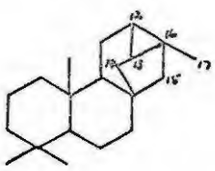

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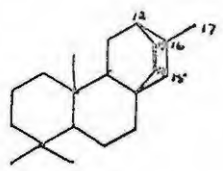
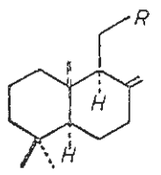


ATISANE

Chart 2 — Bicyclic Systems



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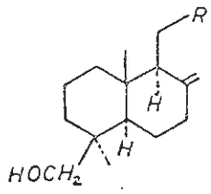

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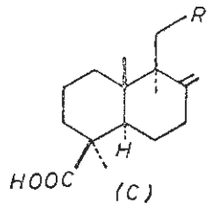

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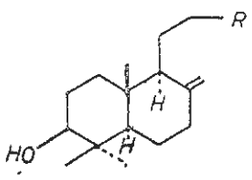

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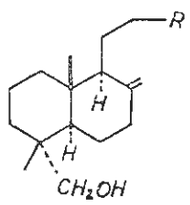

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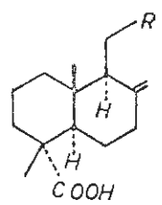

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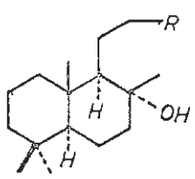

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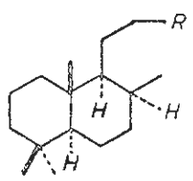

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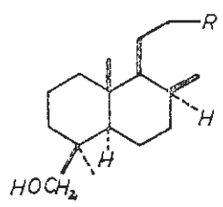

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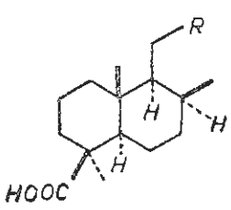

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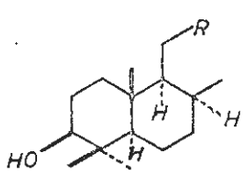

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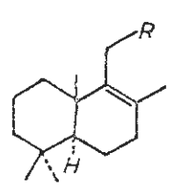

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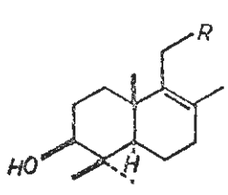

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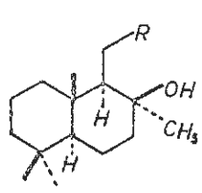

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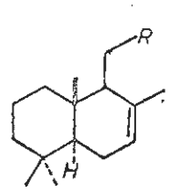

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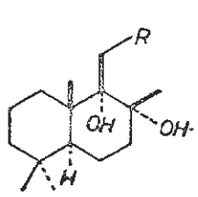

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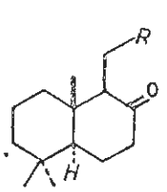

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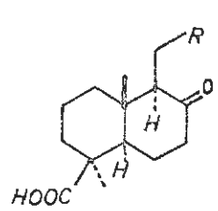

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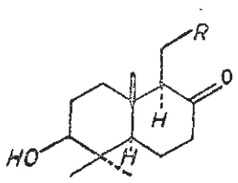

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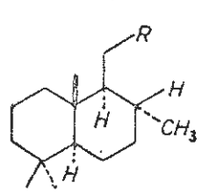

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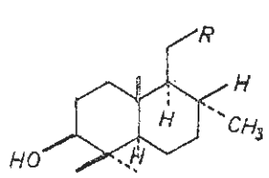

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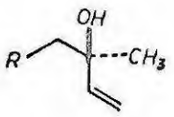
Chart 2

Side-chain Units



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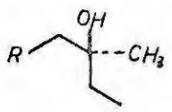

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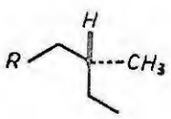

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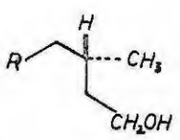

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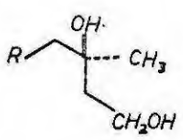

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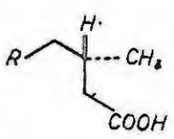

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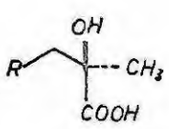

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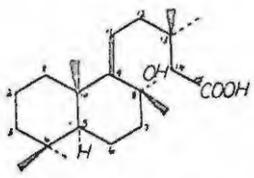
CHART 3



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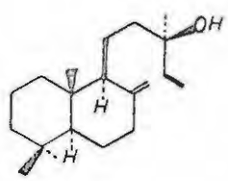

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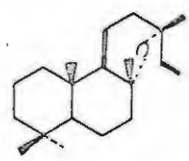

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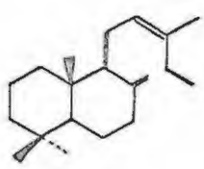

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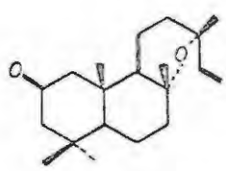
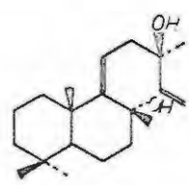


2-KETOMANOYL OXIDE



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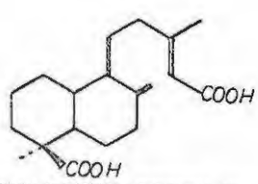

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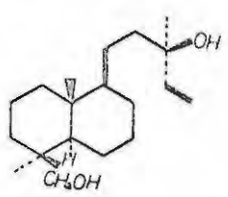

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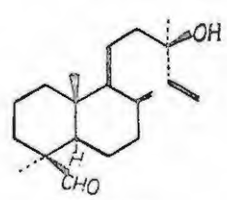

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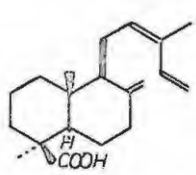

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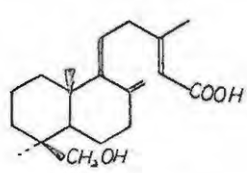

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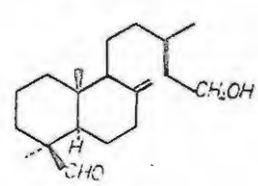

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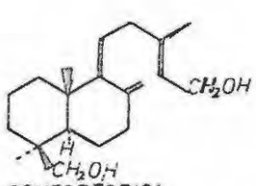

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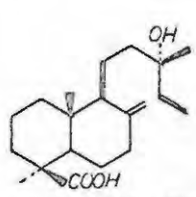
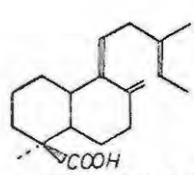
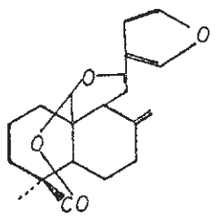


CUPRESSIC ACID

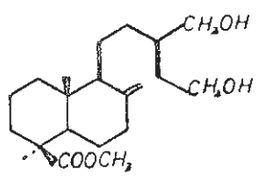


ISO-CUPRESSIC ACID

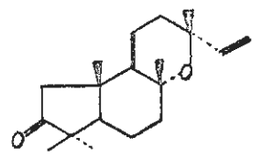
CHART 4.



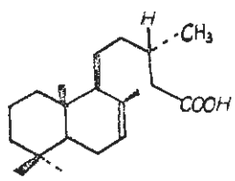
SCIADIN



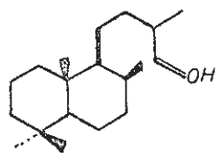
METHYL SCIADOPODATE



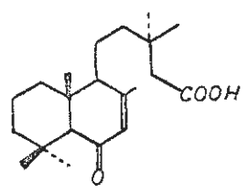
COLENSENONE



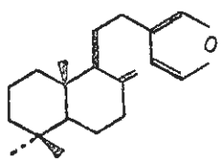
CATIVIC ACID



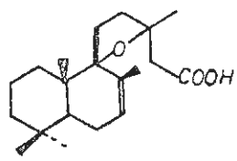
ABIENOL



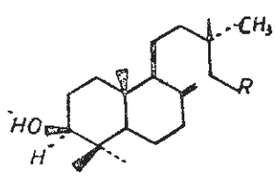
6-OXOCATIVIC ACID



LAMBERTIANIC ACID

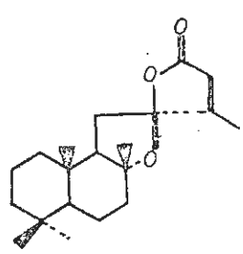


GRINDELIC ACID

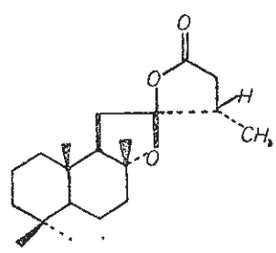


(1) R = CH_2OH
(2) R = CHO
(3) R = COOH

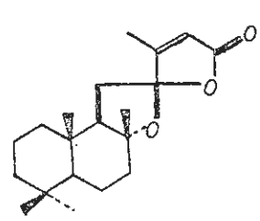
DITERPENES ISOLATED FROM *ARAUCARIA imbricata*



α_1-LEVANTENOLIDE

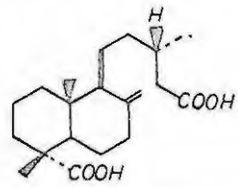


α_2-LEVANTENOLIDE

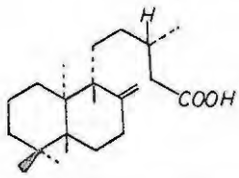


β-LEVANTENOLIDE

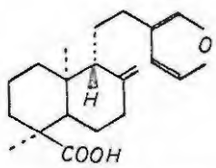
CHART 5



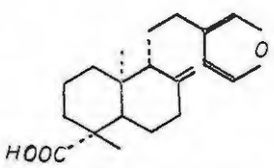
PINIFOLIC ACID



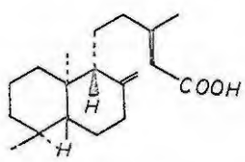
EPERUIC ACID



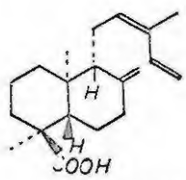
POLYALTHIC ACID



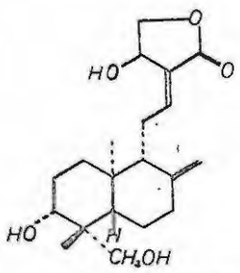
DANIELLIC ACID



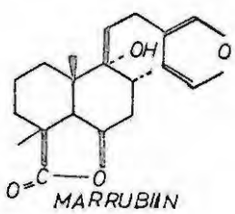
COPALIC ACID



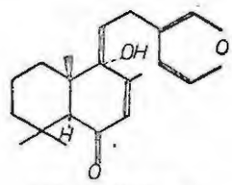
OZIC ACID



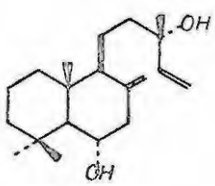
ANDROGRAPHOLIDE



MARRUBIIN



SOLIDAGENONE



LARIXOL

1.1 Solidagenone

Solidagenone (4), $C_{20}H_{28}O_3$, isolated from Solidago canadensis[5,6] was shown to have structure (4) on the basis of its molecular formula, spectrascopic and chemical evidence.

The presence of a β-substituted furan has been deduced from the IR and NMR spectra while in the mass spectrum a strong M-124 peak may indicate scission of a normal terpenoid ring A, which has no oxygen substituents.

The $\alpha\beta$-unsaturated ketone is apparent from the carbonyl absorption at 1678cm^{-1} in the IR spectrum although in the UV spectrum the enone absorption at 1678cm^{-1} is masked in a broad composite band λ_{max} 223mu (ε 10,000). However a narrow band λ_{max} 234mu (ε 9800), results when the furan absorption of a similar compound marrubiin[7] (5), λ_{max} 212mu (5300) is subtracted. The derived maximum is consistent with structure (4) since the γ-hydroxyl group should have a hypsochromic effect.[8,9]

The C_8 methyl group on the double bond appears as a doublet in the NMR spectrum. Its coupling to the α-vinyl proton has been confirmed by doublet irradiation experiments.

The presence of the hydroxyl group in solidagenone in close proximity to the furan ring was suggested by its IR absorption and confirmed by NMR data. Furthermore, this hydroxyl group is tertiary since there is no resonance in the NMR spectrum attributable to a proton of the type H-C-OH. The NMR spectrum also discloses three quarternary methyls and a singlet at τ 7.28 attributed to the proton at C_5.

Structure (4) for solidagenone also readily accomodates the chemical data - thus the failure to form derivatives of the tertiary hydroxyl and the sterically hindered ketone (cf. 6-oxo-gindelic acid (6) and 6-oxo-cativic acid[10] (7) is expected as is the formation[6]

of a saturated ketone on lithium aluminium hydride reduction.

Evidence confirming this structure and leading to an assignment of its stereocheistry is as follows:

Initial approaches envisaged correlation of solidagenone with marrubiin[11] (5) by converting both into dihydrosolidagenone (8). This compound was prepared from marrubiin via the keto-aldehyde[11] (9) by reduction of the derived oily thio-acetal (10) with Raney nickel in acetone.

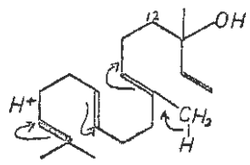
Conditions whereby solidagenone can be reduced to (8) have not been found. The dihydroderivative of solidagenone (11) isolated from $LiAlH_4$ or catalytic reduction has a C_8 axial methyl group. These conditions also gave products of further reduction. However, the major product from Li/NH_3 and Zn/CH_3COOH reduction of solidagenone was the $\beta\gamma$-unsaturated ketone, (12) identical with the more abundant enone from phosphoryl chloride-pyridine dehydration of the keton (8) from marrubiin (5). This along with the observation that the oxidation of dihydrsolidagenone (11) with CrO_3/CH_3COOH gave a keto- γ -lactone (13), confirms the structure of solidagenone and proves the absolute configurations at C_5 and C_{10}.

The configuration at the remaining centre, C_9, was determined by treating the enone (12) with m-chloroperbenzoic acid in chloroform to give (14), a furan containing epoxide which isomerises to yield solidagenone (4) with β-napthalene sulphonic acid in refluxing benzene. Formation of the α-epoxide and ring opening to a 9α -hydroxy group can be predicted on the basis of work[12] carried out on closely analogous compounds.

1.2 Solidagonic Acid

A bitter principle, solidagonic acid (15) was isolated[13] from the roots of Solidago altissima.

Solidagonic acid is a monbasic carboxylic acid, its methyl ester (16)



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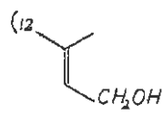

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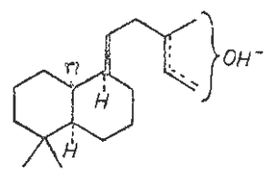

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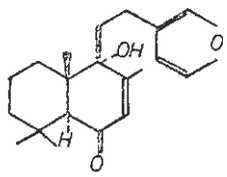

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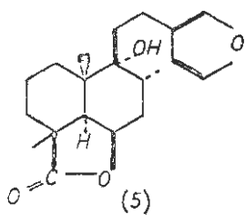

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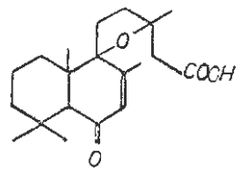

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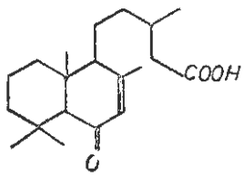

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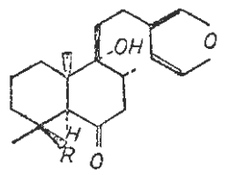
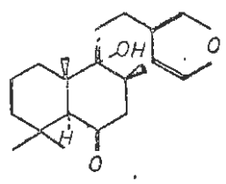


(8)R-CH_3
(9)R-CHO
(10)R=$CH(SCH)_2$



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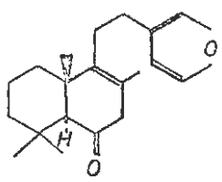

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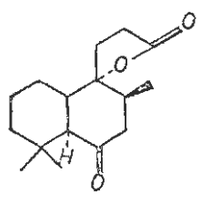

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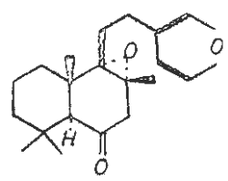


(14)

having an acetylated secondary hydroxyl group determined by NMR spectra. The presence of two tertiary methyl groups a secondary methyl and a $-C(CH_3)=CH$, - group was detected clearly from the NMR spectrum of (16). The conjugation of the carboxyl group and the ethylenic linkage was borne out from the UV and NMR spectra of (16).

Oxidation of (16) with one mole monoperphthalic acid gave a monoepoxide (17) which was reduced with $LiAlH_4$ to afford a triol (18). The diacetate of (18) gave a methyl ketone (19) on ozonolysis. It is clear from these results that solidagonic acid has a $-C(CH_3)=CH-COOH$ group.

Dehydrogenation of the ester (16) with 10% Pd-C resulted in the formation of 1, 2, 5-trimethylnapthalene.

The monoepoxide (17) on treatment with methyl magnesium-bromide gave the triol (2); dehydrogenation of the latter with 10% Pd-C resulted in the formation of 1, 2, 5-tetramethylnapthlene.

A tetrahydroderivative, which has been prepared by hydrogenation of the ester (16) gave the hydroxy acid (21) on saponification. Oxidation of (21) with Jones reagent gave a keto-acid (22) which on treatment with methyl magnesiumbromide followed by dehydrogenation with selenium gave 1, 2, 3, 5-tetramethylnapthalene. A possible structure for solidagonic acid accomodating the above results is illustrated in structure (15). This rearranged bicyclic diterpenoid skeleton is supported by biogenetic considerations.

The structure (15) was confirmed by the NMR spectrum of the derived saturated ketone (23) ($LiAlH_4$ reduction of (16), catalytic hydrogenation and then oxidation with Jones reagent).

The stereochemistry of solidagonic acid has not been defined but its NMR spectrum suggests that the allylic methyl group of the side chain must be <u>cis</u> to the carboxyl group.[16, 17]

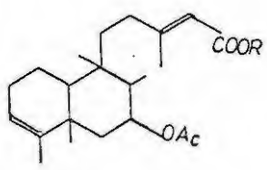
1.3 Furan containing diterpenoids from Solidago serrotina

Several new diterpenoids have been isolated[18] from the roots of Solidago serrotina. In solidagoic acid A (24), $C_{20}H_{28}O_3$, the following structural features were readily recognisable in its NMR spectrum; a β-substituted furan, a proton and a methyl group attached to an olefinic bond, one tertiary and one secondary C-methyl groups. The oily methyl ester of A (32) was readily converted by reduction with $LiALH_4$ into the corresponding alcohol (33). In the alcohol (33) and the derived aldehyde (26), $C_{20}H_{28}O_2$, which also occurs naturally, the $-CH_2OH$ and the CHO resonances show no vicinal spin-spin coupling. The presence of a βγ-unsaturated acid grouping in A (24) was shown by its smooth conversion into the nor-olefin (34), $C_{19}H_{28}O$, which showed three $C-CH_3$ resonances and no vinyl proton signals. Bearing in mind the labdane-related diterpenoid, solidagenone[5,6] (4), the above evidence indicates a rearranged labdane skeleton[19,20,21] for solidagoic acid A and thus the constitutuion (24).

The marked similarity of the NMR spectra of solidagoic acids B (25) and A (24) suggested that the former differed solely in that the vinyl methyl was replaced by an allylic primary alcohol present as its angelate ester.[22] Pyrolysis of acid B afforded angelic acid and the oily γ-lactone (35).

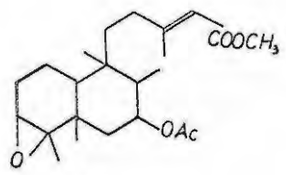
The formation of (35) provided further support for the proposed structure of acid A which has been correlated with B as follows. Reduction of the oily methyl ester (36) of B with $LiAlH_4$ led to the diol (27), $C_{20}H_{30}O_3$, which also occurs naturally. The derived diacetate (37) has also been obtained by direct acetylation of the alcohol (27).

Tentative structures for the hemiacetal (28), $C_{20}H_{28}O_3$, and the dialdehyde (29), $C_{20}H_{26}O_3$, followed from their conversion with



(15) R = H

(16) R = CH_3



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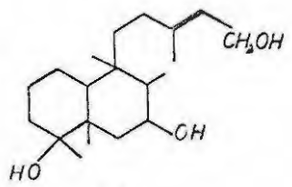

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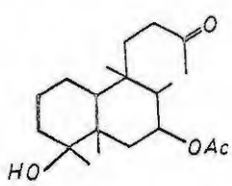

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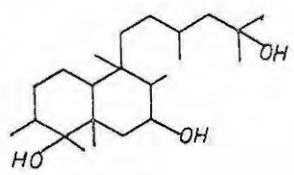

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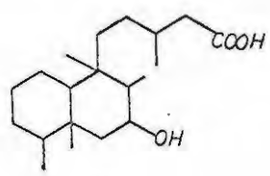

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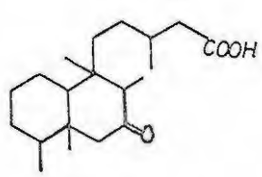

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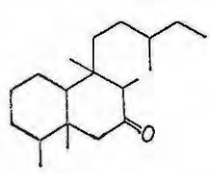

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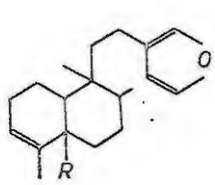
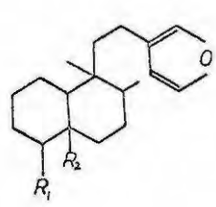


(24) R = COOH

(26) R = CHO

(32) R = COOMe

(33) R = CH_2OH



	R_1	R_2
(25)	CH_2O angeloyl	COOH
(27)	CH_2OH	CH_2OH
(29)	CHO	CHO
(30)	CH_2OH	CH_3
(36)	CH_2O angeloyl	COOMe
(37)	CH_2OAc	CH_2OAc

$LiAlH_4$ into the diol (27), while the chemical and spectroscopic evidence is compatible with structures (32), $C_{20}H_{30}O_2$, and (31), $C_{20}H_{28}O_4$, for the remaining two diterpenoids of natural provenance.

1.4 Labdane diterpenoids from Leonotis leonurus

Compound Y (38), $C_{20}H_{28}O_3$ and X (39), $C_{20}H_{28}O_5$, as well as marrubiin (5), were isolated from the leaves of Leonotis leonurus.[23,24,25]

a) Compound Y

Elemental analysis and mass spectroscopic molecular weight agreed with the molecular formula $C_{20}H_{28}O_3$. The UV and IR spectra were in accordance with the presence of a furan and an $\alpha\beta$-unsaturated keto-group as in structure (38). Compound Y gave a positive reaction for a substituted furan in both the Ehrlich and Liebermann-Burchard tests. That this was a β-substituted furan was clearly indicated by the NMR spectrum.

The co-occurence of marrubiin in the plant suggested that compound Y was closely related to marrubiin, a view supported by its dehydrogenation to 1, 2, 5-trimethylnapthalene. On catalytic hydrogenation the anhydro-compound of Y (obtained by phosphorus trichloride reaction on Y) absorbed 4 moles of hydrogen, one more than compound Y. The UV spectrum of anhydro Y is almost identical with that of compound Y, but the IR band at 1615cm^{-1} is considerably more intense. These facts are accomodated by the presence of a cross-conjugated dienone and suggested that the hydroxyl group in compound Y which resists benzoylation and acetylation is tertiary, and that the $\alpha\beta$-unsaturated keto-group in compound Y is present as Δ^5-7-ketone rather than the alternative Δ^7-6-ketone.[27]

Hexahydro Y was dehydrated with phosphorous trichloride to an oily $\alpha\beta$-unsaturated ketone, probably a mixture of endo- and exo-

cyclic double bonds, which can only arise from the Δ^5-7-ketone and shows that the hydroxyl group is at C_8 or C_9. Treatment of hexahydro-Y with methyl magnesium bromide followed by dehydration afforded 1, 2, 3, 5-tetramethyl napthalene.[28] The absence of a cationic centre precluded the possibility of rearrangement during dehydrogenation[29] and places the keto group unequivocally at C_7.

