"AN EXAMINATION OF THE EXTRACTIVES OF *LEONOTIS* SPECIES"

BY

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A Thesis

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

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SUMMARY

Marrubiin and the other two diterpenoids, compounds X and Y, which had previously been isolated from Leonotus leonurus have been reinvestigated.

Although the structure for marrubiin is well known its stereochemistry has been the subject of protracted discussion and is by no means secure except at C_5 and C_{10}. N.M.R. spectral evidence showed that the lactone ring was cis-fused and β-orientated. Dehydration experiments carried out by previous workers were repeated. In order to resolve the residual uncertainty regarding the stereochemistry at C_9, an attempt was made to prepare iodoacetyl marrubic acid for X-ray crystallographic studies.

Compound Y, $C_{20}H_{28}O_3$, a triply-unsaturated compound was shown by spectral studies to contain a furan ring and an α,β-unsaturated keto-group. It possesses a hydroxyl group incapable of acetylation, but readily removed by alkali and dehydrating agents to yield a tetraunsaturated compound, anhydro-Y; the hydroxyl is thus tertiary. Isolation of 1:2:5-trimethylnaphthalene on dehydrogenation indicated a relationship with the labdane diterpene group and supported the C_{20} formula. The position of the α,β-unsaturated keto-group was resolved by interpretation of the ultraviolet spectra of degradation products and also by isolation of 1:2:3:5-tetramethylnaphthalene on dehydrogenation of a suitable grignard product. The presence of a β-substituted furan was further indicated by colour reactions and confirmed by mass and n.m.r. spectra. The skeleton of compound Y is correlated with marrubiin via "iso-ambreinolide" and its stereochemistry is discussed. Further stereochemical assignments are postulated from the study of the n.m.r. spectra.

Compound X, $C_{20}H_{28}O_5$, was shown by spectral and chemical evidence to be a diterpenoid dilactone containing an ether

bridge between C_9 and C_{13}. Isolation of 1:2:5 trimethyl-naphthalene on dehydrogenation showed it to be closely related to marrubiin. This was supported by n.m.r. spectral results. A structure for compound X is proposed and the stereochemistry discussed.

The aerial portions of Leonotis leonitis were also extracted and shown to contain a new compound, compound R. Preliminary investigation showed that it was a dilactone containing a furan ring. The n.m.r. spectrum of the compound is discussed.

1. INTRODUCTION.

Structural relationships between the labdane diterpenoids.

The name diterpene should strictly speaking be applied only to the hydrocarbon constituents of essential oils containing twenty carbon atoms. However, diterpene hydrocarbons occur rarely in nature and the most important and abundant are the oxygenated derivatives.

The carbon skeletons of diterpenoids (this name covers both the oxygenated and oxygen-free C_{20}-compounds) in general either conform to one of the eight types shown (Chart 1) or can be derived from them by secondary rearrangements in accordance with the Biogenetic Isoprene Rule.