Compound Y was oxidised by chromic acid to a mixture of a γ-lactone (40) containing an $\alpha\beta$-unsaturated keto-group and an acid, both possessing the molecular formula $C_{17}H_{24}O_3$. The acid was shown to contain an extra double bond by hydrogenation and was not decarboxylated at 250°C; it is thus $\gamma\delta$-rather than $\beta\gamma$-unsaturated. The formation of (40) showed that the hydroxyl group in compound Y is attached at C_9 in support of the spectral data from dehydrohexahydro-Y.

The mass spectrum of compound Y is consistent with structure (38).

Compound Y was related to marrubiin (5) through a common degradation product as follows.

Reduction with lithium in ammonia furnished the trans-fused six membered ring ketone (41) which was oxidised with chromic acid to a mixture of an oily acid (42) and a neutral γ-lactone (43) containing a saturated 6 membered ketone. The keto group in (43) was converted into the thioketal which on desulphurisation with Raney nickel afforded a product shown to be identical with a sample of isoambrienolide (44) thus confirming the structure of compound Y (38). The stereochemistry of (44), which has been convincingly established[11,31,32,33,40] and that of compound Y therefore follows for all centres, except C_8, where keto-enol tautomerism could have taken place during lithium-ammonia reduction. The stereochemistry at C_8 follows from observations of solvent shifts in the NMR for the proton and methyl group adjacent to the carbonyl function. In this way, the methyl at C_8 was shown to be equatorial

(α) and the C_8 proton axial (β).

It can be noted that solidagenone (4) is isomeric with compound Y (38) with a Δ^7-6-ketone instead of a Δ^5-7-ketone.

b) Compound X

The suggested molecular formula $C_{20}H_{28}O_5$,[23] is supported by the mass-spectroscopic molecular weight. It is a γ-lactone containing no furan ring and could not be hydrogenated. On hydrolysis 2·1 equivalents of alkali were consumed indicating the presence of two lactone groups. The product afforded a monomethyl ester on treatment with diazomethane. Since marrubic acid is lactonised by diazomethane,[26] this monomethyl ester must be formed at C_{15}. Further, the lactone groups were reduced with lithium aluminium hydride to a tetraol.

Dehydrogenation of compound X yielded 1, 2, 5-trimethylnaphthalene, hence confirming the expected labdane skeleton. From its IR spectrum and its failure to form derivatives, carbonyl and hydroxyl groups are absent and the remaining oxygen atom was thought to be present as an ether. Attempts at fission of this linkage gave only starting material. The above properties are accomodated in structure (39) which is supported by the mass spectrum.

The NMR spectra of compounds X and marrubiin with respect to the ring A/B lactone and the C_{17}-methyl group are very similar, indicating identical stereochemistry at these centres. The proton at C_6 is equatorial (α), giving rise to poorly resolved triplets in compound X and Marrubiin because of one equatorial-equatorial and two equatorial-axial couplings. This lactone ring in X is therefore cis β-fused.[31] The C_{17}-methyl doublets in compound X and marrubiin are shifted upfield in benzene. These facts together with cooccurence of compound X and marrubiin in the plant suggest that X, like marrubiin, has an equatorial (α) C_{17}-methyl group. The

C_{14}- and C_{16}- methylene protons occur as quartets at τ 7.27 (J = 9c/s) respectively. These positions and couplings are as expected for such groups in a lactone ring.

The mass spectroscopic evidence for the C_9, C_{13} oxide is supported by the lack of protons, other than those discussed below τ7.3, showing that neither ether terminus possesses a proton, and also by the fact that the C_{14}- and C_{16}-methylene groups show no vicinal coupling. This ether linkage is also present in grindelic acid and in the precursor of solidagenone.[35]

1.5 Peregrinol

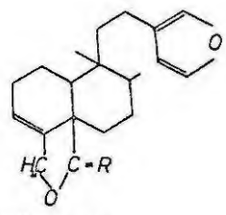
Peregrinol (45), $C_{20}H_{36}O_2$, peregrinine, $C_{20}H_{26}O_5$ (49) and a third compound $C_{19}H_{18}O_6$, were isolated from the dried plants of Marrubiim peregrinum. The structure of peregrinol[37] was determined as 13-labdane-9, 15 diene (45). Hydrogenation afforded dihydroperegrinol (46) and traces of (47). On treating (45) with OsO_4 afforded a tetrol. A monoacetate was obtained on acetylating peregrinol. Chromic acid oxidation of dihydroperegrinol (64) yielded the acid (48). The tosylate of (46) was reduced with $LiAlH_4$ to give (47).

The above chemical evidence together with NMR and IR data postulate structure (45) for peregrinol. The IR spectra of peregrinol and its derivatives and those of the derivatives of sclareol were similar.

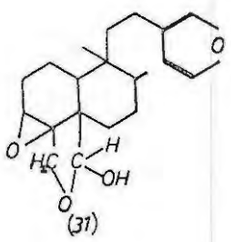
1.6 Peregrinine

Peregrinine (49), $C_{20}H_{26}O_5$ was isolated from Marrubium peregrinum[36] and Marrubium incanum.[39] The IR, NMR, UV and mass spectra indicated the presence of a β-substituted furan in the molecule, and this was also confirmed by a purple colour in the Ehrlich test.

The hydroxyl group of (49) resists both acetylation and



(28) R = H, OH
(35) R = O



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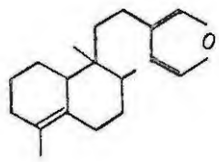

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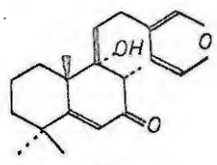

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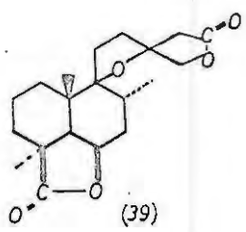

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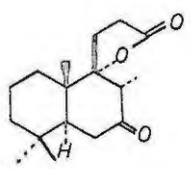

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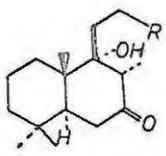
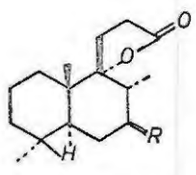
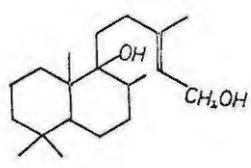


(41) R = β-furyl
(42) R = COOH, Δ8,9



(43) R = O
(44) R = H_2



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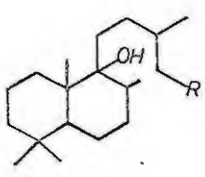
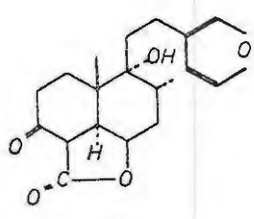
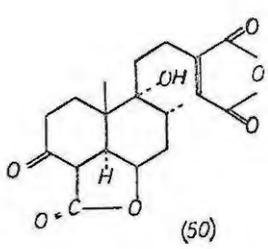


(46) R = CH_2OH
(47) R = Me
(48) R = COOH



(49)



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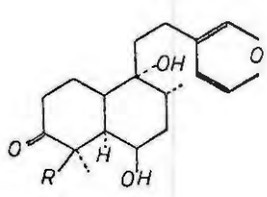
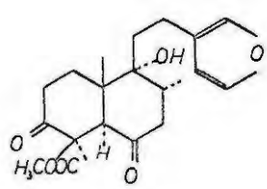


(51) R = COOH
(52) R = $COOCH_3$
(63) R = H



(53)

oxidation with Jones reagent yielded the maleic anhydride (50).

The presence of the γ-lactone in (49) indicated by the IR spectrum was confirmed by alkaline hydrolysis of (49) to the hydroxy acid (51). The latter, on heating with anhydrous solvents reformed the original lactone (49). Esterification of (51) with diazomethane yielded the corresponding methyl ester (52) which on oxidation with chromic acid gave the oily keto-methyl ester (53). This compound (53) lacked resonance in the NMR spectrum attributable to a proton of the type H-C-OH. The presence of a ketone in peregrinine (49) was indicated by its UV and IR spectra and confirmed by the corresponding thiosemicarbazone.

Reduction of (49) with $LiAlH_4$ in tetrahydrofuran gave a tetrol (54) which on acetylation yielded the oily, triacetate (55). Sodium borohydride reduction of (49) afforded the dihydroxylactone (56) which with chromic anhydride in pyridine reformed (49). The compound (56) was converted to a mono-acetate (57) by acetylation at room temperature.

Tosylation of the dihydroxylactone (56) yielded the corresponding sulphonate derivative (59) which when heated in refluxing pyridine yielded the unsaturated compound (58).

The catalytic reduction of (58) with Pd-D in dioxane yielded among other products, a dihydroderivative (60), identical with marrubiin (5), the stereochemistry of which has been established.[11,31,32,33,40]

From the above results, (49) can be formulated as a keto-marrubiin. The presence of the keto-group at position three was established on the basis of the following results.

Ozonolysis of (49) afforded a keto-dilactone (61). Bromination of the latter yielded the corresponding -bromo-ketone (62).

When peregrinine was reacted with N.NaOH in refluxing

oxidation with chromic anhydride in pyridine. However, oxidation with Jones reagent yielded the maleic anhydride (50).

The presence of the γ-lactone in (49) indicated by the IR spectrum was confirmed by alkaline hydrolysis of (49) to the hydroxy acid (51). The latter, on heating with anydrous solvents reformed the original lactone (49). Esterification of (51) with diazomethane yielded the corresponding methyl ester (52) which on oxidation with chromic acid gave the oily keto-methyl ester (53). This compound (53) lacked resonance in the NMR spectrum attributable to a proton of the type H-C-OH. The presence of a ketone in peregrinine (49) was indicated by its UV and IR spectra and confirmed by the corresponding thiosemicarbazone.

Reduction of (49) with $LiAlH_4$ in tetrahydrofuran gave a tetrol (54) which on acetylation yielded the oily, triacetate (55). Sodium borohydride reduction of (49) afforded the dihydroxylactone (56) which with chromic anhydride in pyridine reformed (49). The compound (56) was converted to a mono-acetate (57) by acetylation at room temperature.

Tosylation of the dihydroxylactone (56) yielded the corresponding sulphonate derivative (59) which when heated in refluxing pyridine yielded the unsaturated compound (58).

The catalytic reduction of (58) with Pd-C in dioxane yielded among other products, a dihydroderivative (60), identical with marrubiin (5), the stereochemistry of which has been established. [11,31,32,33,40]

From the above results, (49) can be formulated as a keto-marrubiin. The presence of the keto-group at position three was established on the basis of the following results.

Ozonolysis of (49) afforded a keto-dilactone (61). Bromination of the latter yielded the corresponding α-bromo-ketone (62).

When peregrinine was reacted with N.NaOH in refluxing methanol-water, it afforded the nor-ketone (63) which on oxidation

yielded the corresponding diketone (64). The mono-dehydration of (63) with $POCl_3$ at room temperature followed by acid isomerization of the double bond gave the oily , $\alpha\beta$-unsaturated ketone (65).

On the foregoing evidence, the structure of peregrinine was postulated as shown in (49).

1.7 Diterpenes isolated from Hardwickia pinnata

The oleoresin of Harwickia pinnata has been shown[20] to consist of a series of closely related diterpenoids. Five new compounds have been isolated and four of these reviewed.

a) Hardwickiic Acid

Hardwickiic acid (66), $C_{20}H_{28}O_3$, is a monobasic carboxylic acid. The methyl ester was shown to have a β-mono-substituted furan ring from NMR and IR data. The presence of two quarternary methyls and a $CH_3-\underset{C}{\underset{|}{C}}H-C$ was clear from its NMR spectrum. A triplet assignable to a vinyl proton β to the carboxyl group, was confirmed by the NMR spectrum of the derived alcohol when this triplet as expected shifted upfield. The conjugation of the carboxyl and the ethylenic linkage was further borne out from UV absorption (λ_{max} 213mu).

On quantitative hydrogenation over Rh-C, the ester of hardwickiic acid yielded a tetrahydro-derivative with the conjugated olefinic linkage still intact. Further hydrogenation yielded a hexahydro-derivative which was fully saturated (shown by IR, NMR and tetranitromethane). From the above results it was apparent that hardwickiic acid has only three $C=C$ bonds and consequently must have two carbocyclic rings.

On dehydrogenation with 10% Pd-C, hardwickiic acid yielded 1, 2-dimethyl and 1, 2, 5-trimethylnapthalene. These results can only be accomodated in a rearranged bicyclic diterpenoid skeleton and structure (66) is dictated by biogenetic considerations.[4,41]

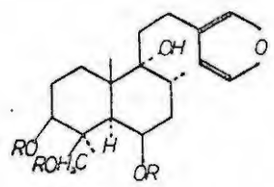
Misra et al[42] provided experimental support for the location of the quarternary methyls and the stereochemistry at the various asymmetric centres thus giving conclusive evidence for the absolute stereostructure for (-) hardwickiic acid. The location of the quarternary methyl at C_5 was evident from its chemical shift data collected for several derivatives of hardwickiic acid in which the olefinic linkage and/or the carboxyl group had been chemically modified.

Ozonolysis of hardwickiic acid, followed by oxidative workup of the ozonide furnished two major products, formulated on the basis of their analytical and spectroscopic data as (67) and (68), in terms of structure (66) for hardwickiic acid. Oxidation of (68) with NaOBr. gave after esterification (67). The methyl ketone ester (68) could be prepared by the ozonolysis of kolavic acid (69).

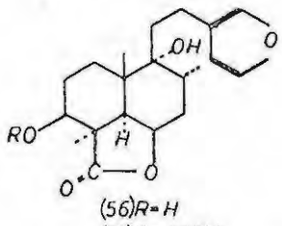
The NMR spectra of both (67) and (68) show one of the quarternary methyls-downfield as required[43] for a methyl group to a carbomethoxy function, thus supporting the presenoe of a quarternary methyl at C_5 in (66).

Bromination[44] of (68) yielded a bromoderivative which on dehydrogenation[45] gave an , $\alpha\beta$-unsaturated ketone (70) confirmed by IR and NMR spectra. The NMR shows two olefinic protons as an A B quartet, a finding consistent only with fully substituted C_9 as shown in (70). The placing of a methyl group at C_9 was further supported by the position of its NMR signal which as required by its being on an allylic carbon has now suffered a downfield shift as compared to (67) and (68). The other methyl remains essentially unchanged.

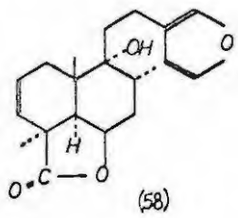
The ring fusion of (66) was shown to be trans-locked, like the C/D rings of an androstan-17-one but with opposite stereochemistry.



(54) R = H
(55) R = $COCH_3$



(56) R = H
(57) R = $COCH_3$
(59) R = $O_3S-C_6H_4-CH_3$



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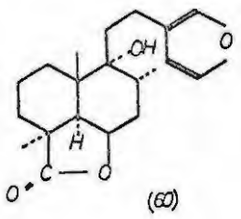

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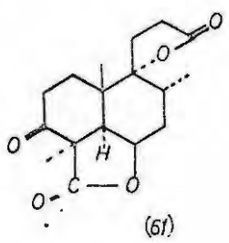

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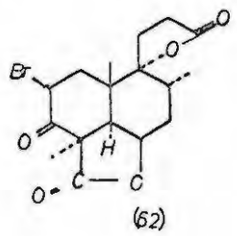

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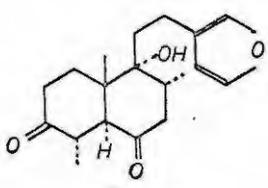

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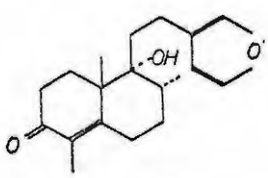

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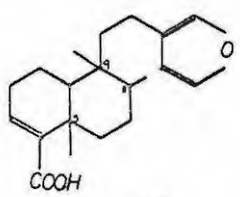

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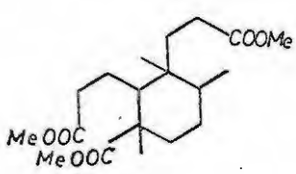

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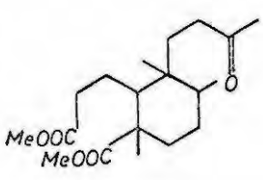

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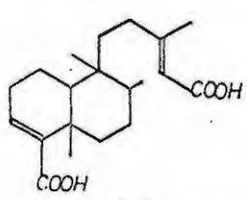
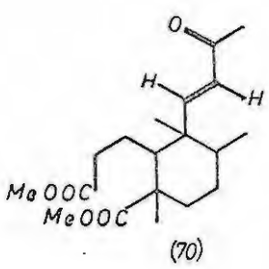


(69)



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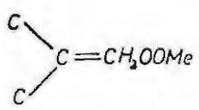

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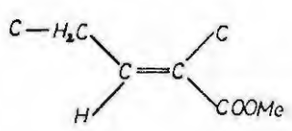


(72)

From NMR studies carried out on the degradation products of hardwickiic acid (66), it was evident that the C_9 methyl was β-orientated and the C_8 methyl equatorial (α).

b) Kolavic Acid

Kolavic acid (69), $C_{20}H_{30}O_4$, is a dicarboxylic acid. The UV absorption of the acid and its dimethyl ester indicated that both the carboxyl functions must be $\alpha\beta$-unsaturated. On catalytic hydrogenation, it yielded a tetrahydro-acid which gave a negative tetranitromethane test. Hence, kolavic acid must contain only two ethylenic linkages. On dehydrogenation with Pd-C, kolavic acid like hardwickiic acid, yielded both 1, 2-dimethyl and 1, 2, 5-trimethylnapthalene indicating that it must be closely related to hardwickiic acid.

The NMR spectrum of dimethyl kolavate showed the presence of two quarternary methyls, a CH_3-$\underset{C}{C}H$-C grouping, one methyl on an olefinic linkage and two - COOMe groups. The nature of the olefinic linkages were shown to be either (71) or (72) consistent with the signals in the vinyl proton region of the NMR spectrum. Bearing in mind the structure of hardwickiic acid, the above data for kolavic acid lead to its formulation as (69). The stereochemistry of the side chain was deduced from the chemical shift of the allylic methyl group which must be <u>cis</u> to the carboxyl group.[46,47] *Kolavic acid was then correlated with hardwickiic acid as follows. Methyl hardwickiate on hydrogenation over Pt. in acetic acid, absorbed 3.3 moles of hydrogen to furnish an ester alcohol, which on $LiAlH_4$ reduction yielded a mixture of a solid and a liquid glycol in approximately equal quantities, the latter was converted into its diacetate and chromatographed to yield a pure diacetate which was found to be identical with (73), obtainable from tetrahydrokolavic acid.*

c) Kolavenic Acid

Kolavenic acid (74) was isolated as its methyl ester (75). From the spectral properties of the ester and bearing in mind the spectral characteristics of hardwickiic acid, structure (74) was confirmed by its direct correlation with kolavic acid through the hydrocarbon (76).

From the NMR spectrum it was shown that the shift of the quarternary methyl signal from 76 c/s in hardwickiic acid to 59 c/s in kolavenic ester is in accordance with the proximity of the methyl to the C_4 carboxyl function.

As expected[48] the signal is shifted to 47 c/s in tetrahydro-kolavenic ester.

d) Kolavenol

From spectral characteristics, kolavenol was assigned structure (77) which is consistent with biogenetic considerations. The assigned structure was confirmed by its preparation from kolavenic acid by $LiAlH_4$ reduction.

1.8 Dextrarotatory Hardwickiic Acid

A dextrarotatory diterpene acid (66), $C_{20}H_{28}O_3$ was isolated[21] from a legroin extract of Copaifera officinalis. IR spectra were characteristic of an $\alpha\beta$-unsaturated acid and of a furan.[49] The acid gave an ester with diazomethane, the NMR spectrum of which showed proton resonances characteristic of a furan ring, a triplet assigned to the ringed hydrogen of the system - C-(H)= C-COOR while a quartet and a triplet corresponded to the methylene and methyl protons of the ethoxy carbonyl group; singlets at τ8.72 and 9.22 were assigned to the two quarternary methyl groups whilst a doublet at τ9.14 corresponded to a methyl group α to a methylene group in the system CH_3-$\dot{C}$H-CH_2-. Hydrogenation of the ester over Adams catalyst gave a liquid product lacking furan bands in the

IR but showed the presence of -C≡C-COOEt group.

Dehydrogenation of the ester with Pd-C afforded a mixture of 1, 2-dimethyl and 1, 2, 5-trimethylnapthalene. The spectra and chemical properties of the new acid were very similar to those of (-) hardwickiic acid.[20]

Mixed melting points of the two acids was some 50° higher than the melting points of the components, a property typical of a racemic compound. The IR spectra of the two compounds were superimposable. A trace of (-) hardwickiic acid when treated with diazomethane gave an ester indistinguishable from the ester obtained from this new acid. Hence, the acid from Copaifera officinalis is (+) hardwickiic acid. The spectra and chemical properties of the two enantiomers support the assigned[20] structure (66) and biogenetic considerations also lend support to it.

1.9 Cascarillin

Cascarillin (78), $C_{22}H_{32}O_7$, the bitter principle from the bark of Croton eleuteria[50] was shown by Halsall et al[51] to be a diterpenoid monoacetate. Chemical and spectroscopic evidence suggested that cascarillin belonged to the same group of diterpenes as clerodin.[52] On treatment with acid, deacetylcascarillin formed a stable acetal which was converted into a crystalline iodoacetate. X-ray single crystal analysis[53] of this iodacetate was then undertaken to determine the structure of cascarillin. The crystal structure was solved by means of the phase determining heavy atom method.[54] The carbon and oxygen atoms were located in three dimensional electron-density distributions. The absolute configuration for cascarillin as in (78) was deduced by Bijvoet's anamalous dispersion method.[55] Optical rotatory dispersion measurements are in agreement with this stereochemical assignment.[56]

1.10 Diterpenes of the Cascarillin group from Dodonaea species

These diterpenes possess a rearranged enantio-labdane skeketon of the type exhibited by the cascarillins.[56]

a) Diterpenes from Dodonaea attenuata

The ether extract of D. attenuata[19] gave the acetoxy-hydroxy-acid (79), $C_{22}H_{30}O_6$, as the major acidic constituent. From NMR studies a β-furan ring, a β-vinyl proton in an $\alpha\beta$-unsaturated carbonyl grouping having a methylene group adjacent to the vinyl proton, a tertiary methyl and an acetate group were determined.

Saponification or $LiBH_4$ reduction of the natural acid gave a dihydroxy acid (80) which lactonized when heated with N,N-di-cyclohexylcarbodiimide[57] in pyridine, to give the hydroxy-lactone (82). Similar treatment of the natural acid (79) or acetylation of (82) with acetic anhydride gave the acetoxy lactone (83) establishing chemically that the ester grouping in (79) was an acetate. The IR spectra of (83) confirmed the presence of an $\alpha\beta$-unsaturated - γ-lactone and acetate groups.

Reduction of (79) with sodium in ethanol saturated the Δ^3 olefinic linkage and gave a mixture of the hydroxy-lactone (85) and the dihydroxy-acid (90). These two products were formulated as epimers at C_4 since methylation of (90) gave the methyl ester (91), which gave the lactone (85) after treatment with sodium methoxide in methanol and saponification. Oxidation of (85) with chromic acid in pyridine gave the lactonic acid (89) and the aldehyde (86).

The NMR spectra of (85) indicated that the C_{19}-methylene group was attached to a fully substituted carbon atom and that the carbon atom adjacent to the C_{17}-protons bears one proton.

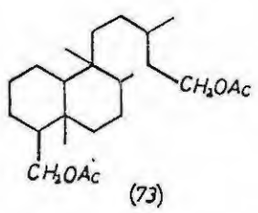
The relative position of the acetoxymethyl group and the - substituted furan ring in (79) was established as follows. Acetylation of the hydroxylactone (85) gave the acetate (87) which, when oxidised with ozone followed by Jones[58] reagent gave the

tris-nor-acetoxy acid (92). Saponification of (92) gave the hydroxy-acid (93) which was oxidised with Jones reagent to the corresponding diacid, heated with acetic anhydride and then at 280° to give the keto-lactone (94) (shown by IR to be γ-lactone, cyclopentanone[59]).

Treatment of the lactone aldehyde (86) with boiling Ac_2O/NaOAc followed by ozonolysis and Jones oxidation of the resulting enol acetate gave the lactone-keto-acid (95). Methylation of the latter gave (96) which showed IR absorptions attributed to γ-lactone, ester and cyclohexanone groupings.[59] Bromination and dehydrobromination of (96) gave the conjugated ketone (97). The NMR spectrum of (97) showed two olefinic protons as an AB quartet indicating a cis-disubstituted olefinic linkage. Treatment of (97) with NaOH liberated formaldelhyde presumably by a retro-aldol fission of the C_5 substituent since the keto-lactone (96) did not react in this manner. This indicates the relationship between the lactone and conjugated ketone functions in (97) and hence the number and nature of the carbon atoms linking the hydromethyl and acetoxymethyl groups in (79).

Evidence of the side chain in (79) followed from a Barbier-Wieland type degradation of a suitably protected tris-nor-acid (98). Evidence for the decalin ring system was obtained by dehydrogenation of the lactone (88) with selenium to give 1, 2-dimethylnapthalene isolated as the trinitrobenzene adduct. This result together with the evidence summarized in partial structure (99) requires the natural acid to have the constitution (79). The absolute stereochemistry of the above compound was also established through degradative and spectroscopic studies of compound (79).

The lactone (84), $C_{20}H_{26}O_3$, was obtained from methylated acidic extract of D. attenuata. Saponification gave the hydroxy



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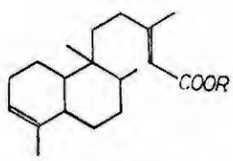
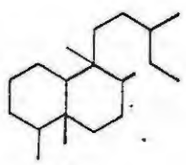


(74) R = H
(75) R = CH_3



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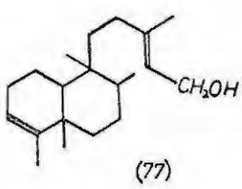
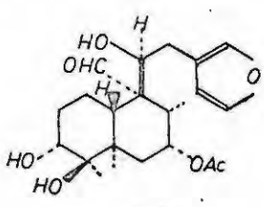
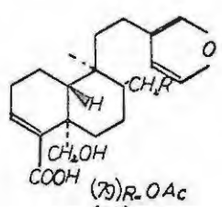


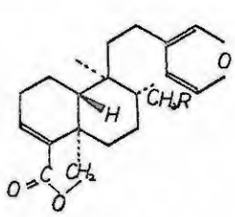
(77)



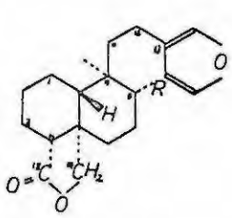
(78)



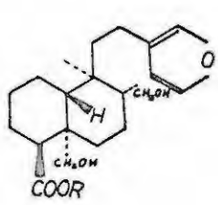
(79) R = OAc
(80) R = OH
(81) R = H



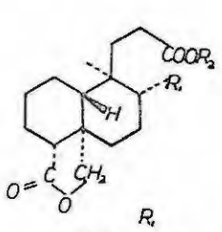
(82) R = OH
(83) R = OAc
(84) R = H



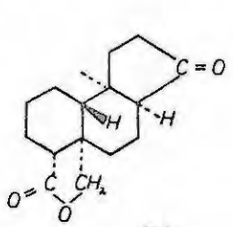
(85) R = CH_2OH
(86) R = CHO
(87) R = CH_2OAc
(88) R = CH_3
(89) R = COOH



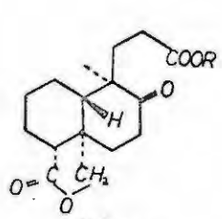
(90) R = H
(91) R = CH_3



R_1	R_2
(92) CH_2OAc	H
(93) CH_2OH	H



(94)



(95) R = H
(96) R = CH_3

acid (81). The physical constants of (81) and the lactone (84) suggests that the compounds are identical with hautriwaic acid from Dodonaea viscosa[60] and its lactone respectively. Reduction of the hydroxy acid (81) with sodium-ethanol gave the lactone identical with that prepared from (79) thus establishing the structure and stereochemistry of the lactone (84) and the acid (81).

b) Acidic Constituents of Dodonaea lobulata

The ether extract of the dried leaves and branchlets of Dodonaea lobulata[61] afforded a monohydroxyacid (100), $C_{20}H_{36}O_4$, and two dihydroxyacids (101), $C_{20}H_{36}O_4$, and (102), $C_{20}H_{36}O_4$. These three acids were shown to be the first examples of the enantio-labdane[56] skeleton.

The methyl ester (103) of the monohydroxy acid (100) had an IR spectrum identical with that of 8α-hydroxy-labdan-15-oate. The physical properties of the methyl ester (103) was dehydrated with phosphorus oxychloride in pyridine, to give a mixture of exocyclic isomer (108), the 7 (8) trisubstituted isomer and the remainder presumably being the tetra-substituted 8 (9) isomer.

The exocyclic isomer (108) was hydrolysed to the acid (109). Ozonolysis of the ester (108) and saponification of the product, gave the nor-keto acid (110), also obtained by ozonolysis of the acid (109). Isomerisation of (108) was effected with methanolic H_2SO_4, to give after hydrogenation, the saturated ester (112).

A comparison of the physical constants of the crystalline compounds described above, with values published for corresponding labdane derivatives, clearly establishes the structure of the monohydroxyacid (100) as 8β-hydroxy-enantio-ladan-15-oic acid.

The diol acids (101) and (102) were separated as their methyl ester acetates, and their skeletons established by degradative relationship with (103) and (111) respectively. The NMR spectrum of

the methyl ester (104) exhibited peaks corresponding to three quaternary methyl peaks, a methyl group on a carbon atom bearing oxygen and a doublet corresponding to a secondary methyl group. A peak at $\tau 6.36$ was attributed to the carbomethoxy function. Jones oxidation of the methyl ester (104) affords a ketol ester (113), in which the β-hydroxyketone function was established by forming the conjugated ketone (114).

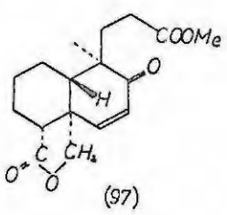
The location of the 1:3 diol system at the 6 and 8 positions as in (101) was suggested by the physical constants of the conjugated ketone (114), and the saturated keto-acid (115), which correspond to those expected for enaniomers of 6-oxo-catavic acid and 6-oxo-labdan-15-oic acid.

Wolf-Kishner reduction of the keto acid (115) gave an acid, which after methylation with diazomethane was found to be identical to methyl enantiolabdan-15-oate (112).

The stereochemistry at C_8 in the diol (104) was established by dehydration of the acetoxy-ester (105) to give a mixture of double bond isomers, whose NMR spectrum showed it to consist of at least 60% exocyclic 8 (20)-olefin, and so the hydroxyl function at C_8 may be assigned the equatorial 8β-configuration.[62] The C_6-hydroxyl must be also in an equatorial configuration since it readily forms an acetate under standard conditions.[63]

The skeleton of the second dihydroxy acid (102) was established as follows.

The diol methyl ester (106) afforded the ketol-ester (116) on oxidation with Jones reagent. Treatment of (116) with ethane-1, 2 dithiol and boron trifluoride ethereate in acetic acid at room temperature yielded the ethylene thioketal (117) which was desulpurized with Raney nickel. The product was identified as methyl- 8β-hydroxy-enantio-labdan-15 oate (103).



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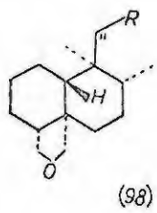

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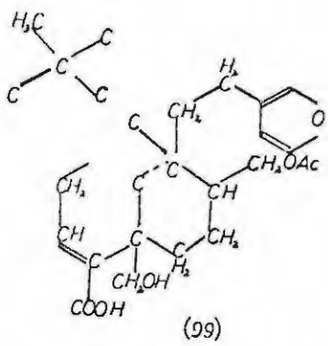

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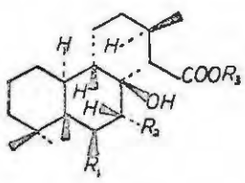
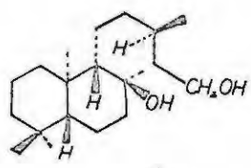


	R_1	R_2	R_3
(100)	H	H	H
(101)	OH	H	H
(102)	H	OH	H
(103)	H	H	Me
(104)	OH	H	Me
(105)	OAc	H	Me
(106)	H	OH	Me



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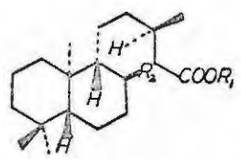
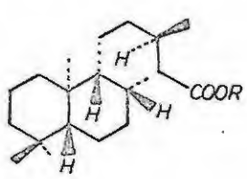
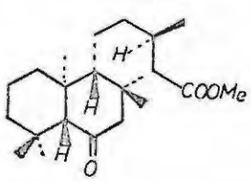


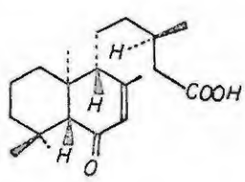
	R_1	R_2
(108)	Me	CH_2
(109)	H	CH_2
(110)	H	O



(111) R = H
(112) R = Me



(113)



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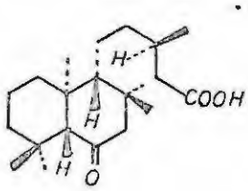
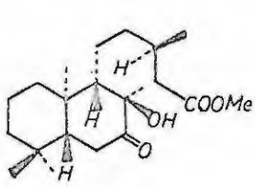
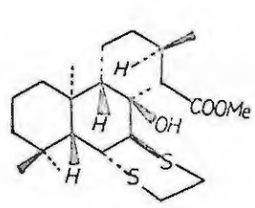


(115)



(116)



(117)

The location of the secondary alcohol in the diol ester (106) at C_7 followed from the latter's oxidation by 1 mole of periodic acid[64] to yield a product which gave an iodoform reaction and which exhibited peaks for CH_3-C(=O)- , $-CH_2$-CHO and $-COOCH_3$ groups expected for the oxidation of (106). The C_7 hydroxyl is equatorial since it was readily acetylated[63] and since reduction[72] with $LiAlH_4$ either of the ketol ester (116) or of the diol ester (106) furnished the same triol.

1.11 Crotonin

Crotonin (119), $C_{19}H_{24}O_4$, a furanoid norditerpene was isolated[65] from the leaves and twigs of Croton eleuteria. The presence of a γ-lactone, a β-substituted furan, ketone, and two secondary methyls was shown by IR and NMR spectroscopy.

Hydrogenation of (119) gave the acid, hexahydrocrotonin (120) and tetrahydrocrotonin. This result defines the relation of the furan ring to the lactone. The acid (120) was converted into the keto alcohol (121) which on oxidation yielded a mixture of (120) and (122). In the NMR of (122) the aldehyde proton shows up as a singlet.

Selenium dioxide oxidation of (119) yielded an amorphous phenol (123). The methyl ether (124) had NMR signals for a secondary methyl, an aromatic methyl, methoxyl and metacoupled aromatic protons. Chromic acid oxidation of (124) in acetic acid, gave (125) which had UV absorption characteristic for that of a substituted p-methoxyacetophenone.[66] The NMR confirms the position of a methyl group at C_4.

The furfurylidine derivative (126) has UV absorption peaks in good agreement with the furfurylidine derivative of p-methoxyacetophenone. In the NMR the C_8 proton appears as a quartet and the methyl at C_8 as a doublet.

The above evidence establishes the gross structure (119) for

crotonin, which is structurally related to the cascarillins isolated from Croton eleuteria.[67]

1.12 Ozic Acid

Extraction of the dry wood of Daniella ogea[68,69] yielded ozic acid (127), $C_{20}H_{30}O_2$, the NMR spectrum of which showed two tertiary methyl groups, one vinyl methyl group and six vinyl protons. The UV and IR spectrums showed the presence of a conjugated diene. The compound absorbed 3 moles of hydrogen on hydrogenation. The above facts lead to the general structure (127) in agreement with the observed principal peaks in the mass spectrum.[68,70]

The stereochemistry at C_5, C_9 and C_{10} were inferred by analogy with daniellic acid (128) and confirmed by correlation with neoabietic acid. Ozic acid must therefore have the antipodal stereochemistry with respect to the steroids, as does daniellic acid.

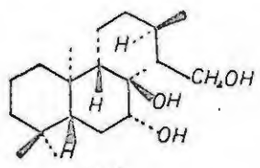
The 4-carboxylic acid group was assigned an equatorial configuration.[71]

The configuration of the 12, 13-double bond in the side chain of ozic acid was arrived at from UV and NMR measurements, with ethanol and carbon tetrachloride respectively as solvents. The results for ozic acid are consistent with a cis-configuration about the 12, 13-double bond.[73] The complete structure and stereochemistry of ozic acid was thus established as in (127).

Ozic acid is similar to communic (129) and zanzibaric (130) acids.[74] Both cis-[75] and trans-communic acids[76], which have not been isolated as solids owing to their lability, have the normal stereochemistry with respect to the steroids, and have axial 4-carboxy groups.

1.13 Zanzibaric Acid

Zanzibaric acid (130), was extracted as the acetate (131) from Trachylobium verrucosum.[69,77] The structure of (130) was determined by chemical degradation and spectral data.



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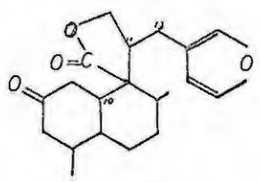

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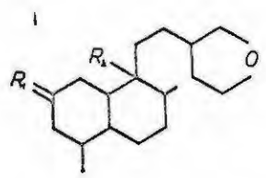
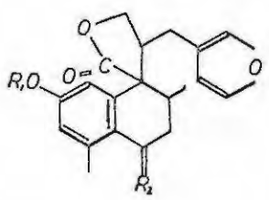
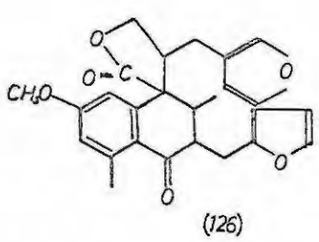


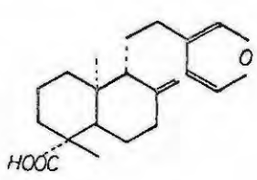
	R_1	R_2
(120)	O	COOH
(121)	O	CH_2OH
(122)	O	CHO



	R_1	R_2
(123)	H	H_2
(124)	Me	H_2
(125)	Me	O



(126)



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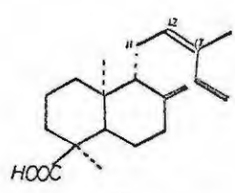

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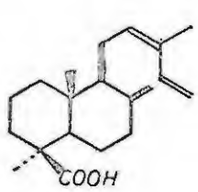

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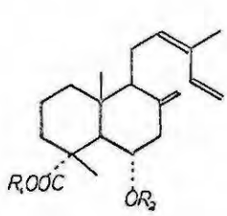
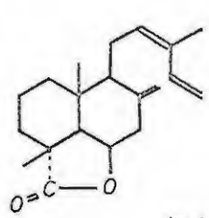


	R_1	R_2
(130)	OH	H
(131)	OH	Ac
(132)	OMe	Ac
(133)	OMe	H



(134)

The acetate (131) when methylated with diazomethane yielded (132) which on catalytic hydrogenation yielded a mixture of C_{13}- and C_8-epimers of (135). Addition of maleic anhydride to (131) yielded an anhydride. Treatment of the methoxyacetate (132) with sodium in refluxing propanol yielded the 12, 15-dihydro derivative, the ozonide of which was reduced with zinc in acetic acid to give a diketo-derivative (137) which when refluxed with sodium ethoxide in ethanol gave the conjugated compound (138). Alkaline hydrolysis of (132) gave zanzibaric acid (130) which on treatment with N.N-dicyclocarbodiimide gave a high yield of the lactone (134). The methylated product (133) of zanzibaric acid gave (136) on catalytic hydrogenation. The product was oxidised with Jones reagent to give the 6-oxo-derivative.

The above chemical evidence together with spectroscopic evidence postulate structure (130) for zanzibaric acid.

Zanzibaric acid, like ozic acid, has the antipodal stereochemistry and like trans-communic acid, has a trans-configuration at the 12, 13-double bond on the side chain.

1.14 Aplysin-20

Aplysin-20, (139), $C_{20}H_{35}O_2Br.$, was isolated from Aplysia kurodai.[78,79] IR and NMR data indicated that there were two hydroxyl groups, a double bond and 5 quarternary methyl groups. Aplysin-20 was treated with acetic anhydride-pyridine to give a monoacetate which could be converted into starting material with methanolic potassium hydroxide. In a comparison of the NMR spectra of aplysin-20 and its mono-acetate, the presence of partial structure (140) was arrived at. Considering (140) together with the presence of two hydroxyl groups, and the appearance of a pair of strong peaks, coupled with the strongest peak at m/e191 in the mass spectrum suggested the presence of partial structure (141). The structure of aplysin-20 was then determined by X-ray analysis using the multiple

film technique and Ni-filtered Cu-K_α methods.

The spectral and chemical evidence for the structure of aplysin-20 are in agreement with the results obtained by X-ray analysis. Aplysin-20 is the first bicyclic diterpene which has the axial hydroxyl group at C_8.

1.15 Psiadol

Psiadol (142), $C_{20}H_{30}O_3$, was isolated[80] from the leaves of Psiadia altissima.

The following deductions were made from its IR, NMR and mass spectra: the presence of a β-substituted furan, two quarternary methyls, $-CH_2OH$ and $-C=CH_2$ groups. Acetylation of (142) yielded a diacetate (143) while Jones oxidation yielded a ketoaldehyde (144). The UV and NMR spectral features of psiadiol (142) and its derivatives agree well with partial structure (145), assuming the normal labdane diterpene skeleton for psiadol.

That the hydroxyl group was at C_4 and not at C_{10} was indicated by both the reaction of Psiadol with acetonide and by the configuration of the C_6-hydroxyl group. The six-line pattern at δ 5 and δ 11 in the NMR spectrum of (143) and the coupling constants between the protons at C_5, C_6 and C_7 establishes the equatorial configuration of the hydroxyl at C_6. More direct evidence concerning the configuration at C_6 was obtained as follows. The keto-aldehyde (144) on sodium borohydride reduction afforded an amorphous dihydroxy compound (146) different from (142). Acetylation of (146) gave a monoacetate (147). Therefore the C_6-hydroxyl in (146) must have the axial configuration since the complex hydride reduction of (144) is sterically controlled by the 1, 3 axial substituents in C_4 and in C_{10}.

From a study of NMR spectra of diterpenes,[81,82,83] containing both a C_4-methyl and a C_4-hydroxymethyl (or acetoxy-methyl) group, the axial configuration was assigned to the C_4 acetoxymethyl

group of Psiadol derivative (143).

The above results are rationalized in terms of structure (142).

Confirmation of the structure (142) as well as proof of the stereochemistry was provided by correlation with a lambertianic acid derivative (148) of established structure and configuration.[84]

Psiadol diacetate (143) was oxidised with Jones reagent and a maleic anhydride (149) was obtained. The anhydride was ozonised and the product worked up oxidatively to yield (150). This compound on treatment with potassium hydroxide in methanol-water and reacetylation yielded an $\alpha\beta$-unsaturated ketone (151). Catalytic reduction of this ketone in ethanol on Pd-C yielded (152) whose IR and NMR were identical to the acid (148) obtained from the lambertianic acid. The specific rotations of the acid (152) was of the same magnitude as (148) but opposite in sign, revealing an antipodal relationship between the acids.

Hence the absolute configuration depicted for psiadol in (142) may be taken as proved.

1.16 Plathyterpol

Plathyterpol[85] (153), $C_{20}H_{34}O$, a liquid diterpene was extracted from the heartwood of Plathymenia reticulata. A tertiary hydroxyl group, and four olefinic protons attached to two double bonds (estimated by catalytic reduction) one of which is present as a vinyl group, was estimated by spectroscopic data. Jones oxidation gave a ketone (154) which was formulated in accordance with its NMR spectrum.

Huang-Minlon reduction of this ketone gave a liquid olefin, which on oxidation with sodium dichromate-acetic acid gave an $\alpha\beta$-unsaturated ketone.

Dehydrogenation of (153) and (154) afforded 1, 2, 5-trimethylnapthalene confirming that the natural product is a terpene with a reduced napthalene ring system. The above evidence leads to

structure (153) for plathyterpol.

The stereochemistry of plathyterpol is not defined but the NMR spectrum of the ketone suggests that the proton at C_{10} is equatorial to ring A and hence the ring fusion is cis as in columbin.[86]

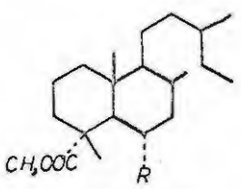
1.17 Olearin

Olearin (155), $C_{20}H_{26}O_5$, a neutral diterpene dilactone of the columbin type[86] was isolated[87], from Olearia heterocarpa. The UV and IR spectrum indicated the presence of two $\alpha\beta$-unsaturated ester groupings. The carbonyl absorption of tetra hydro-olearin showed that both carbonyl groups are present in γ-lactone rings. The fifth oxygen of olearin is present in a readily acetylatable hydroxyl group which is secondary since tetrahydro-olearin may be oxidised to a ketone. (156).

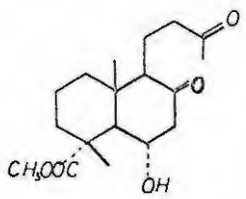
Partial structure (157) was established in the following way. Treatment of the ketone (156) with alkali gave rise to formaldehyde, presumably via the retro-aldol condensation. Thus the hydroxyl of the opened lactone must be β to the keto group. In addition, strong acid or dissolution in dilute alkali followed by acidification converts tetrahydro-olearin to an isomer (158) which also contains two γ-lactone rings and which may be oxidised to a carboxylic acid (159).

The NMR spectrum of olearin is consistent[88] with a β-substituted butenolide structure. The remainder of the proposed structure is supported by NMR data, which indicate the presence of one tertiary methyl, one secondary methyl, one vinyl proton adjacent to a $-CH_2-$ group, and the grouping $-CH_2-O$ attached to a fully substituted carbon atom.

On heating the methane sulphonate of olearin in dimethyl sulphoxide anhydro-olearin (160) is formed. The trans-assignment for the introduced double bond follows from its NMR spectrum. Both protons of this trans-ethylenic link appear as doublets, hence C_9 in olearin does not bear a proton, indicating a rearranged labdane skeleton.



(135) R = OAc
(136) R = OH



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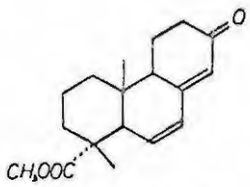

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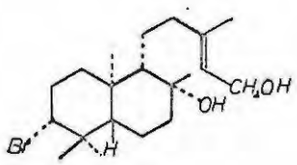


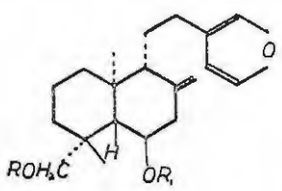
(139)

$-C(CH_3)=CH-CH_2OH$

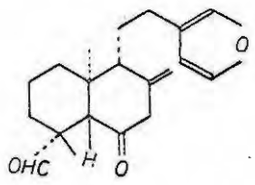
(140)

$-CH_2-CH_2-C(CH_3)=CH-CH_2OH$

(141)



(142) R = H
(143) R = Ac



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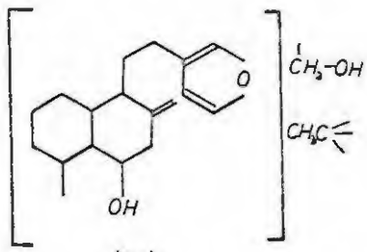

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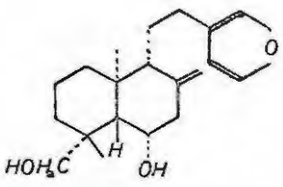

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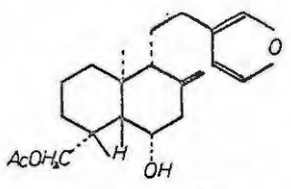

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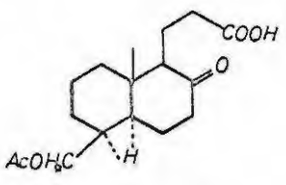

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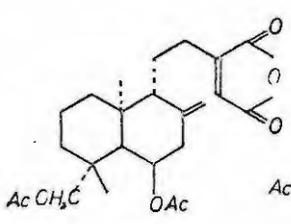

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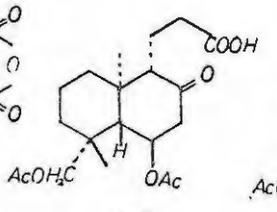

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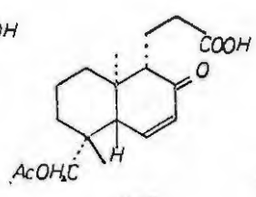

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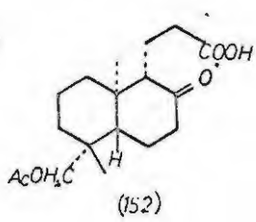


(152)

Olearin readily adds on bromine to yield dibromo-olearin, the NMR spectrum of which shows a vinyl proton indicating that the butenolide double bond is retained. Oxidation of dibromo-olearin with potassium permanganate in acetone produces a dibromo-acid which on refluxing with alkali gave formaldehyde and a diene (161).

Dehydrogenation of the dimethyl ester of the diene (161) with dichlorodicyanoquinone, followed by hydrolysis gives the tetralin dicarboxylic acid (162). The structure assigned to (162) was consistent with the NMR spectrum of the dimethyl ester. In support of the structures proposed for the above compounds, the diene (161), on dehydrogenation over 10% Pd-C yielded 1, 2-dimethylnapthalene.

1.18 Diterpenes of Oxystigma oxyphyllum

The extract from the fresh wood of Oxystigma oxyphyllum[89] when chromatographed yielded two acid fractions, (A) and (B). These acidic fractions were then rechromatographed to give components (A - 1), (A - 2) and (B - 1), (B - 2) as their methyl esters.

The spectral properties of (A - 1) methyl ester, $C_{21}H_{34}O_2$, was consistent with the structure of methyl labda-8 (20), 13-dien-15-oate[90] (163). $LiAlH_4$ reduction of (A - 1) methyl ester gave a hydroxy ketone (166).

The hydroxy ketone was dehydrated to an α,β-unsaturated ketone, in agreement with the synthetic racemate[91] of (167). This in conjunction with the observed optical rotation of (A - 1) methyl ester suggests that (A - 1) is mainly (165) but contains its enantiomer, copalic acid[92] (see chart 5). This was confirmed as follows.

The methyl ester of (A - 1) was ozonised to the diketo-compound which was cyclised with alkali to the hydroxy-ketone (166). Dehydration of the hydroxy-ketone gave the racemate and (167).

The methyl ester of (A - 2) gave IR and NMR spectra which

suggested that it may be the $\Delta^{7,13}$ isomer of (A -1), the negative optical rotation of its methyl ester indicated a relationship to copalic acid,[92] rather than to its enantiomer (163).

In support of this, hydrogenation of (A - 2) methyl ester afforded a laevorotatory tetrahydro-derivative (c.f. hydrogenation of methyl eperuate[93] and methyl cativate[94]). Compound (A - 2) is thus eperua-7, 13-dien-15-oic acid (168).

The p-phenylphenacyl esters of the acid fraction (A) gave the racemate (169).

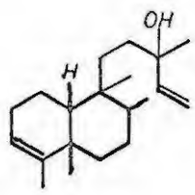
The IR and NMR characteristics of (B - 1) methyl ester were consistent with it being methyl eperuate (170) and ozonolysis of (B - 1) afforded the known keto-acid[93] (171). The spectral properties of (B - 2) methyl ester were similar to those of (B - 1) methyl ester except that they indicated a trisubstituted double bond. Compound (B - 2) is therefore the Δ^{7}-isomer (171). This was confirmed by hydrogenation of its methyl ester to give a laevorotatory dihydro-derivative, the properties of which were in agreement with those of methyl dihydro-eperuate.[93]

Of the five diterpene acids characterised, one is of the normal stereochemical series with respect to the steroids, while four belong to the antipodal labdane series. It is interesting to note that this is the first example of co-occurence of the two series.

1.19 Agathalic Acid

In addition to the diterpenoids cis- and trans-communic acids, abietic acid, neoabietic acid, and monomethylagathalic acid, isolated[95] from the oleoresin of Black kauri, the acid portion of the oleoresin also yielded a new aldehydic diterpenoid,[96] agathalic acid (172), $C_{20}H_{30}O_3$, (trans-19-oxolabda-8 (14), 13 (15)-dien-16-oic acid), isolated through Girard Reagent P.

The structure of the compound followed from NMR and chemical evidence. Reduction of agathalic acid (172) with $LiAlH_4$ gave



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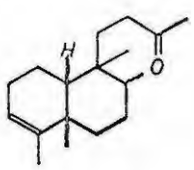

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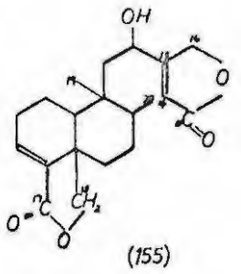

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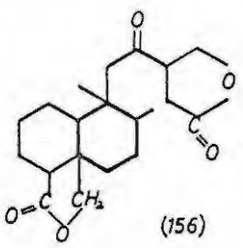

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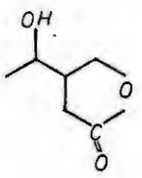

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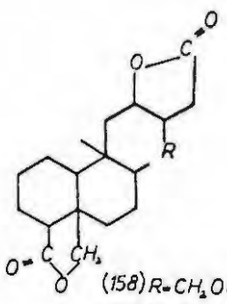
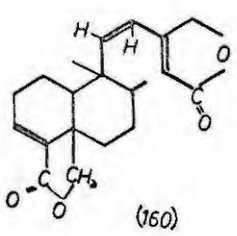


(158) R = CH_2OH

(159) R = COOH



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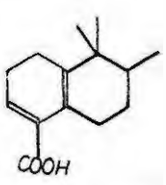
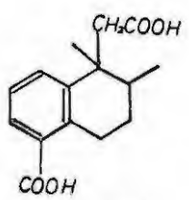
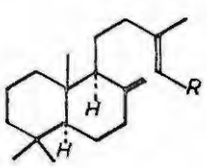


(161)



(162)

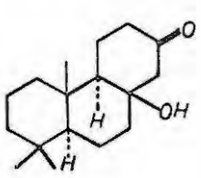


(163) R = COOMe

(164) R = CH_2OH

(165) R = COOH

(169) R = $COOCH_2COC_6H_4Ph$



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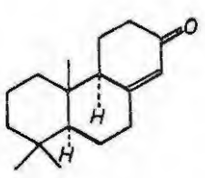
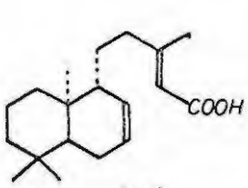
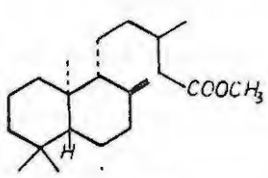


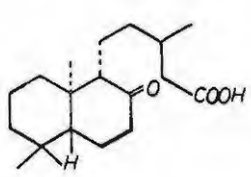
(167)



(168)



(170)



(171)

agathadiol (173), while reduction with sodium borohydride gave agatholic acid (174). Agathalic acid was synthesized from agatholic acid (174), (previously synthesized[97] from agathic acid (176) by CrO_3 oxidation). The oxime (175) from the synthetic compound was identical in all respects with the oxime from the natural product.

1.20 A hydroxy-acid from Juniperus phoenicea

Hydroxy acid (177), $C_{20}H_{34}O_3$, which occurs as a mixture of erythro-threo isomers was isolated from Juniperus phoenicea.[98] IR and NMR analysis on the ester (178) showed the presence of two quarternary methyls, one secondary and one primary methyl groups, an ester function and a hydroxyl group. The UV spectra showed no conjugation. The above values were consistent with bicyclic terpenes bearing a terminal methylene group $\Delta^{8(20)}$ and an axial ester function at C_4 e.g. methyl agathate and methyl communate,[99] both of which have already been isolated from Juniperus species.

Following the above evidence, partial structure (119) was formulated for the compound (177). The hydroxyl group was shown to be secondary and confirmed by forming an acetate.

The structure of the hydroxy acid (178) was confirmed by its correlation with tetrahydro-agathadiol (181) as follows. The dihydro-compound (180) of the hydroxy acid (177) was dehydrated with thionyl chloride-pyridine to give an anhydro-compound which was then reduced with $LiAlH_4$ to give tetrahydro-agathadiol (181), whose structure and stereochemistry has already been established.

It is apparent from the above chemical evidence that the acid belongs to the labdane series bearing a C_4 acid.

The alcohol function was shown to be in the C_{12} position by chromic acid oxidation of (178) to the ketone (182). The ketone (182) showed no absroption in the UV region, a fact which eliminates the C_{11} position and it not being a methyl ketone eliminates

position C_{14}. Hence the structure of the acid is as shown in (177).

1.21 Diosbulbine-A, -B and -C

Diosbulbine-B (183a) $C_{19}H_{21}O_6$: -A (184a) $C_{19}H_{22}O_7$, -C (185a), $C_{20}H_{24}O_7$ was isolated from Dioscorea bulbifera. Dehydrogenation of the lithium aluminium hydride product of (183a) gave 1, 2, 5-trimethyl napthalene.

Following from spectroscopic, chemical and physical properties partial structures (183b), (184b) and (185b) were formulated for diosbulbines-B, -A and -C respectively.

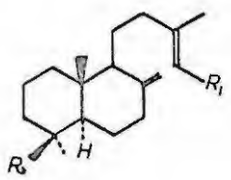
The NMR and IR spectrums of diosbulbine-B and -C when compared showed that one of the γ-lactones of diosbulbine-B has split to form -C (185a). Esterification of (185a) with diazomethane formed a methyl ester which was identical to (184a). Hence it follows that diosbulbine-A (184a) must be the methyl ester of diosbulbine-C.

Hydrogenation of diosbulbine-B yielded tetrahydrodiosbulbine-B (186), hexahydrodiosbulbine-B (187) and a small quantity of octahydro-diosbulbine-B.

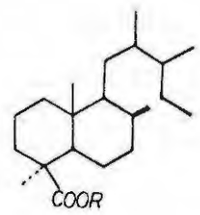
Two epimers of (186), regarded epimeric on the β-carbon of the tetrahydro-furan ring were isolated. Saponification of (186) gave (188) which on esterification yielded (189).

The secondary hydroxyl group of diosbulbine-A (184a) could not be acetylated and chromic acid oxidation yielded predominantly starting material together with a small amount of the product resulting from the oxidation of the hydroxyl group and the furan ring. Acetylation of (189) also yielded starting material. This information lead to the conclusion that (184a) and (189), possessed a sterically hindered secondary axial hydroxyl group.

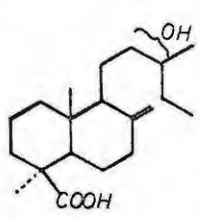
The ketone (190) of (189) showed no bands in the IR spectrum due to hydroxyl absorption.



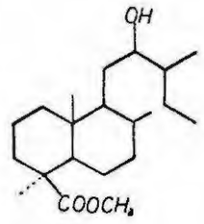
	R_1	R_2
(172)	COOH	CHO
(173)	CH_2OH	CH_2OH
(174)	COOH	CH_2OH
(175)	COOH	CH = NOH
(176)	COOH	COOH



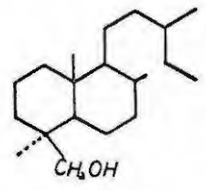
(177) R = H
(178) $R = CH_3$



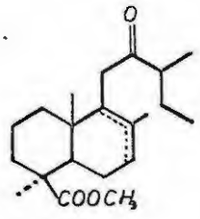
(179)



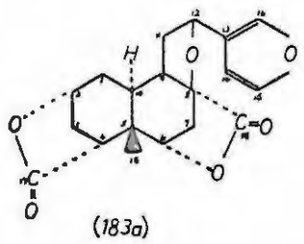
(180)



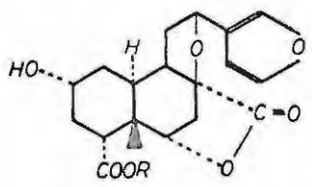
(181)



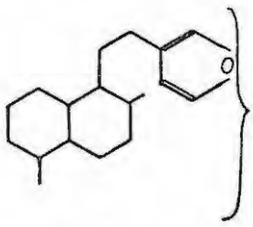
(182)



(183a)

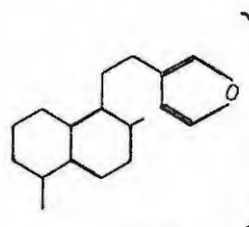


(184a) R = H
(185a) $R = CH_3$



1 tert. CH_3
2 γ-lactones
1 Ether

(183b)



1 tert CH_3
1 γ-lactone
1 OH
1 COOR
1 Ether

(184b) R = H
(185b) $R = CH_3$

De hydration of (189) gave (191) and $LiAlH_4$ reduction of (189) gave a tetrol which could be acetylated to form a triacetate. From the above chemical evidence, together with spectroscopical data it was concluded that the two carbonyl groups of the γ-lactones of (183a) were in the C_4 and C_8 positions. These conclusions then left three possibilities i.e. C_2, C_6 and C_{11} positions for the attachment of the two lactone ether oxygens. The positions of the ether linkage were shown as follows.

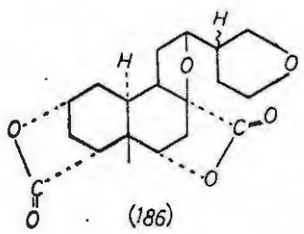
In the IR of hexahydrodiosbulbine-B (187) the ether band at $1064cm^{-1}$ that was present in the IR of (183a), (184a), (185a) and (186) was missing. The appearance of an hydroxyl which could not be acetylated shows that an ether group results in the formation of a new hydroxyl group. These results were consistent with the mass and NMR spectra of (187).

It was assumed from spectral and chemical evidence that the newly formed tertiary hydroxyl group was equatorial. $LiAlH_4$ reduction of (187) at 0°C gave a pentahydroxyderivative which when oxidised with $NaIO_4$ gave a carbonyl derivative (6 membered cyclic ketone). It was then conslusive that the tertiary hydroxyl group of (187) resulted from the ether oxygen and that it must be adjacent to the carbonyl group of the lactone.

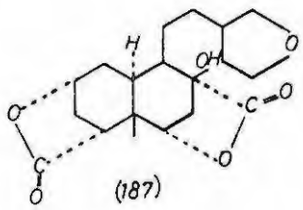
The second bonding position for the ether linkage was shown to be at C_{12} from the NMR spectra of products obtained by ozonolysis of (183a).

From the foregoing evidence, it was concluded that the two γ-lactone rings occur at carbon atoms C_8-C_6 and C_4-C_2 while the ether group occurs at the C_4 and C_{12} positions.

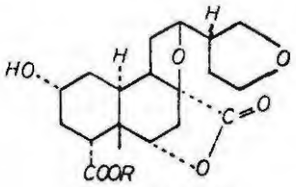
It was determined from NMR spectra that the carbon atom adjacent to the tertiary methyl group does not bear a carboxyl group or an ether linkage. This conclusion then left three possibilities for



(186)

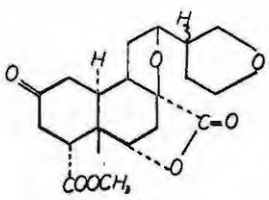


(187)

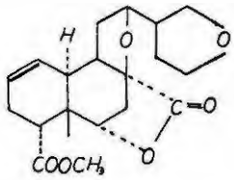


(188) R = H

(189) R = CH_3



(190)



(191)

the placing of the tertiary methyl group i.e. C_5, C_9, C_{10} positions. Further, the proton of the grouping $H-\overset{1}{C}-O-\overset{1}{C}=O$ of (184a), (185a), (189), (190) and (191) appears as a doublet, therefore the tertiary methyl of Diosbulbine-B must be in the C_5 position, as the dihedral angle between a proton in the C_6 or C_7 position can only be at 90°.

The above evidence together with spin-coupling experiments of (185a) and (189) as well as the Overhauser-effect[101] shows diosbulbine-B to have structure (138a). The structures (184a) and (185a) for diosbulbines-A and -C therefore follow from that for diosbulbine-B.

The stereochemistry of diosbulbine-B as in (183a) follows from IR, NMR, Mass and X-ray analysis.

2. EXPERIMENTAL

General experimental details

Melting points are corrected and $[\alpha]_D$ *values refer to chloroform solutions (unless otherwise stated) at room temperature, measured on a Bellingham and Stanley, No.542201 polarimeter and a Perkin-Elmer model 141 digital read-out polarimeter. Alumina for chromatography was acid-washed, neutralised and activated by heating at 170° for 18 hours. IR spectra were determined on a Beckman IR8 in chloroform solutions and in KBr; UV spectra were measured in ethanol with a Unicam SP.800 spectrophotometer. NMR spectra were determined on Perkin-Elmer model R12 and Varian HA-100 instruments in* $CDCl_3$ *(unless otherwise stated) using approximately 0.3M solutions and tetramethylsilane as internal standard. Mass spectra were obtained on an A.E.I. MS9 mass spectrometer.*

TLC was carried out on Merck Kieselgel G, using hexane-ethyl acetate as the mobile phase followed by development with iodine.

2.1 The extraction of Leonotis dysophylla (Benth.)

The plant material was collected near East London during April 1968-1969. It was air dried in the shade for approximately 4 weeks and extracted. The following experiment is typical.

Dried material (817g.), collected on the 26th April 1969, was steeped in acetone (120L.) at room temperature for 5 days, the acetone run off and the plant material washed with a further 60L. of acetone. The combined acetone extracts were concentrated by flash distillation to approximately 10 litres, and stirred with decolourising charcoal (B.D.H. 400g. and then 200g.) at room temperature for 4 hours. The solution was filtered through a celite pad and evaporated to a gum on a rotary evaporator. Addition of ethyl alcohol (75ml.) afforded crystals which were filtered off, washed with ethyl alcohol (75ml.) and dried in a vacuum dissicator. (Yield 36.0g., 0.44%) TLC in ethyl acetate-hexane (7:3) showed that this material consisted almost entirely of one substance (8-hydroxymarrubiin (192)).

These crystals were dissolved in dry chloroform (500ml.) and boiled with decolourising charcoal (15g.). The solution was filtered through a celite pad and extracted with water (3x 200ml.) to remove inorganic material. The combined water washings were washed with chloroform (2 x 75ml.) and the combined chloroform solutions were dried (Na_2SO_4) and evaporated to a gum which dissolved in refluxing ethyl acetate (400ml.). The resulting crystals were filtered off, washed with a little cold ethyl acetate and dried in a vacuum dessicator, (Yield 20.2g.) m.p. 173° raised to 176° on recrystallisation from the same solvent.

The filtrate was concentrated to approximately 150ml., addition of hexane afforded a further crop of crystals (192) (5.2g.), m.p. 174° $[\alpha]_D^{24}$ + 33° (C 1.0).

Found:

C 69.04%

H 8.00%

Molecular Weight (Mass spectrum) 348

Calculated for $C_{20}H_{28}O_5$:

C 68.94%

H 8.10%

2.2 Colour tests on marrubiin, 8-hydroxymarrubiin and leonitin

In each of the following tests marrubiin was used as the standard compound. Results are recorded in Table 1a,b,c.

a) Lieberman-Burchard test.[26,102]

Marrubiin, 8-hydroxymarrubiin and leonitin (10-15mg. each) were separately dissolved in chloroform (2ml.); acetic anhydride (10 drops) and concentrated sulphuric acid (2 drops).

b) Action of chromic acid

The above compounds (25mg. each) were separately dissolved in a solution of nitromethane in chloroform.

Table 1 a

COMPOUND	RESULT	CONCLUSION
Marrubiin	Green colour produced	Furan ring present
8-hydroxymarrubiin	Green colour produced	Furan ring present
leonitin	No colour change	No furan ring present

Table 1 b

Marrubiin	Green colour produced	Tertiary hydroxyl group
8-hydroxymarrubiin	Green colour produced	Tertiary hydroxyl group
leonitin	No colour change	No tertiary hydroxyl group

(c) Action of nitromethane

The compounds (15mg. each) were separately dissolved in a solution of nitromethane in chloroform.

Table 1 c

COMPOUND	RESULT	CONCLUSION
Marrubiin	Green colour produced	Unsaturation present
8-hydroxymarrubiin	Green colour produced	Unsaturation present
leonitin	No colour change	No unsaturation present

2.3 Quantitative alkaline hydrolysis experiments
(Results are recorded in table 2)

Marrubiin (134mg.) was refluxed with a standard ethanolic solution (0.2022N) of potassium hydroxyide (10ml.). The hot solution was then back titrated with standard hydrochloric acid solution (0.08704N) to determine the excess alkali present. The number of moles alkali consumed per mole of compound was then calculated. 8-Hydroxymarrubiin (130mg.) and leonitin (133mg.) were then treated as above.

Table 2

COMPOUND	Moles of alkali consumed per mole of compound	INFERENCE
Marrubiin	1.00	Indicates one $-\overset{O}{\overset{\|}{C}}-O-$ group accounting for two oxygens in the molecule.
8-hydroxymarrubiin	0.96	Indicates one $-\overset{O}{\overset{\|}{C}}-O-$ group accounting for two oxygens in the molecule.
Leonitin	2.85	Indicates three $-\overset{O}{\overset{\|}{C}}-O-$ groups accounting for six oxygens in the molecule.

2.4 Attempted acetylation of 8-hydroxymarrubiin

The compound (30mg.) was refluxed on a glycerine bath with acetic anhydride (2ml.) and pyridine (0.15ml.) for two hours. The solvents were removed under reduced pressure, methyl alcohol (5ml.) added and the solution allowed to stand at room temperature

for one hour. The methanol was removed under reduced pressure, benzene added to the crystalline residue and traces of pyridine distilled off with the benzene. The residue crystallised from ethyl acetate-hexane as shiny prisms (25mg.) m.p. 174-175° unchanged on admixture with starting material.

2.5 Attempted dehydrogenation of 8-hydroxymarrubiin

Compound (192) (2.00g.) was intimately mixed with 10% palladised charcoal (2.00G) in a 25ml. flask fitted with an air condensor 30cm. in length. The apparatus was flushed with pure nitrogen and then heated at 300-320° for three hours when approximately 280ml. of gas was evolved chiefly during the first $1\frac{1}{2}$ hours. The reaction product was extracted with boiling hexane (10 x 20ml.) filtered through a celite pad and dried over Na_2SO_4. The filtered solution was evaporated to approximately 30ml., poured into a column (2cm. diameter) of neutral alumina (35g.) and eluted with dry hexane. The first 300ml. were collected (50ml. eluates). In each case, removal of the solvent yielded gums which failed to form crystalline 1, 3, 5-trinitrobenzene or 1. 3, 5-trinitrotoluene adducts, oils resulting in each case.

2.6a) Hydrogenation of 8-hydroxymarrubiin.

A mixture of the compound (2.3g.), 10% palladium hydroxide-barium sulphate (2.3g.) and ethyl alcohol (150ml.) was shaken under hydrogen for three hours when 297ml. of hydrogen at N.T.P. (2.06 moles) were absorbed. The solution was filtered through a celite pad and evaporated to a gum (1.1g.) which was shown to be a single compound on TLC. This compound, however, could not be induced to crystallise.

b) Phosphorus trichloride dehydration of hydrogenation product of 8-hydroxymarrubiin

To a solution of the gum (300mg.) in dry pyridine (30ml.) was added freshly distilled phosphorus trichloride (3ml.) in dry pyridine (5ml.). The solution was refluxed for one hour, cooled and

poured into ice-cold water (100ml.). The pyridine was removed under reduced pressure and the aqueous solution extracted with ether (3 x 100ml.). The combined ethereal extracts were washed with 5% hydrochloric acid (2 x 50ml.), water (2 x 50ml.), sodium bicarbonate solution (2 x 50ml.), water (2 x 50ml.) and dried over Na_2SO_4. Removal of the solvent afforded a gum (210mg.) shown by TLC to be a complex mixture which could not be separated.

2.7 Saponification of 8-hydroxymarrubiin

A solution of the compound (192) (500mg.) in ethanolic sodium hydroxide (50ml., 0.4N) was refluxed for 30 hours. Water (50ml.) was added, the alcohol removed under reduced pressure and the aqueous solution acidified with dilute hydrochloric acid. The resulting solution was extracted with ether (4 x 50ml.). The combined ethereal extracts were washed with sodium bicarbonate solution (2 x 50ml.), water (2 x 50ml.) and dried over Na_2SO_4. Removal of the solvent yielded a gum which crystallised from ethyl acetate-hexane as prisms to yield 8-hydroxymarrubic acid (193) (410mg.) m.p. 93-95°, $[\alpha]_D^{24}$ + 23° (C 1.0).

Found:

C 64·45

H 8·44

Calculated for $C_{20}H_{30}O_6$:

C 65.59

H 8.19

2.8 Lithium aluminium hydride reduction of 8-hydroxymarrubiin

A solution of compound (192) (2.5g.) in 50ml. of tetrahydrofuran (dried over sodium and redistilled over lithium aluminium hydride) was refluxed with lithium aluminium hydride (3.0g.) on a glycerine bath. After 6 hours, a further quantity of lithium aluminium hydride (1.0g.) was added and the mixture refluxed for

a further 18 hours. The solution was cooled in ice, the excess hydride decomposed by adding wet ether (100ml.), followed by dilute sulphuric acid (100ml.) and the layers separated. The tetrahydrofuran was removed on a rotary evaporator and the aqueous solution extracted with ether (4 x 50ml.). The combined ethereal extracts were washed with sodium bicarbonate solution (3 x 50ml.), water (3 x 50ml.) and dried over Na_2SO_4. Removal of the solvent afforded a gum (2.3g.) (shown by TLC to be a single compound which crystallised from ethyl acetate-hexane as needles (2.15g) to yield 8-hydroxymarrubenol (195) m.p. 153°, $[\alpha]_D^{24}$ + 33° (C 1.0).

Found:

C 68.15%

H 9.40%

Calculated for $C_{20}H_{32}O_5$:

C 68.15%

H 9.15%

2.9 Tosylation of 8-hydroxymarrubenol (192)

An ice-cold solution of p-toluene sulphonyl chloride (0.75g.) in pyridine (3ml.) was added to compound (192) (300mg.) in pyridine (5ml.) at 0° and the mixture kept at 0° for 4 days. The solution was poured into ice-water and the oily precipitate extracted with ether (4 x 25ml.). The combined ethereal extracts were washed successively with cold 5% hydrochloric acid (2 x 20ml.), water (2 x 20ml.) and dried over Na_2SO_4. Removal of the solvent afforded a crystalline precipitate (140mg.) which crystallised from ethyl acetate as prisms (196) (110mg.) m.p. 126°. Recrystallisation from the same solvent afforded prisms m.p. 128°, which gave a positive test for the presence of sulphur.

Found:

C 64.28%

H 7.52%

Calculated for $C_{27}H_{38}O_7S$

C 64.03%

H 7.51%

2.10 Periodate oxidation of 8-hydroxymarrubiin

8-Hydroxymarrubiin (0.18g.) m.p. 176° was dissolved in ethyl alcohol (50ml.) in a 100ml. volumetric flask, 0.1M aqueous sodium periodate (20.0ml.) added and the solution made up to volume with water. A blank experiment was run concurrently. At time intervals of 1, $3\frac{1}{2}$ and 48 hours, 10ml. aliquots were withdrawn and water (30ml.) was added to each and the alcohol removed on a rotary evaporator. To each was added sodium bicarbonate (5g.), 0.0101M sodium arsenite (40ml.), potassium iodide (5g.) and the mixture allowed to stand at room temperature for 10 minutes and then titrated against 0.0099N iodine solution. Results (see Table 3) showed that no periodate had been consumed even after 48 hours.

Table 3

TIME INTERVALS	SAMPLE		BLANK	
	Arsenite	I_2	Arsenite	I_2
1 Hour	40ml.	1.1	40ml.	1.2
$3\frac{1}{2}$ Hours	40ml.	1.95	40ml.	1.95
48 Hours	40ml.	1.95	40ml.	2.00

2.11 Lead tetra-acetate oxidation of 8-hydroxymarrubiin

The compound (192) (1.0g.) was dissolved in benzene (150ml.). Lead tetra-acetate (1.5g.) was added over a period of 20 minutes and the mixture stirred vigorously at 30° for 1 hour.

The crystalline lead acetate was filtered off followed by removal of the solvent to afford a gum which was shown by TLC to consist predominantly of starting material together with a slight trace of a new compound. Attempts to isolate the new compound by column chromatography and preparative TLC failed to yield separations.

This experiment was repeated using different solvent systems viz. methanol, acetic acid, pyridine, 25% acetic acid-pyridine and 25% acetic acid-methanol. Although the concentration of the new component was slightly increased in methanol and 25% acetic acid-methanol, all attempts at isolation failed.

2.12 Phosphorus trichloride dehydration experiments of 8-hydroxymarrubiin

a) In Benzene

To a boiling solution of the compound (500mg.) in dry benzene (30ml.) was added phosphorus trichloride (0.25ml.) in benzene (2ml.). The solution was heated for 30 minutes under reflux, cooled and poured into water (50ml.). The aqueous layer was separated off and extracted with benzene (3 x 20ml.). The combined benzene extracts were washed with 10% sodium hydroxide solution (3 x 50ml.), water (3 x 50ml.) and dried (Na_2SO_4). Removal of the solvent afforded a gum (450mg.) which crystalised from ethyl acetate-hexane, m.p. 173-4, undepressed on admixture with 8-hydroxymarrubiin. Thin layer chromatography revealed starting material. The IR spectra ($CHCl_3$) were also identical.

b) In Pyridine

To a boiling solution of 8-hydroxymarrubiin (2g.) in dry pyridine (30ml.) was added freshly redistilled phosphorus trichloride (3ml.). The solution was refluxed for three hours; cooled and poured into ice-cold water (100ml.). The pyridine was removed under reduced pressure and the solution extracted with chloroform (3 x 100ml.). The combined chloroform extracts were washed successively with cold 5% HCl (3 x 50ml.); water (2 x 50ml.), sodium bicarbonate solution (2 x 50ml.), water (2 x 50ml.) and dried (Na_2SO_4).

Removal of the solvent afforded a gum (1.58g.). Thin layer chromatography in ethyl acetate hexane (3:7) revealed the absence

of starting material but two new compounds (R_f's 0.54, 0.64) were shown to be present.

Chromatography of the above gum on neutral alumina (35g.) yielded on elution with ethyl acetate-hexane (1:20) the more polar component as an oily gum (198a and 198b) which did not crystallise and a gum (R_f 0.54) which crystallised from dry hexane as needles (525mg.) m.p. 113° raised on recrystallisation to yield the epoxide (196) m.p. 114°, $[\alpha]_D^{24}$ + 51° (C 1.0)

Found:

C 72.37%

H 8.07%

Calculated for $C_{20}H_{26}O_4$:

C 72.70%

H 7.93%

2.13a) Hydrogenation of the epoxide (197)

A mixture of compound (197) (450mg.), 10% palladium hydroxide-barium sulphate (500mg.) and ethyl alcohol (100ml.) was shaken under hydrogen for two hours when approximately 2 moles of hydrogen at N.T.P were absorbed. Filtration and removal of the solvent afforded a gum (400mg.) shown by TLC to be a mixture of 3 components (R_f's 0.28, 0.12, 0.05) none of which corresponded to starting material.

The gum was chromatographed on neutral alumina (12g.) and the column eluted initially with benzene and then increasing concentrations of ethyl acetate. Benzene eluted a gum (220mg.) which crystallised from hexane as fine needles (199) (158mg.) m.p. 111° $[\alpha]_D^{24}$ + 58° (C 1.0).

Found:

C 71.67%

H 9.12%

Calculated for $C_{20}H_{30}O_4$:

C 71.85%

H 8.89%

Elution with 20% ethyl acetate-benzene afforded a gum (70mg.) which could not be induced to crystallise. Further elution with 50% ethyl acetate-benzene afforded the third compound (100mg.) which crystallised from hexane as needles m.p. 118°.

b) Hydrogenation of the oily gum (198a and 198b) obtained from the dehydration of 8-hydroxymarrubiin

The oily gum (200mg.), in ethanol (50ml.) was hydrogenated over 10% palladium hydroxide-barium sulphate (200mg.) and the product worked up as above. The gum obtained was shown by TLC in ethyl acetate-hexane (3:7) to be a mixture of mainly two components (200 a and b) (R_f's 0.44, 0.36) and a trace of starting material.

2.14 Lithium-aluminium hydride reduction of the epoxide (197)

A solution of the epoxide (197) (340mg.) in 25ml. of tetrahydrofuran (dried over sodium and redistilled over lithium aluminium hydride) was refluxed with lithium aluminium hydride (700mg.) for 17 hours. The solution was cooled in ice and the excess hydride decomposed by adding wet ether (30ml.), followed by dilute sulphuric acid (30ml.) and the layers separated. The tetrahydrofuran was removed on a rotary evaporator and the aqueous solution extracted with ether (3 x 50ml.). The combined ethereal extracts were washed with sodium bicarbonate solution (2 x 25ml.), water (2 x 25ml.) and dried over Na_2SO_4. Removal of the solvent afforded a gum (338mg.) which crystallised from ethyl acetate-hexane as needles (210mgm) to yield (202), m.p. 143-135°, $[\alpha]_D^{22}$ + 18° (C 1.0) identical to an authentic sample of marrubenol (R_f, m.p. and mixed m.p., IR, NMR, and $[\alpha]_D$).

2.15 Action of p-napthalene sulphonic acid on the epoxide (197)

A saturated solution of p-napthalene sulphonic acid in benzene was added to a solution of the epoxide (250mg.) in benzene (50ml.). After 15 minutes at 80-85°, the solution was cooled and filtered through a short column of alumina. Removal of the solvent yielded a gum (245mg.) which was shown on TLC in ethyl acetate-hexane (3:7) to contain starting material (R_f 0.54) together with three other components (R_f's 0.64, 0.30 and 0.19). Column chromatography of the gum on neutral alumina using hexane as the solvent yielded the more polar component (R_f 0.64) as an oily gum (109mg.) which failed to crystallise. On TLC this gum corresponded to the oily component (198a and 198b) obtained from the dehydration of 8-hydrcxymarrubiin (192) (see expt. 2.12b). Elution with 5% ethyl acetate-hexane yielded starting material followed by component (R_f 0.30) which crystallised from benzene-hexane as plates (60mg.) m.p. 115°. Further elution with 5% ethyl acetate-hexane yielded component (R_f 0.19) which crystallised from benzene-hexane as needles (19mg.) m.p. 155°.

2.16 Chromic acid oxidation of marrubiin (5)

Marrubiin (5) (1.5g.) in acetic acid (15ml.) at 0° was treated with chromic acid (3.75g.) in water (6ml.) and acetic acid (20ml.) and the solution left at room temperature for 4 days. The acetic acid was removed on a rotary evaporator, water (100ml.) added and the solution extracted with ether (4 x 50ml.). The combined ethereal extracts were washed successively with water (3 x 50ml.), sodium carbonate solution (2 x 50ml.), water (2 x 50ml.) and dried over Na_2SO_4. Removal of the solvent afforded a gum (350mg.) which crystallised from benzene-hexane to yield the dilactone as needles (207a) (300mgm.) m.p. 161-162° $[\alpha]_D^{24}$ + 31° (C 1.0).

Found:

C 66.27%

H 7.64%

Calculated for $C_{17}H_{24}O_5$:

C 66.21%

H 7.84%

Further elution with increasing percentages of ethyl acetate yielded the third component (R_f 0.42) together with component R_f 0.53. This mixture was eventually separated by repeated column chromatography using ethyl acetate-benzene as the solvent to give the third component (95mg.) which crystallised from ethyl acetate-hexane as prisms (205) m.p. 247°; $[\alpha]_D^{24}$ + 68°, (C 1.0).

Found:

C 65.98%

H 7.52%

Calculated for $C_{17}H_{24}O_5$:

C 66.21%

H 7.84%

2.18 Phosphorus trichloride dehydration of the γ-dilactone (204)

The compound (204) (550mg.) m.p. 195° in dry pyridine (30ml.) was treated with redistilled phosphorus trichloride (3ml.) in dry pyridine (5ml.) and the solution refluxed for 2 hours. The reaction mixture was poured on ice and the pyridine azeotropically removed. The remaining solution was extracted with chloroform (5 x 50ml.); the combined chloroform extracts were washed successively with 5% hydrochloric acid (2 x 50ml.) water (2 x 50ml.), sodium bicarbonate (2 x 50ml.), water (2 x 50ml.) and dried over Na_2SO_4. Removal of the solvent afforded a gum (510mg.) shown by TLC in ethylacetate-hexane (7:3) to be a mixture of 3 components (R_f's 0.96, 0.74, 0.14).

The gum was chromatographed on neutral alumina (15g.) and the column was eluted with benzene followed by increasing concentrations of ethyl acetate. The early fractions in benzene afforded a gum (R_f 0.96) which crystallised from benzene-hexane as needles (206) m.p. 164°.

Found:

C 70.33%

H 7.75%

Calculated for $C_{17}H_{22}O_4$:

C 70.32%

H 7.64%

Later fractions from the column on elution with 5% ethyl acetate-benzene afforded a small amount of gum (R_f 0.74) (25mg.) which crystallised from benzene-hexane as prisms m.p. 275°. This compound was identical in all respects (m.p. and mixed m.p., IR, NMR and UV) with that obtained by chromic acid oxidation (expt. 2.17).

Found:

C 69.61%

H 7.36%

Fractions from the column eluted with ethyl acetate afforded the third component (R_f 0.14) (110mgm.) which crystallised from ethyl acetate-hexane as prisms m.p. 185°.

Found:

C 61.45%

H 7.31%

2.19 Hydrogenation experiments on the dehydration products of the γ-dilactone (204)

a) Hydrogenation of Compound (206) m.p. 164°

The compound (122mg.) m.p. 164° in ethanol (50ml.) was hydrogenated over 10% palladium hydroxide-barium sulphate (250mg.).

Filtration and removal of the solvent gave a product (45mg.) shown by TLC to consist of one compound with an identical R_f value to that of the dilactone of marrubiin (207a). Crystallisation from benzene-hexane afforded feathery needles (33mg.) (207a and 207b) m.p. 145°, $[\alpha]_D^{24}$ + 31.4° (C 0.5).

Found:

C 70.00%

H 8.27%

Calculated for $C_{17}H_{24}O_4$:

C 69.86%

H 8.21%

b) Hydrogenation of Compound m.p. 275°

The compound (16mg.) m.p. 275° in ethanol (20ml.) was hydrogenated over 10% palladium hydroxide-barium sulphate (30mg.) when 1.2 moles of hydrogen absorbed. Filtration and removal of the solvent afforded a gum (18mg.) which crystallised from benzene-hexane as needles (5mg.) m.p. 245°.

Found:

C 69.14%

H 7.50%

c) Attempted hydrogenation of compound m.p.185°

The compound (50mg.) m.p. 185°, 10% palladium hydroxide-barium sulphate (100mg.) and acetic acid (30ml.) were shaken under hydrogen for 2 hours. No uptake of hydrogen was observed.

2.20 The extraction of Leonotis leonitis

The plant material was collected in different areas around Grahamstown during November 1968 and March 1969. It was air dried in the shade for approximately 4 weeks and extracted. The following experiment is typical.

Dried material (5400g.) collected on the 13th March 1969, 6 miles from Grahamstown was steeped in acetone (90L) at room

temperature for 4 days, the acetone run off and the plant material washed with a further 50L of acetone. The combined acetone extracts were concentrated by flash distillation to approximately 5L and stirred with decolourising charcoal (B.D.H.; 150g. then 100g.) at room temperature for 4 hours. The solution was filtered through a celite pad and evaporated to a gum on a rotary evaporator. The residual gum was dissolved in ethyl alcohol (300ml.) and allowed to stand at room temperature for 4 days whereupon crystallisation took place. The crystals were filtered off, washed with a little cold ethyl alcohol and dried in a vacuum dessicator. (Yield 16.2g., 0.3%) TLC in ethyl acetate-hexane (7:3) showed that this material consisted almost entirely of one substance (Leonitin) (210).

These crystals were dissolved in dry chloroform (500ml.) and boiled with decolourising charcoal (15g.). The solution was filtered through a celite pad and extracted with water (3 x 100ml.) to remove inorganic material. The combined water washings were washed with chloroform (3 x 50ml.) and the combined chloroform solutions were dried (Na_2SO_4) and evaporated to a gum which was dissolved in refluxing ethyl acetate (400ml.). The resulting crystals were filtered off, washed with a little cold ethyl acetate and dried in a vacuum dessicator (Yield 10.0g.) m.p. 241-243° $[\alpha]_D$ 0° (C 1.0).

The filtrate was concentrated to approximately 100ml., and allowed to stand at room temperature for 24 hours whereupon a further crop of crystals (4.8g.) m.p. 241-243° settled out.

Found:

C 65.43%

H 7.44%

M.W. (Mass spectrum) 406

Calculated for $C_{22}H_{30}O_7$:

C 65.01%

H 7.44%

3.21 Attempted acetylation of leonitin (210)

A solution of leonitin (50mg.) in freshly redistilled acetic anhydride (1.5ml.) and dry pyridine (0.1ml.) was refluxed on a glycerine bath for 12 hours. The solvents were removed under reduced pressure, methyl alcohol (5ml.) added and the solution allowed to stand at room temperature for one hour.

Removal of methanol, addition of dry benzene and subsequent azeotropic distillation to remove traces of pyridine yielded a crystalline residue which crystallised from methanol as needles (40mg.) m.p. 241° undepressed on admixture with starting material.

2.22 Attempted dehydration of leonitin (210)

To a solution of leonitin (100mg.) in dry pyridine (3ml.) was added freshly redistilled phosphorus trichloride (0.5ml.) in dry pyridine (1.0ml.). The solution was refluxed for two hours and poured into ice-cold water (50ml.). The pyridine was removed under reduced pressure and the solution extracted with chloroform (3 x 25ml.). The combined chloroform extracts were washed successively with cold 5% hydrochloric acid (2 x 20ml.), water (2 x 20ml.), sodium bicarbonate solution (2 x 20ml.), water (2 x 20ml.) and dried (Na_2SO_4). Removal of the solvent afforded a gum (96mg.) which crystallised from methanol as needles (91mg.) m.p. 238°, raised on recrystallization from the same solvent to 241°, undepressed on admixture with starting material.

2.23 Attempted dehydrogenation of Leonitin (210)

Leonitin (2.00g.) was intimately mixed with 10% palladised charcoal (2.00g.) in a 25ml. flask fitted with an air condensor 30cm. in length. The apparatus was flushed with pure nitrogen and

then heated at 300-320° for three hours when approximately 260ml. of gas was evolved, chiefly during the first 1½ hours. The reaction product was extracted with boiling hexane (10 x 20ml.), filtered through a celite pad and dried (Na_2SO_4). The filtered solution was evaporated to about 30ml., poured onto a column (2cm. diameter) of neutral alumina (35g.) and eluted with dry hexane. The first 300ml. were collected (50ml. eluates). In each case, removal of the solvent yielded gums which failed to form crystalline 1, 3, 5-trinitrobenzene or 1, 3, 5-trinitrotoluene adducts, oils resulting in each case.

2.24 Quantitative alkaline hydrolysis of leonitin and isolation of acetic acid

Leonitin (133.1mg.) was refluxed with an ethanolic solution of potassium hydroxide (10ml.) of known normality for 16 hours. The hot solution was neutralised with hydrochloric acid, of known normality, using phenolthalein. Results are tabulated below.

Weight of leonitin	133.1mg.
Normality of hydrochloric acid	0.0870N
Normality of potassium hydroxide	0.2022N
Volume of hydrochloric acid required for back titration	12.20ml.
Volume of potassium hydroxide	10.00ml.
Volume of acid in blank	22.90ml.
Moles of alkali consumed/mole of compound	2.85

The above solution was made alkaline with a few drops of potassium hydroxide (0.2022N), water (60ml.) added and the alcohol removed under reduced pressure. The remaining solution was acidified with 5N hydrochloric acid (2ml.) and distilled using a glycerine bath. The distillate, neutralised with 1.3ml. 0.2022N KOH, was evaporated to a small volume, transferred to a 5ml. flask, and acidified with one drop of 0.1N hydrochloric acid,

p-Bromophenacyl bromide (57mg.) in ethyl alcohol (2ml.) was added and the solution refluxed for 4 hours. The alcohol was removed, water (10ml.) added and the precipitate filtered off and dried in a vacuum dessicator. The residue (m.p. 71°-79°) was dissolved in benzene and passed through a short column of neutral alumina to yield a crystalline residue (27mg.) which crystallised from methyl alcohol-water to yield the p-bromophenacyl ester m.p. 83-84° undepressed on admixture with an authentic specimen of p-bromophenacyl acetate (m.p. 83-83°).

2.25 Saponification of Leonitin

A solution of Leonitin (248mg.) in ethanolic sodium hydroxide (35ml., 0.3N) was refluxed for 15 hours. Water (50ml.) was added, the alcohol removed under reduced pressure and the solution divided into 2 portions (A) and (B).

Solution (A) was acidified with 5N hydrochloric acid to a pH of 1-2, and heated on a water bath for one hour, extracted with chloroform (3 x 40ml.). The combined chloroform extracts were washed with water (2 x 25ml.) and dried (Na_2SO_4). Removal of the solvent yielded a gum (130mgm.) which crystallised from benzene-hexane as prisms to yield the , 86-dilactone (2ii) m.p. 227°, $[\alpha]_D^{24}$ + 39° (C 1.0). Recrystallisation from the same solvent raised the m.p. to 228-229°.

Solution (B) was acidified with 5N hydrochloric acid to a pH of 1-2 and extracted immediately with chloroform (3 x 40ml.). The combined chloroform extracts were washed with water and dried (Na_2SO_4). Removal of the solvent yielded a gum (132mg.) which crystallised from benzene-hexane as prisms m.p. 228°. TLC of these 2 compounds showed them to be identical.

Found:

C 65.8%

H 8.0%

Molecular weight (mass spectrum) 364

Calculated for $C_{20}H_{28}O_6$:

C 65.92%

H 7.7%

2.26 Acetylation of the γδ-dilactone (211)

A solution of compound (211) (100mg.) in freshly redistilled acetic anhydride (3ml.) and dry pyridine (0.2ml.) was refluxed on a glycerine bath for 12 hours. The solvents were removed under reduced pressure, methyl alcohol (5ml.) added and the solution allowed to stand at room temperature for 1 hour. Removal of methanol, addition of dry benzene and subsequent azeotropic distillation to remove traces of pyridine yielded a solid residue which crystallised from chloroform-hexane to yield the acetate (212) as rhombs (50mg.) m.p. 301° $[\alpha]_D^{22}$ + 26° (C 0.8).

Found:

C 64.51%

H 7.53%

Molecular weight (Mass spectrum) 406

Calculated for $C_{22}H_{30}O_7$:

C 65.01%

H 7.44%

2.27 Oxidation of the γδ-dilactone (211)

a) 8N CrO/H_2SO_4 (Jones oxidation)

b) CrO_3/pyridine (Sarretts oxidation)

a) A solution of the γδ-dilactone (211) (300mg.) in acetone (15ml.) (distilled over potassium permanganate) was cooled in ice and 8N CrO_3/H_2SO_4 (0.1ml.-1ml.) was added from a microburette until a persistant orange colour was obtained. (After 5 minutes the colour of the solution changed from orange to green). Water (60ml.) was added and the solution allowed to stand at 5°

for 2 hours. As no precipitate formed, the solution was extracted with chloroform (3 x 50ml.). The combined chloroform extracts were washed with water (2 x 40ml.), dried (Na_2SO_4) and evaporated to a gum (120mg.), shown by TLC to be a mixture of 2 compounds (R_f's 0.228, 0.93 respectively) one of which proved to be a starting material (R_f 0.228).

Attempts to isolate the second compound by column chromatography and preparative TLC, however failed to yield a crystalline compound.

b) CrO_3 (100mg.) was dissolved slowly, with stirring, in ice-cold pyridine (3ml.) to give a bright yellow complex. To this was added a solution of the 8β-dilactone (211) (100mg.) dissolved in a minimum of dry pyridine (2ml.). The mixture was stirred at 0° for 1 hour, left overnight at room temperature, the pyridine removed under reduced pressure, water (100ml.) added and the solution extracted with chloroform (5 x 50ml.). The combined chloroform extracts were washed successively with 5N hydrochloric acid (4 x 25ml.) water (4 x 25ml.), sodium bicarbonate (3 x 25ml.), water (3 x 25ml.) and dried (Na_2SO_4). Removal of the solvent yielded a gum (95mg.) which crystallised from chloroform-hexane as needles (80mg.) to give the keto- 8β-dilactone (213), m.p. 124° $[\alpha]_D^{24}$ - 2°, (C 1.0).

Found:

C 52.79%, 52.48%, 52.89%

H 5.63%, 5.68%, 5.68%

Molecular weight (Mass spectrum) 362

Calculated for $C_{20}H_{26}O_6$:

C 66.29%

H 7.18%

2.28 *Attempted preparation of 2, 4-dinitrophenylhydrazone of the keto- δ-dilactone (213)*

A solution of 2, 4-dinitrophenylhydrazine (20mg.) in hot ethyl alcohol (0.5ml.) was added to a solution of the oxidation product (213) (20mg.) in ethyl alcohol (0.5ml.). One drop of concentrated hydrochloric acid was added, the solution was refluxed for 1 hour, allowed to stand at room temperature and finally in the refrigerator. No crystallisation took place.

2.29 *Lithium Aluminium hydride reduction of leonitin*

A solution of leonitin (210) (1.5g.) in 80ml. of tetrahydrofuran (dried over sodium and redistilled over lithium aluminium hydride) was refluxed with lithium aluminium hydride (3.0g) on a glycerine bath. After 6 hours, a further quantity of lithium aluminium hydride (1.0g) was added and the mixture refluxed for a further 18 hours. The solution was cooled in ice, the excess hydride decomposed by adding wet ether (100ml.), followed by dilute sulphuric acid (100ml.) and the layers separated. The aqueous layer was extracted continuously with ether for 24 hours. The combined ethereal extracts became cloudy and was allowed to stand in the refrigerator overnight. The resulting white precipitate was filtered off and crystallised from ethyl acetate to yield the alcohol (216) as prisms (800mg.) m.p. 156-7°. Recrystallisation from the same solvent raised the m.p. to 158°, $[\alpha]_D^{24}$ - 2° (C 1.0 in ethanol).

Found:

C 64.00%

H 10.00%

Calculated for $C_{20}H_{36}O_6$:

C 64.49%

H 9.74%

2.30 *Tosylation of lithium aluminium hydride reduction product of compound X*

An ice-cold solution of p-toluenesulphonyl chloride (1.6g.) in dry pyridine (2ml.) was added to the lithium aluminium hydride reduction product (214) of compound X (39) (300mg.) in pyridine (3ml.) at 0°C and the mixture kept at 0°C for 4 days. The solution was poured into ice-water and the oily precipitate extracted with ether (3 x 30ml.). The combined ethereal extracts were washed successively with cold 5% hydrochloric acid (2 x 10ml.), water (2 x 10ml.), sodium bicarbonate solution (2 x 10ml.), water (2 x 10ml.) and dried (Na_2SO_4). Removal of the solvent yielded a gum (310mg.) shown by TLC to be a single compound (R_f 0.72) differing from starting material (R_f 0.50) and giving a negative test for the presence of sulphur.

Column chromatography of this gum over neutral alumina (12g.) in benzene afforded in the early fractions a compound (R_f 0.72) which deposited as needles on removal of the solvent. Attempts to recrystallise this compound, however, proved unsucessful and it was sublimed at 110-140°/0.05mm. to yield (215) as needles (35mg.) m.p. 115°.

Found:

C 74.5%

H 9.68%

Calculated for $C_{20}H_{32}O_3$:

C 75.00%

H 10.00%

2.31 Tosylation of the alcohol (216)

An ice-cold solution of p-toluenesulphonyl chloride (5.0g.) in dry pyridine (10ml.) was added to the alcohol (216) (1.0g.) in pyridine (9ml.) at 0°, and the mixture kept at 0° for 8 days, care being taken to see that no moisture entered the flask. The solution was poured into ice-water and the oily precipitate extracted with

ether (4 x 50ml.). The combined ethereal extracts were washed successively with 5% hydrochloric acid (2 x 30ml.), water (2 x 30ml.), sodium bicarbonate solution (2 x 30ml.), water (2 x 30ml.) and dried (Na_2SO_4). Removal of the solvent yielded a gum (0.994g.) shown by TLC to consist predominately of 2 components (R_f 0.80 and 0.68) differing from starting material and giving a positive test for the presence of sulphur. Repeated column chromatography in benzene over neutral alumina yielded the one component (R_f 0.80) as an oil which failed to crystallise. Attempts to isolate the second compound however failed.

2.32 Lithium aluminium hydride reduction of the tosylate of the alcohol (216)

A solution of the crude tosylate (0.88g.) in 100 ml. tetrahydrofuran (dried over sodium and redistilled over lithium aluminium hydride) was refluxed with lithium aluminium hydride (2.0g.) on a glycerine bath. After 6 hours a further quantity of lithium aluminium hydride (0.75g.) was added and the mixture refluxed for a further 18 hours. The solution was cooled in ice, the excess hydride decomposed by adding wet ether (100ml.), followed by dilute sulphuric acid (100ml.). The aqueous layer was separated, the tetrahydrofuran removed on a rotary evaporator, and extracted with ether (3 x 50ml.). The combined ethereal extracts were washed successively with water (2 x 50ml.), sodium bicarbonate solution (2 x 50ml.), water (2 x 50ml.) and dried (Na_2SO_4). Removal of the solvent yielded a gum (0.50g.) giving a negative test for the presence of sulphur, shown by TLC to be a mixture of at least 6 components, one of which had an identical R_f value to that of compound (215). These components were eluted with benzene-ethyl acetate on an alumina column (12g.) with virtually no separation from one another. Repeated preparative TLC (ethyl acetate-hexane (1:1)) also failed to achieve separation. The gum was distilled at

110-114°/0.05mm., to yield a colourless oil which failed to crystallise, and shown by TLC to be a mixture.

2.33 Controlled hydrolysis of leonitin (210)

A variety of reagents were used in an attempt to hydrolyse the ester function without attacking the lactone rings. These included potassium hydroxide, potassium carbonate, methanolic hydrochloric acid and sodium borohydride.

In each case solutions of leonitin (210) (50mg. ≡ 0.123mm.) in ethanol (40ml.) were treated with varying concentrations of the above reagents ranging from ½ mm.-16mm. per millimole of compound (see Table 4).

Table 4

Leonitin	KOH	$KHCO_3$	K_2CO_3	HCl	$NaBH_4$	Conc. per mm. of leonitin
50mg.	3.5mg.	6mg.	8.5mg.	2.25mg.	2.3mg.	½
50mg.	7.0mg.	12mg.	17mg.	4.5mg.	4.6mg.	1
50mg.	14.0mg.	24mg.	34mg.	9mg.	9.2mg.	2
50mg.	21.0mg.	48mg.	68mg.	18mg.	18.4mg.	4
50mg.	28.0mg.	96mg.	136mg.	36mg.	36.8mg.	8
50mg.	56.0mg.	192mg.	272mg.	72mg.	73.6mg.	16

The solutions were shaken for 48 hours and the reaction followed by TLC. No reaction took place at room temperature. Gentle heating of the solutions resulted only in the formation of the γδ-dilactone (211) and starting material (210).

2.34 Sodium methoxide hydrolysis of leonitin

Leonitin (210) (600mg.≡ 1.48mm.) was added to a solution of sodium (340mg. ≡ 14.8mm.) in dry methanol (10ml.) and the mixture refluxed for two hours. The solution was then cooled, water (25ml.) added and after acidifying with hydrochloric acid the methanol was removed under reduced pressure. The solution was extracted with chloroform (3 x 25ml.), and the combined chloroform

extracts were washed with water (3 x 25ml.) and dried (Na_2SO_4).

Removal of the solvent yielded a gum which was shown by TLC to consist predominately of the 18-dilactone (211), together with slight traces of three other components.

3. DISCUSSION

3.1 8-Hydroxymarrubiin

Elemental analysis of 8-hydroxymarrubiin, (192) m.p. 176°, $[\alpha]_D^{17}$ + 33°, agrees with the molecular formula, $C_{20}H_{28}O_5$, and is consistent with the mass spectral parent peak at m/e 348.

The IR spectrum (KBr) displayed a band at 3620cm^{-1} due to a free hydroxyl group(s). Bands at 1770 and 875cm^{-1} suggested the presence of a γ-lactone and a furan ring respectively. The presence of a β-substituted furan moiety is supported by positive Ehrlich and Lieberman-Burchard tests and more specifically by the NMR spectrum (Fig.4) which displayed a doublet (1H) at τ3.76 (J = 1c/s) due to a β-substituted proton and one proton multiplets at τ2.80 and τ2.67 due to the α-substituted protons of the furan ring. Furthermore. the UV spectrum showed a maximum at 213 mμ (ε 5,100) similar to that found in related furan derivatives[1].

The NMR spectrum closely resembled that of marrubiin (Table 5, Fig.3) and showed bands at:

A A singlet (3H) at τ8.71 attributed to the tertiary methyl group at C_{20}.

B A singlet (3H) at τ8.61 attributed to a tertiary methyl group at C_{18}.

C A singlet (3H) at τ8.91 attributed to a tertiary methyl group at C_{17}.

D A poorly resolved triplet (1H) at τ5.23 attributed to the 6α-hydrogen.

E A multiple (1H) at τ3.76 attributed to a β-hydrogen (at C_{14}) on a furan ring.

F A multiplet (1H) at τ2.76 attributed to an α-hydrogen (at C_{16}) on a furan ring.

G A multiplet (1H) at τ2.64 attributed to an α-hydrogen (at C_{13}) on a furan ring.

Table 5

Comparison of the NMR spectra (100Mc/s) in $CDCl_3$ of marrubiin and 8-hydroxymarrubiin

GROUP	MARRUBIIN	8-HYDROXY-MARRUBIIN
20-Me	τ8.96 (s)	τ8.71(s)
18-Me	8.72 (s)	8.61(s)
17-Me	9.04(d)(J= 6.5c/s)	8.91(s)
6-H	5.28(t)(W= 10c/s)	5.23(t)(W= 10c/s)
14-H	3.72(d)(J= 1c/s)	3.76(d)(J= 1c/s)
15-H	2.64(m)	2.64(m)
16-H	2.76(m)	2.76(m)

s = singlet; d = doublet; t = triplet; m = multiplet.

It may be observed from Table 1 that the C_{17}-methyl singlet at τ8.91 in 8-hydroxymarrubiin appears as a doublet at τ9.04 (J=6.5c/s) in marrubiin. Accordingly it is likely that the extra oxygen atom in 8-hydroxymarrubiin occurs as a hydroxyl group at C_8.

Furthermore, the signal at τ5.23 arises from an equatorial proton at C_6 having one equatorial-equatorial and two axial-equatorial spin-spin couplings[11] indicating the 19,6 β-stereochemistry for the A/B lactone as in marrubiin. The oxygen function at C_6 is therefore β-orientated.

The mass spectrum of 8-hydroxymarrubiin besides confirming the molecular formula (m/e 348), showed significant peaks at m/e 81, 95, 109 and 179. The base peak (m/e 81) arises from cleavage of the bond β to the furan ring (i.e. cleavage between C_{11} and C_{12}). The significant peak (m/e 95) 14 mass units higher indicates that the cleavage of the C_9-C_{11} bond becomes important as would be expected from the presence of the C_9-hydroxyl group. Some of the possible fragmentation mechanisms are depicted in scheme 1.

Dehydrogenation of 8-hydroxymarrubiin over Pd-C catalyst

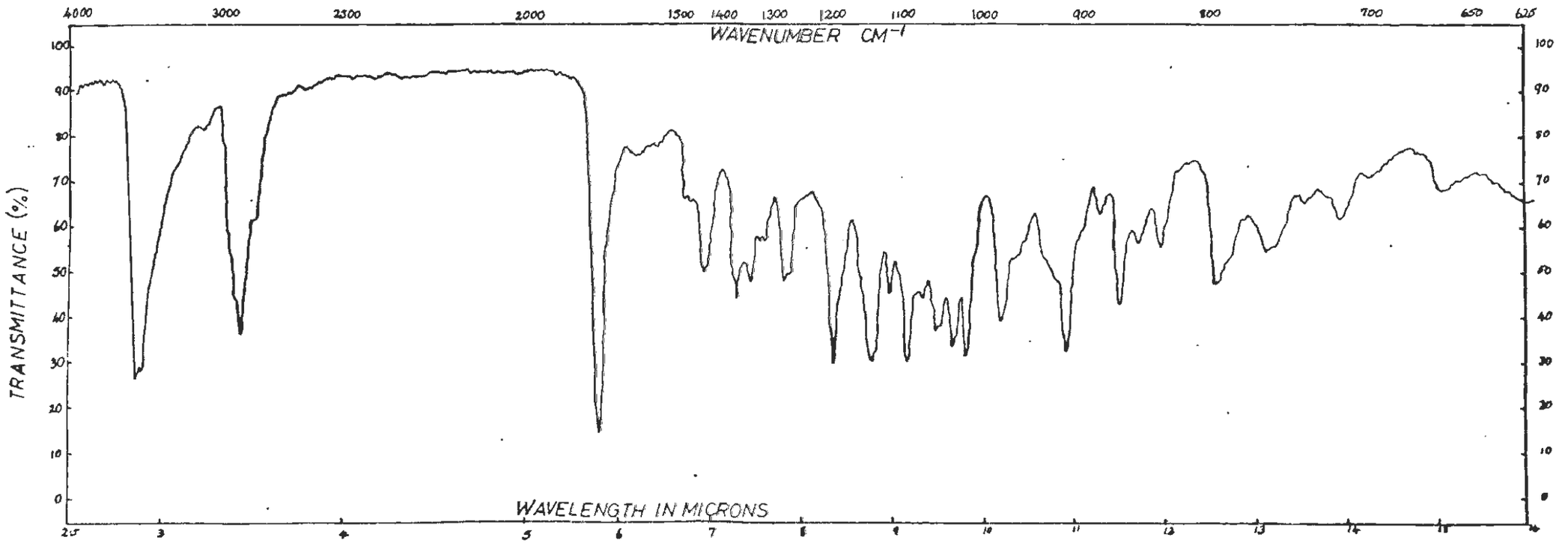


FIG. 1 INFRARED SPECTRUM OF 8 – HYDROXYMARRUBIIN (KBr)

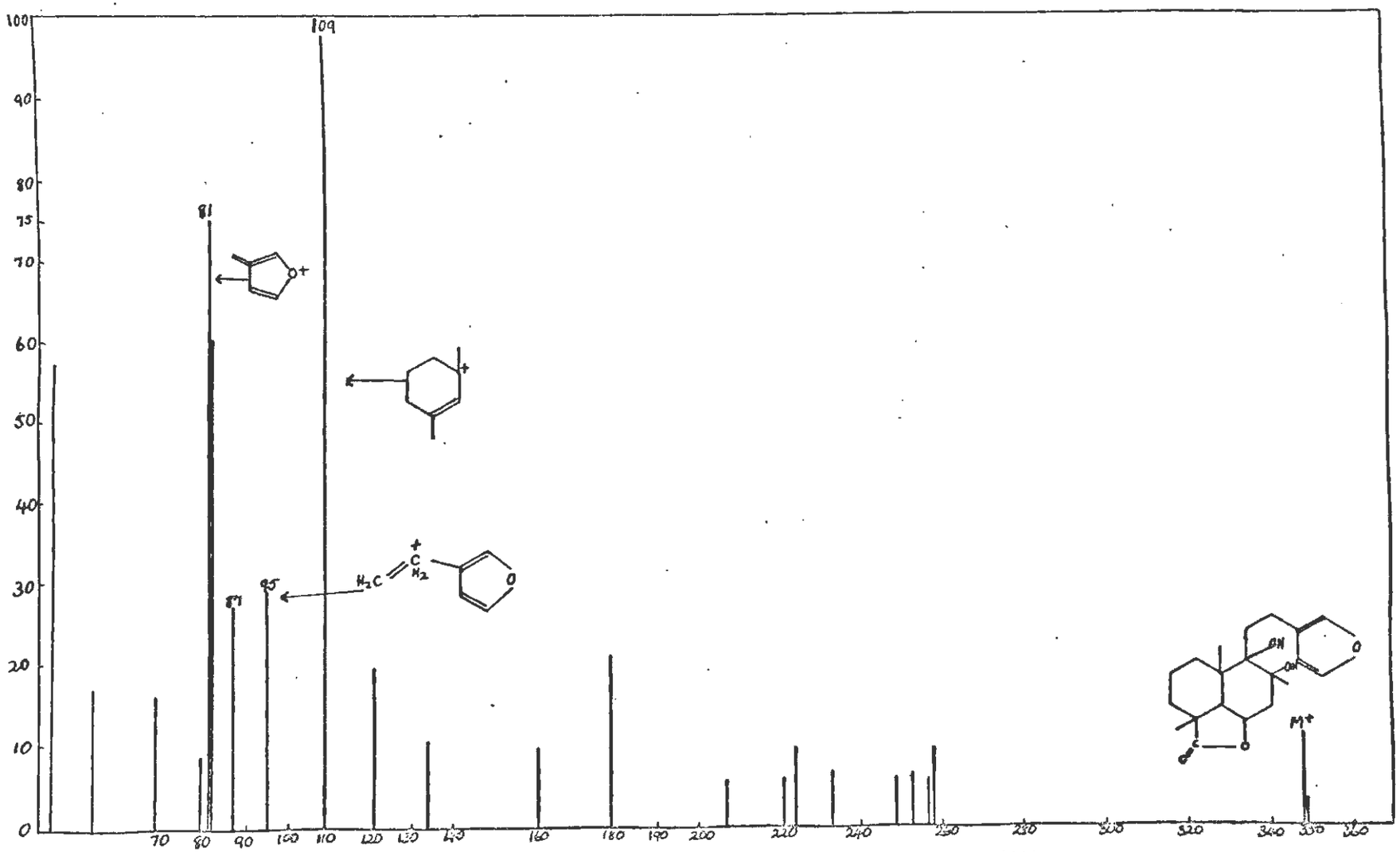
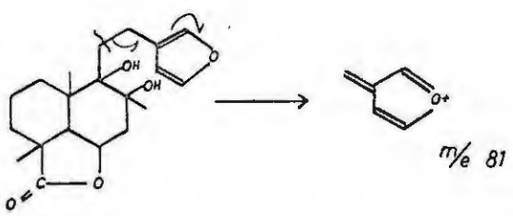


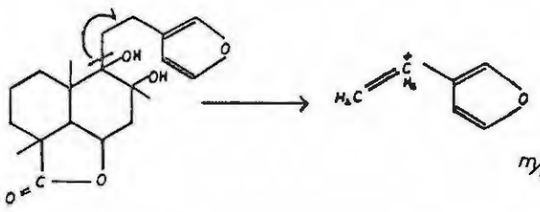
FIG. 2 MASS SPECTRUM OF 8-HYDROXYMARRUBIIN

Scheme 1

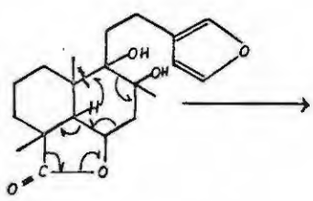
Possible fragmentation mechanism for the mass spectrum of 8-hydroxymarrubiin



m/e 81

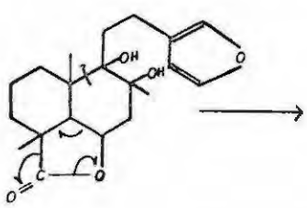


m/e 95



m/e 109

OR



m/e 109

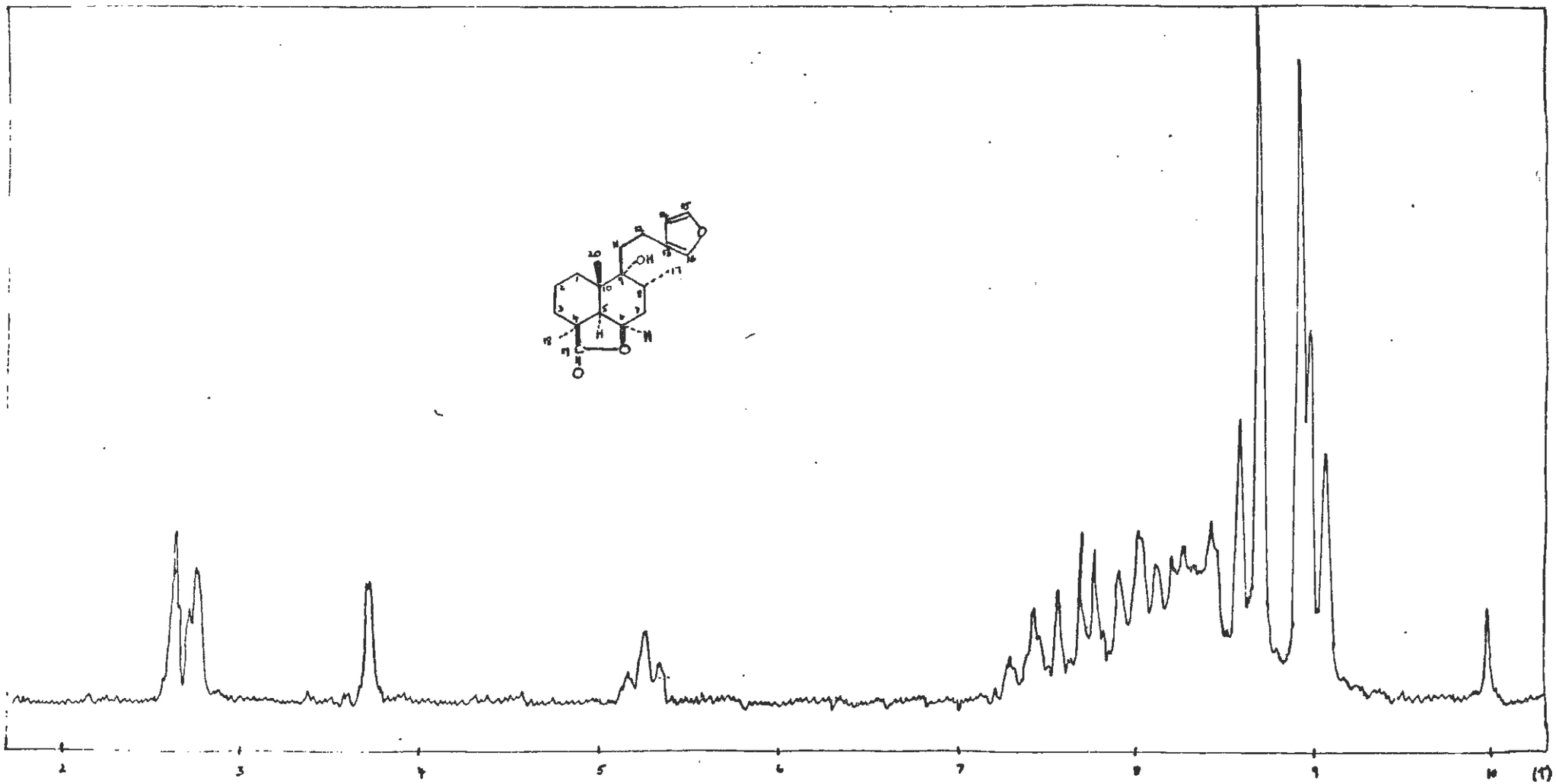


FIG 3. N M R SPECTRUM OF MARRUBIIN.

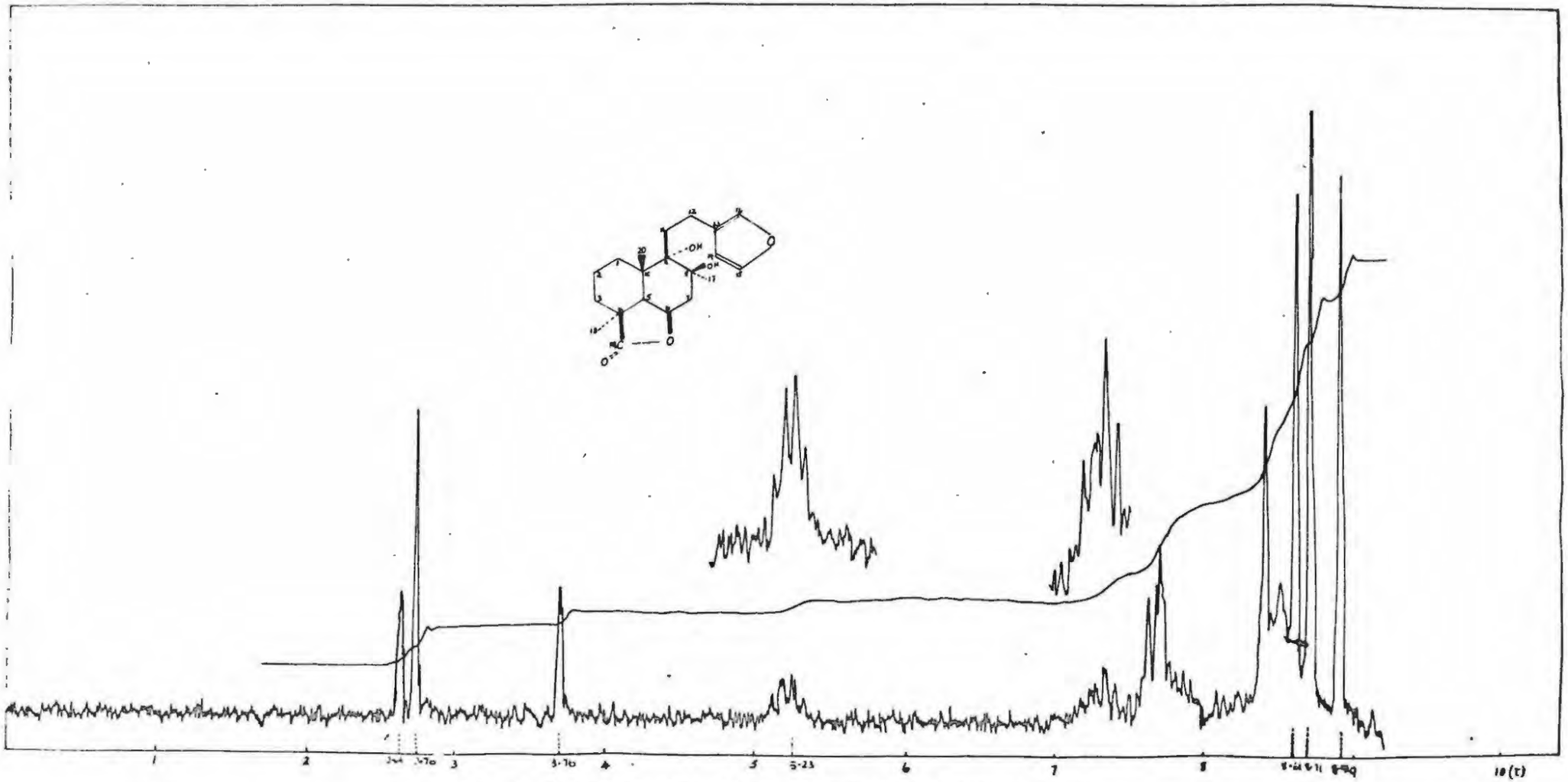


FIG. 4. NMR SPECTRUM OF 8-HYDROXY MARRUBIIN

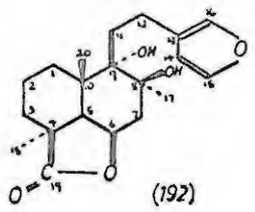
failed to give 1, 2, 5-trimethylnapthalene which is readily obtained from marrubiin.[24]

8-Hydroxymarrubiin failed to react with boiling acetic anhydride-pyridine which is consistent with the hydroxyl groups being tertiary. The presence of such hydroxyl groups is supported by a positive test with chromic acid (Table 1b).

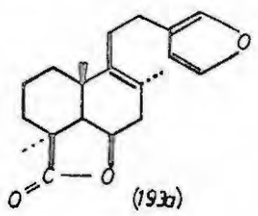
Hydrogenation of 8-hydroxymarrubiin over 10% palladium hydroxide-barium sulphate in ethanol resulted in the absorption of 2.06 moles of hydrogen indicating that there was no unsaturation at any other centre of the molecule besides the furan ring. The gum obtained was shown by TLC to be a single component. Since it could not be induced to crytallise the gum is probably an epimeric mixture as it afforded after reaction with phosphorus trichloride in refluxing pyridine a complex mixture which could not be separated. Had it been possible to isolate a single product after dehydration it was intended to relate 8-hydroxymarrubiin to the hydrogenated product (193b) of anhydro-marrubiin (193a).

Saponification of 8-hydroxymarrubiin in 0.4N ethanolic sodium hydroxide afforded 8-hydroxymarrubic acid (194), m.p. 93-95°, ν_{max} ($CHCl_3$) 3620(OH), 1770 (γ-lactone) and 875cm^{-1} (furan). Reduction of 8-hydroxymarrubiin with lithium aluminium hydride gave 8-hydroxymarrubenol (194), ν_{max} ($CHCl_3$) 3640 (free hydroxyl) and 870cm^{-1} (furan). On treating (195) with p-toluenesulphonyl chloride in pyridine at 0°, the expected ether (196) corresponding to the ether from marrubiin did not result. Instead a monotosylate, $C_{27}H_{38}O_7S$, was formed.

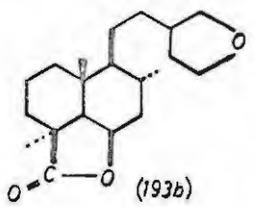
Dehydration of (192) with phosphorus trichloride in benzene yielded starting material. However, dehydration with phosphorus trichloride in pyridine yielded the epoxide (197), m.p. 114°, ν_{max} ($CHCl_3$) 1760 (γ-lactone) and 875cm^{-1} (furan), and an oily gum. Lithium aluminium hydride reduction of the epoxide (197) gave



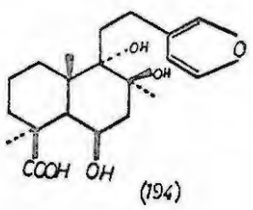
(192)



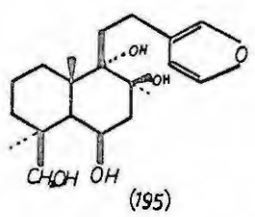
(193a)



(193b)



(194)



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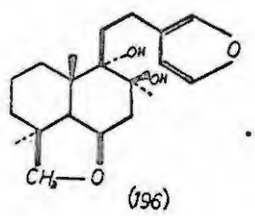
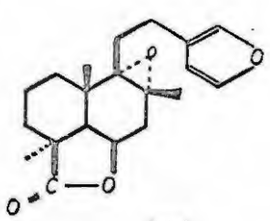
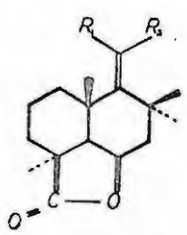


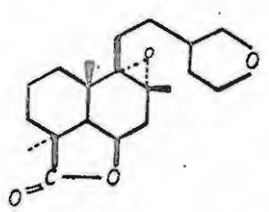
(196)



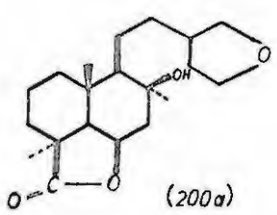
(197)



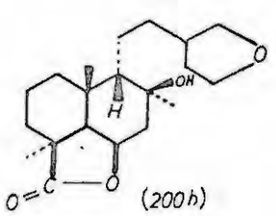
	R	R_2
(198a)	H	CH_2Fu
(198b)	CH_2Fu	H



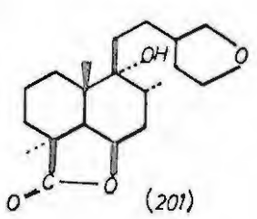
(199)



(200a)



(200b)



(201)

marrubenol (202) identical (m.p. and mixed m.p., IR, NMR and $[\alpha]_D$) with authentic marrubenol[26], thus establishing the structure and stereochemistry of 8-hydroxymarrubiin at all centres excepting C_8.

TLC of the oily gum (from dehydration of 8-hydroxymarrubiin) showed that it consisted of two almost superimposable components for which structures (198a) and (198b) are proposed. Hydrogenation of this mixture afforded two products (TLC), presumably (200a) and (200b) neither of which corresponded to tetrahydromarrubiin (201).

Chromic acid oxidation of 8-hydroxymarrubiin yielded the γ-dilactone (204), ν_{max} 3620 (free hydroxyl) and 1770cm^{-1} (γ-lactone) together with the $\gamma\delta$-dilactone (205), ν_{max} 3630 (free hydroxyl), 1765 (γ-lactone) and 1740cm^{-1} (δ-lactone) and a compound, m.p. 275°, ν_{max} 1770cm^{-1} (γ-lactone). Formation of the $\gamma\delta$-dilactone (205) is consistent with structure (192) for 8-hydroxymarrubiin.

On refluxing with phosphorus trichloride in pyridine the γ-dilactone (204) afforded a mixture of three components (TLC), which were separated by chromatography on alumina. The more polar component, m.p. 164°, corresponded to (206), ν_{max} ($CHCl_3$) 1770 (γ-lactone) and 885cm^{-1} ($H_2C = CR_1R_2$). This structure is supported by the NMR spectrum. The methyl singlet due to the C_{17}-methyl group has disappeared and has been replaced by singlets at τ4.88 and τ4.97 due to the vinylidine group. Compounds, m.p. 275°, ν_{max}($CHCl_3$) 1770cm^{-1} (γ-lactone) and m.p. 185°, ν_{max} 3520 (free hydroxyl) and 1770cm^{-1} (γ-lactone) were also isolated from the mixture. It may be noted that the compound, m.p. 275°, was obtained by two routes (scheme 2).

Hydrogenation of compound m.p. 275°, over 10% palladium hydroxide-barium sulphate catalyst gave a product, m.p. 245°. The fact that the dilactone of marrubiin (207a) or its epimer (207b) was not produced eliminated the possibility of the structure being (203).[12]

Compound, m.p. 185°, was not hydrogenated over 10% palladium hydroxide-barium sulphate.

Catalytic hydrogenation of (206) afforded a product, m.p. 145°, ν_{max} 1770cm^{-1} (γ-lactone), identical by TLC with the dilactone (207a) of marrubiin. Their NMR (Table 6) and IR spectra were virtually identical indicating that the product, m.p. 145°, was a mixture of the C_8-epimers (207a) and (207b). Furthermore, on admixture with the dilactone (207a), m.p. 161-162°, it still melted at 145° and $[\alpha]_D$ values were in close agreement.

Table 6

Comparison of the NMR spectra (100Mc/s) in $CDCl_3$ of the dilactone (207a) of marrubiin and the product m.p. 145°

GROUP	DILACTONE (207a) OF MARRUBIIN	PRODUCT m.p. 145
20-Me	8.95(s)	8.96(s)
18-Me	8.73(s)	8.73(s), 8.76(s)
17-Me	9.11(d, J= 6 c/s)	9.11(d, J= 6c/s), 9.13(d, J= 4c/s)
6-H	5.28(t, W= 12.6c/s)	5.29(t, W= 12.6c/s)

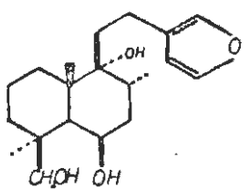
s = singlet; d=doublet.

An analogous case has been described by Adinolfi and Mangoni[12] who obtained a mixture of the C_8-epimers (209a) and (209b) on hydrogenation of compound (208).

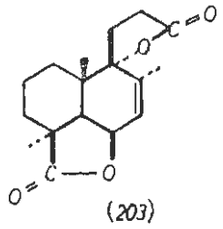
The isolation of marrubenol (202) by trans-diaxial opening of the epoxide ring proves that the epoxide is α and is in favour of a β-configuration for the C_8-hydroxyl group in 8-hydroxymarrubiin. The presence of a trans-diaxial gem-diol grouping is supported by the failure of 8-hydroxymarrubiin to react with either periodate or lead tetra-acetate. The observed reductive ring opening of the epoxide (197) to give exclusively the 9- α-ol, presumably by steric control, finds close analogy.[103]

The above evidence conslusively prove that the compound, m.p.

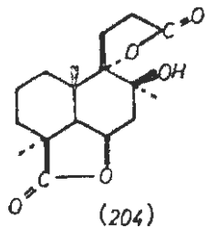
176°. from L. dysophylla is 8β-hydroxymarrubiin. Recently the same substance has been isolated by White et al[104] from L. nepetaefolia (From Trinidad). A sample kindly provided by Professor White was identical in all respects (mixed m.p., IR, NMR and mass spectra) with our own.



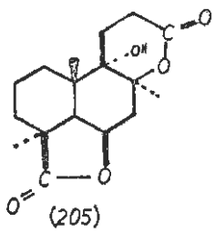
(202)



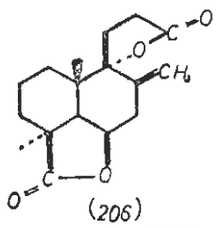
(203)



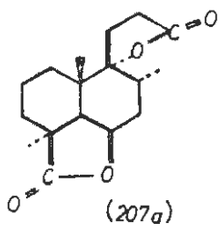
(204)



(205)

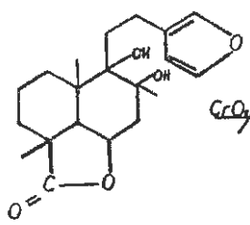


(206)



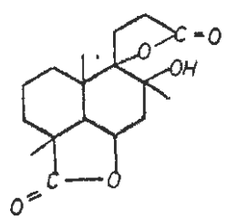
(207a)

Scheme 2

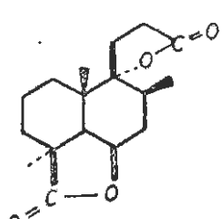


(192)

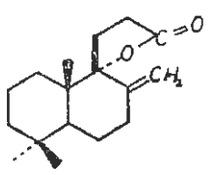
CrO_3/Pyridine, 0° → COMPOUND m.p. 275° ← Refluxing PCl_5/Pyridine



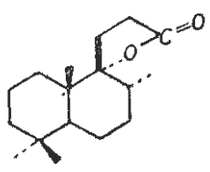
(204)



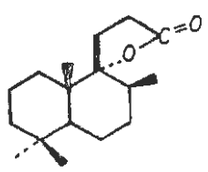
(207b)



(208)



(209a)



(209b)

3.2 Leonitin

Leonitin (210), m.p. 243°, $[\alpha]_D 0°$, isolated from Leonotis leonitis was shown by elemental analysis and mass spectroscopic molecular weight (M^+ 406) to have the molecular formula $C_{22}H_{30}O_7$.

The IR spectrum ($CHCl_3$) exhibited a strong band at 1770cm^{-1} (γ-lactone) with a shoulder at 1730cm^{-1} (ester carbonyl) but no bands at 875cm^{-1} due to a furan. Leonitin is transparent in the UV: the absence of absorption at 210-220 mμ confirms the absence of a β-furyl moiety. In addition it failed to absorb hydrogen on catalytic reduction and gave negative Ehrlich, Lieberman-Burchard and tetromethane reactions (Table 1).

The nature of six of the oxygen atoms was shown on alkali treatment when 2.85 equivalents of alkali were consumed indicating the presence of two γ-lactone groups and an ester function (NMR). As judged from its IR spectrum and the failure to form derivatives (it resists benzoylation, acetylation and dehydration) leonitin does not possess a hydroxyl group. In addition it does not react with any carbonyl reagents and therefore the remaining oxygen atom is probably present as an ether.

The NMR spectrum ($CDCl_3$) of leonitin having several features in common with compound X (39) (see Table 7) possed the following signals:

A A doublet (3H) at τ9.12 (J= 6c/s), attributed to a secondary methyl group at C_{17}.

B A singlet (3H) at τ8.73, attributed to a tertiary methyl group at C_{18}.

C A singlet (3H) at τ7.98, attributed to the C_{22}-methyl group of the ester function.

D A quartet (2H) at τ7.25, attributed to the C_{14}-methylene group.

E A quartet (2H) at τ5.83 (J= 9c/s) attributed to the C_{16}-methylene group.

F A quartet (2H) at τ5.77 (J=7c/s) attributed to the C_{20}-methylene group.

G A poorly resolved triplet (1H) at τ5.32, attributed to the C_6-equatorial (α) proton.

Table 7

Comparison of the NMR spectra (100Mc/s) in $CDCl_3$ of compound X and leonitin

GROUP	COMPOUND X	LEONITIN
17-Me	τ9.16(d)(J=6.5c/s)	τ9.12(d)(J=6c/s)
18-Me	8.72(s)	8.73(s)
22-Me	-	7.98(s)
14-CH_2	7.27(q)(J=17c/s)	7.25(q)(J=18c/s)
16-CH_2	5.81(q)(J=9c/s)	5.83(q)(J=9c/s)
20-CH_2	-	5.77(q)(J=7c/s)
6-H	5.32(t)	5.32(t)

s = singlet; d = doublet; t = triplet; q = quartet.

The singlet (3H) in compound X at τ8.96 is absent in leonitin and is replaced by a singlet (3H) at τ7.89 attributed to the methyl group of the acetoxy function. A pair of overlapping quartets (4H) at 5.83(J=9c/s) and 5.77(J=7c/s) in leonitin accounts for the C_{16}- and C_{20}-methylene groups respectively.

The NMR spectra of compound X (39), marrubiin (5) and leonitin (210) are almost identical with repsect to the 19, 6 β-lactone. The assignment of the 19, 6 -stereochemistry to the ring A/B lactone in leonitin follows from the NMR signal at τ5.32 arising from an equatorial (α) proton at C_6 having one equatorial-equatorial and two axial-equatorial spin-spin couplings as in marrubiin[11] and compound X[1,24]. The very large coupling constant (J=18c/s) for the C_{14}-methylene protons, virtually identical to compound X, is typical of geminal protons in a 5-membered ring next to a carbonyl group and that for the C_{16}- and C_{20}-methylene protons is normal for the type of system shown.[1,24] (See Table 7). Evidence for

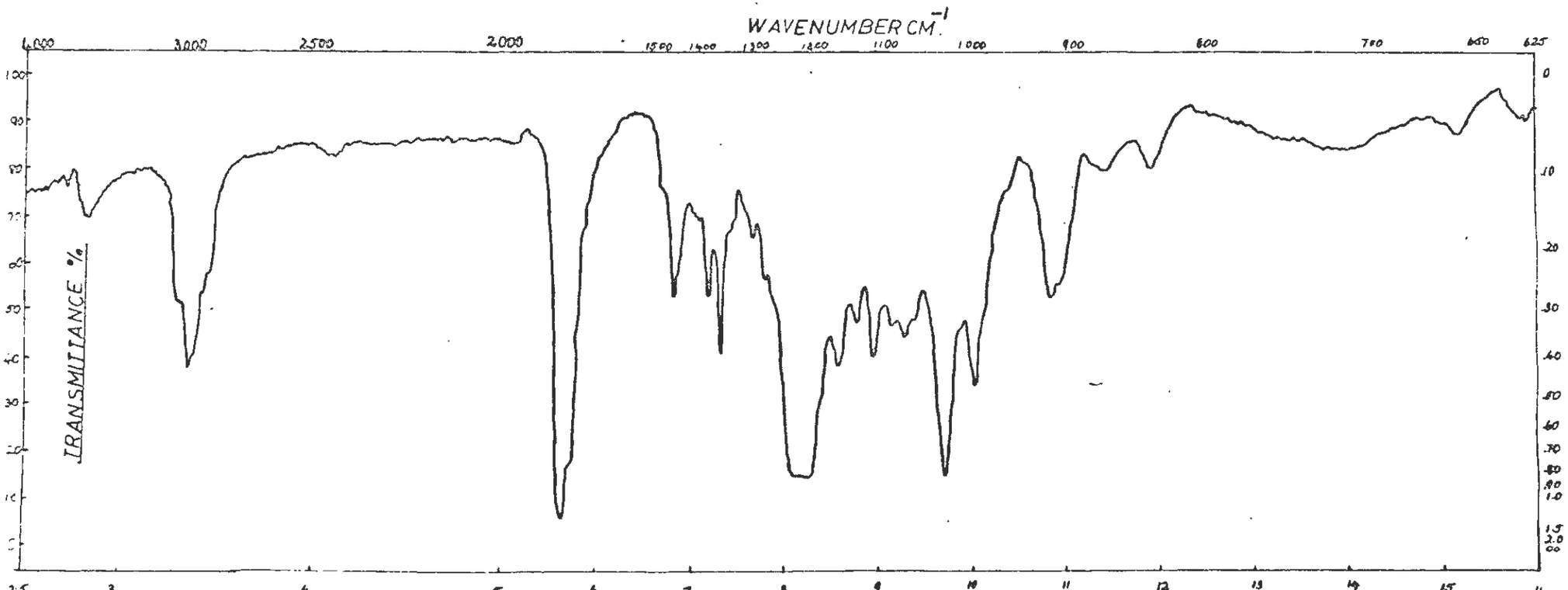


FIG. 5 INFRARED SPECTRUM OF LEONITIN ($CHCl_3$)

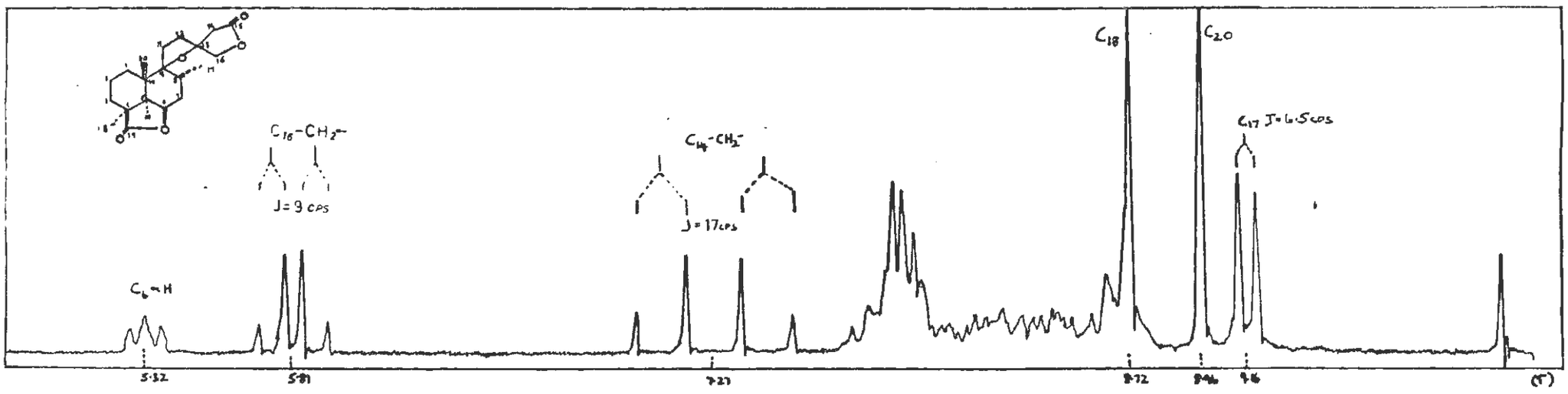


FIG. 6 N M R SPECTRUM OF COMPOUND X.

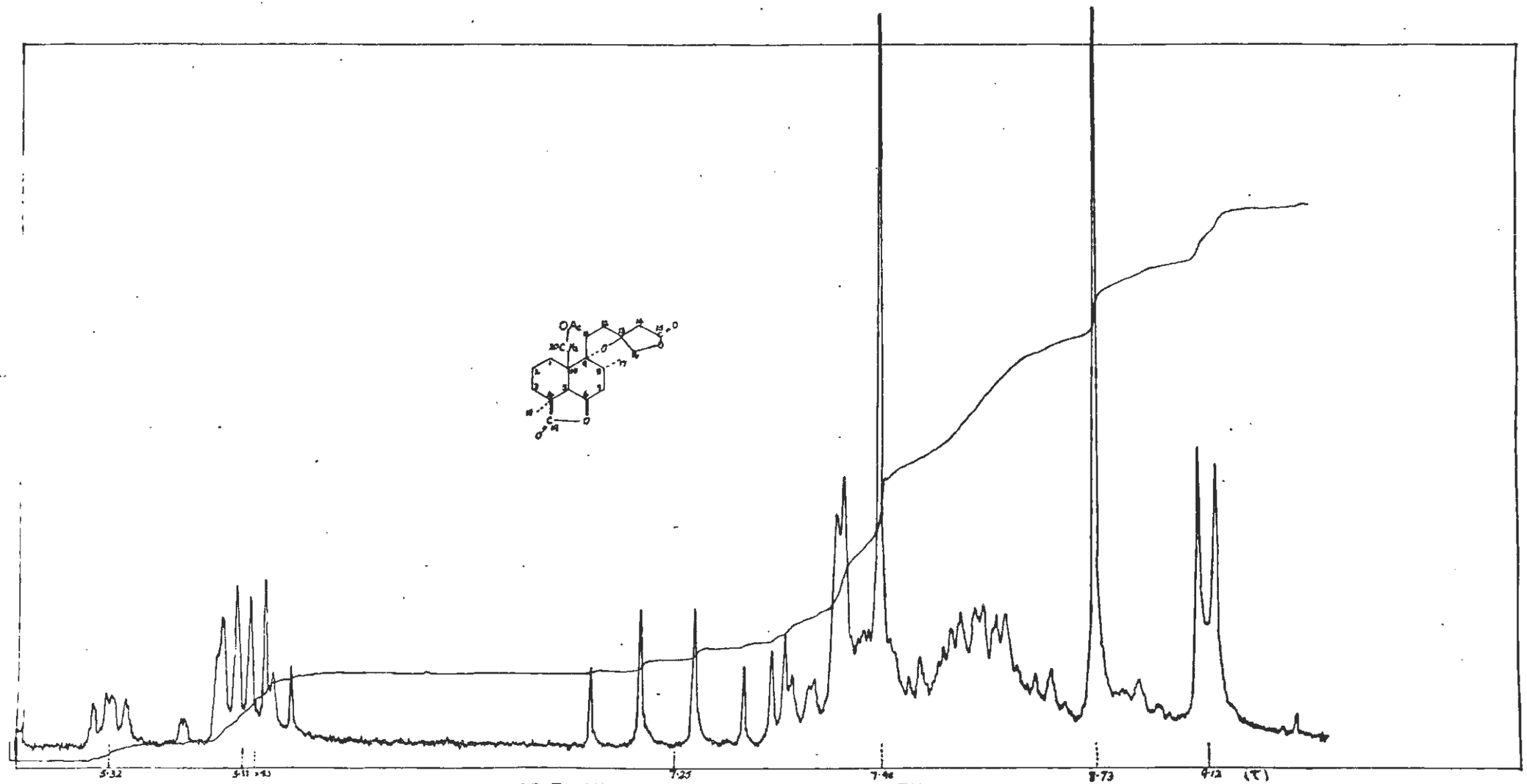


FIG. 7 NMR SPECTRUM OF LEONITIN

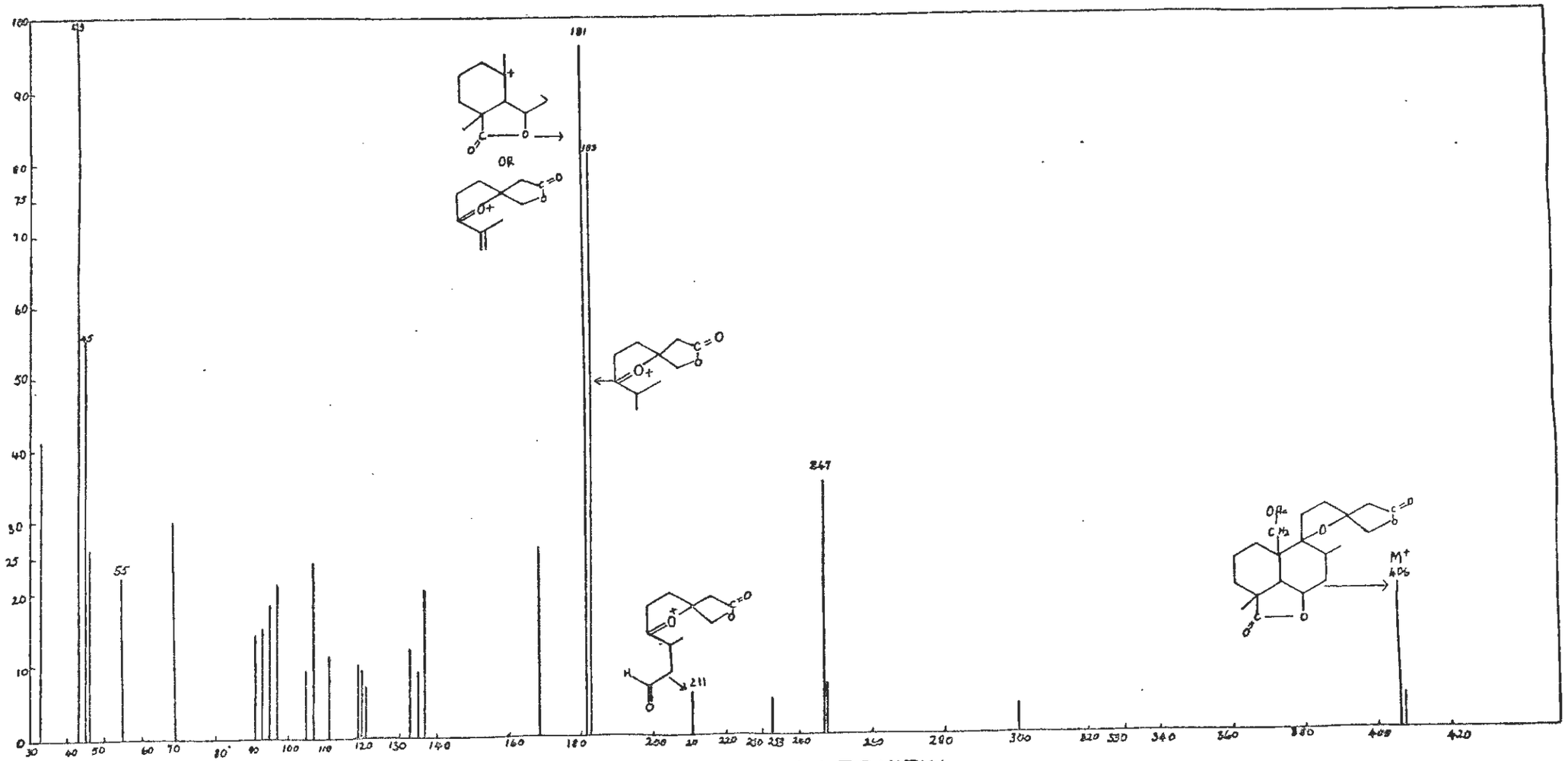
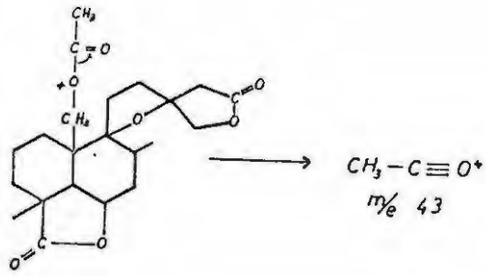
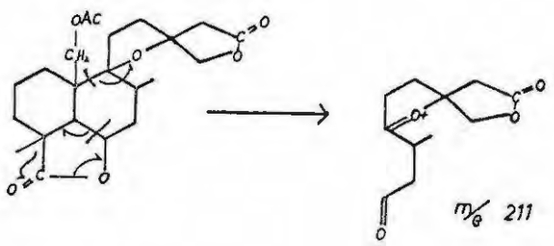


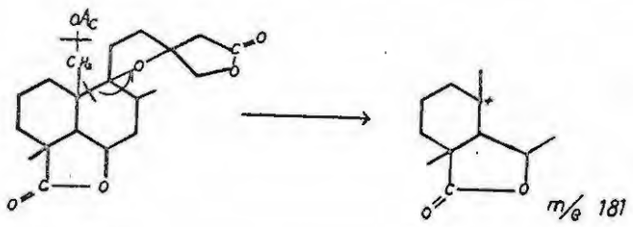
FIG 8. MASS SPECTRUM OF LEONITIN

Scheme 3

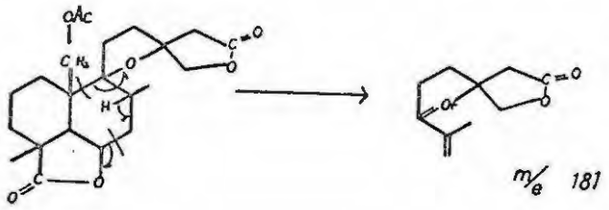
Possible fragmentation mechanism for the mass spectrum of leonitin

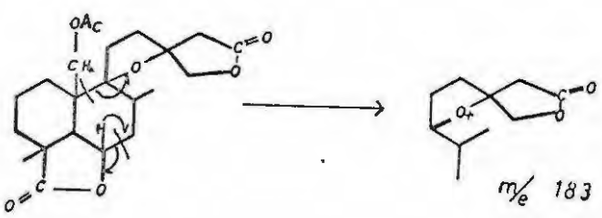






OR





the C_9-C_{13} oxide is supported by the lack of protons, other than those discussed, below τ 7.25, showing that neither ether terminus possesses a proton and also by the fact that the C_{14}- and C_{16}-methylene groups show no vicinal coupling. (It can be noted that this ether linkage is also present in compound X,[24] grindelic acid[105] and in the precursor of solidagenone.[35]).

The presence of a M-59 peak in the mass spectrum of leonitin as well as the isolation of acetic acid (as the p-bromophenacyl ester) on saponification supports the NMR evidence for the presence of an acetoxy function in leonitin. The mass spectrum (M^+ 406) of leonitin is consistent with the proposed structure (210). Peaks at m/e 211, 183, 181 (base peak) and 43 are accounted for in scheme 3.

The $\gamma\delta$-dilactone (211), $C_{20}H_{28}O_6$, (M^+ 364), obtained on saponification exhibited in its IR spectrum ($CHCl_3$) absorption bands at 3750, 1770 and 1720cm^{-1} attributed to a free hydroxyl group, a γ-lactone and a δ-lactone respectively. Relactonization of one of the acid groups formed on hydrolysis to reform the γ-lactone is not surprising as compound X[1,24] behaves in a similar manner. The formation of the δ-lactone and the appearance of a free hydroxyl group at C_6 is readily explained by the structure proposed.

Oxidation of (211) with Sarret reagent afforded the keto-dilactone (213) (M^+362), ν_{max} (KBr.) 1770 (γ-lactone) and 1735cm^{-1} (δ-lactone and carbonyl group).

The analytical results for (213), however, are anomalous and do not agree with the spectral evidence obtained. The compound (213) failed to react with hydroxylamine hydrochloride or 2, 4-dinitrophenylhydrazone. (Related 6-keto compounds eg. 6-oxocativic acid[105] are known to be unreactive due to steric hindrance). Absence of the signal (τ 5.36) due to the C_6-equatorial (α) proton in the NMR spectrum of (213) further lends support for the structure proposed.

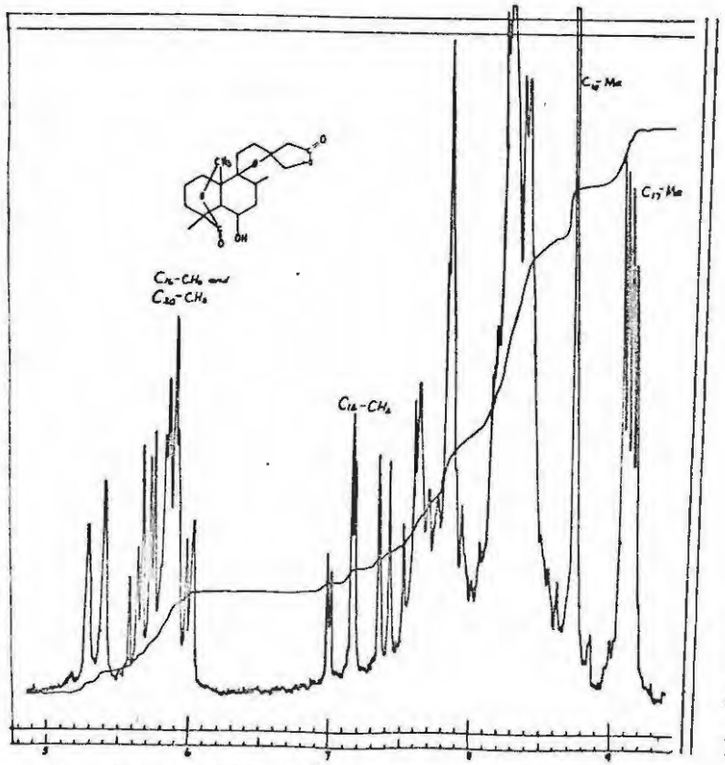


Fig 9a NMR SPECTRUM OF 8β-DILACTONE (211)

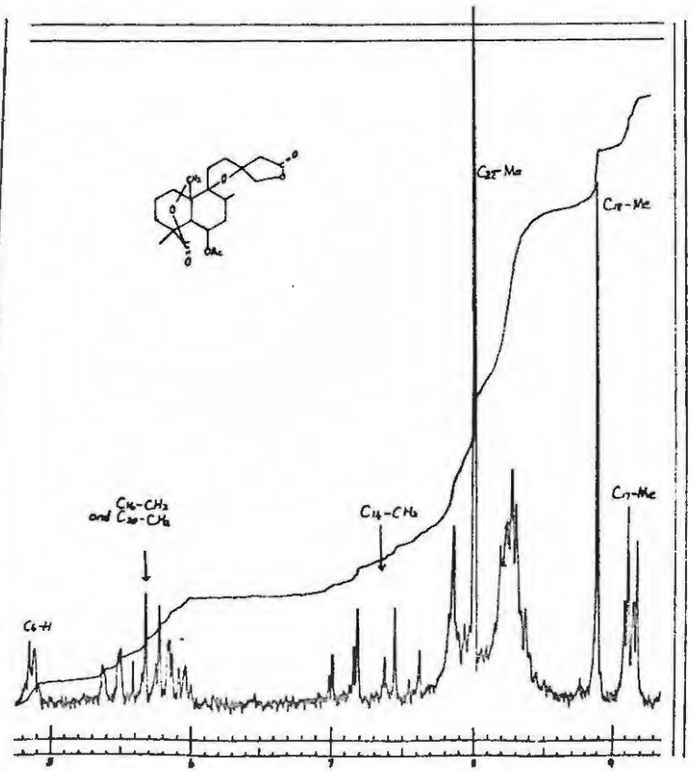


Fig 9b NMR SPECTRUM OF ACETOXY-DILACTONE (212)

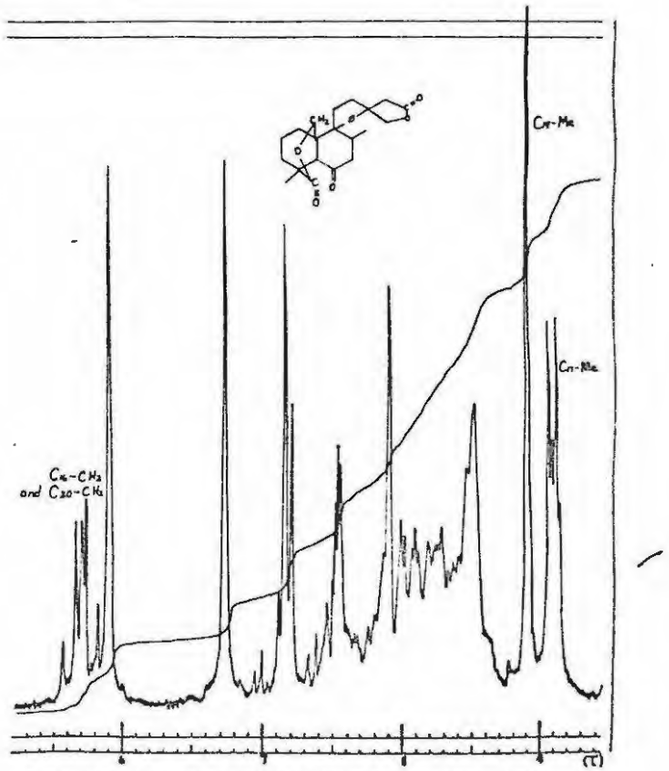


Fig 9c NMR SPECTRUM (in DMSO) OF KETO-DILACTONE (213)

Acetylation of (211) with acetic anhydride-pyridine afforded the acetoxy-dilactone (212), (M^+ 406), ν_{max}(KBr.) 1775 (γ-lactone) and 1730cm^{-1} (δ-lactone and ester). In the NMR spectrum the singlet (3H) at τ8.01 is attributed to the acetoxymethyl group. The NMR spectra of (211), (212) and (213) (see Table 8 and fig.9) displayed certain features in common.

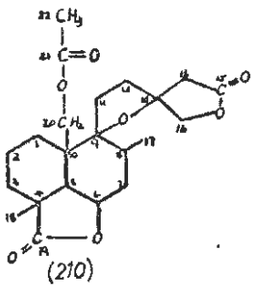
Table 8

Comparison of NMR spectra of $\gamma\delta$-dilactone (211), acetoxy-dilactone (212) in $CDCl_3$ and keto-$\gamma\delta$-dilactone (213) in DMSO

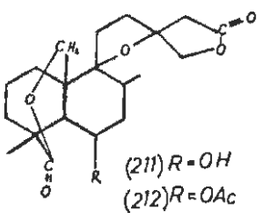
	$\gamma\delta$-dilactone	acetoxy-dilactone	keto-$\gamma\delta$-dilactone
17-Me	9.12(d)(J=6c/s)	9.16(d)(J=6c/s)	9.1(d)(J=6c/s)
	9.09(d)(J=6c/s)	9.12(d)(J=6c/s)	9.07(d)(J=6c/s)
18-Me	8.76(s)	8.91(s)	8.89(s)
22-Me	-	8.01(s)	-
14-CH_2	7.27(m)(W=55c/s)	7.32(m)(W=60c/s)	7.34(m)(W=48c/s)
16-CH_2 and 20-CH_2	5.82(m)(W=48c/s)	5.66(m)(W=60c/s)	5.77(m)(W=42c/s)
6-H		4.86(m)(W=12c/s)	-

s = singlet; d = doublet; m = multiplet.

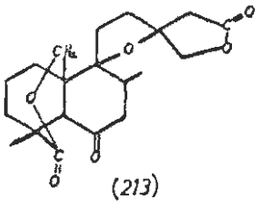
The most remarkable property of the spectra, recorded in $CDCl_3$ for (211) and (212) and in DMSO for (213) (at 25° in all cases), was the appearance of two sets of signals in each spectrum. The doublet (3H) in leonitin, attributed to the C_{17}-methyl, now appeared as a pair of doublets (3H) resembling a quartet. Signals at τ7.25, 5.83 and 5.77, which appeared as quartets in leonitin (see Table 8) now resonated as complicated multiplets. The above compounds (211), (212) and (213) appeared to be pure (TLC) and their NMR spectra suggest that they exist in solution at room temperature as mixtures of conformation isomers. A similar case of a compound existing in solution as a mixture of two conformers has recently been reported by Yoskioka and Mabry for the germacronolide dilactone, isabelin.[109]



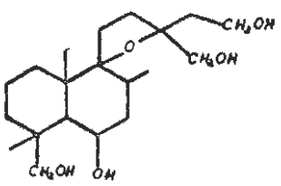
(210)



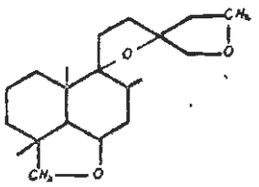
(211) R = OH
(212) R = OAc



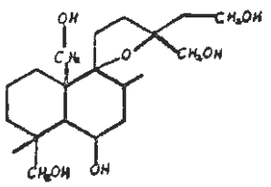
(213)



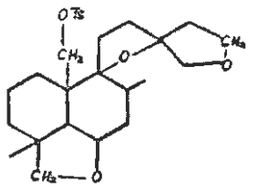
(214)



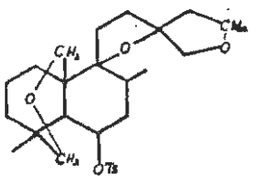
(215)



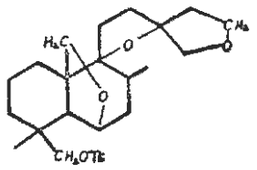
(216)



(217)

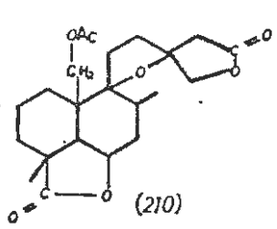


(218)



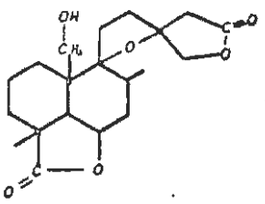
(219)

Scheme 4

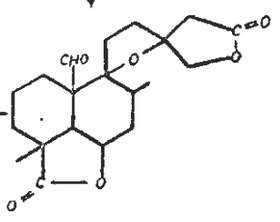


(210)

controlled hydrolysis →



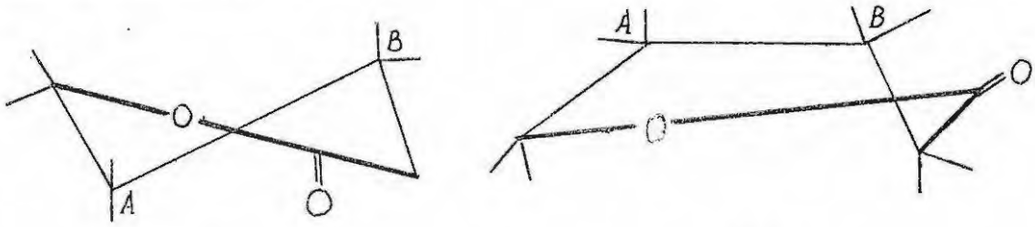
oxidation ↓



← ① Thioketal
② Raney Nickel

Compound X (39)

X-ray crystallographic analyses of several δ-dilactones have shown that the lactone group C-C(=O)-O-C is planar and models suggest that there are in fact two conformations that satisfy the condition of planarity.[86,106]



(1) (2)

(1) the half-chair in which carbon atoms A and B are situated one on each side of the plane and (2) the half-boat where both A and B are displaced to the same side of the plane of the lactone function.

Calculations[107] based on data available from several X-ray analyses of natural products containing δ-lactones demonstrate the existence of both conformations.

It is therefore assumed from their NMR data that compounds (211), (212) and (213) occur in solution in the half-chair and halr-boat conformations. Variable NMR temperature studies are presently being carried out in an attempt to determine that this is the case.

The nature of the carbon skeleton of leonitin could not be proved conclusively by dehydrogenation as in compound X[24] (39), due to the fact that no napthalene product could be isolated. Attempts at fission of the ether linkage in leonitin yielded only starting material (similar results were obtained in compound X).

An attempt was made to relate leonitin and compound X through a common degradation product in the following manner. Reduction of compound X (39) with lithium aluminium hydride afforded a crystalline tetrol (214) which on treatment with p-toluene-sulphonyl chloride afforded the di-ether (215). Leonitin was

reduced in an analagous manner to the alcohol (216), $C_{20}H_{36}O_6$, the infrared spectrum of which exhibited a strong absorption band at 3400cm^{-1} (hydroxyl) but showed no absorption bands at 1765 (γ-lactone) and 1730cm^{-1} (ester carbonyl). Compound (216) on treatment with p-toluenesulphonyl chloride afforded an oil, shown by TLC to be a mixture presumably of the ethers (217, 218, 219).

Attempts to separate the oil however proved unsuccesful and lithium aluminium hydride reduction of this oil, yielded a complex mixture.

(It can be noted that mixtures of compounds due to rearrangement, result on $LiAlH_4$ reduction of tosylates in which the functional group is in an unusual environment.[108])

One of the components of the complex mixture obtained corresponded to (215) on TLC but could not be isolated as a single component either by preparative TLC or column chromatography.

An attempt to convert leonitin to compound X (see scheme 4) proved abortive since the ester could not be split by alkali, acid, sodium methoxide or sodium borohydride, without attacking the lactone groups. Due to steric effects it is doubted whether the formation of the thioketal from the aldehyde would result.

On the foregoing evidence the structure (210) was proposed for leonitin.

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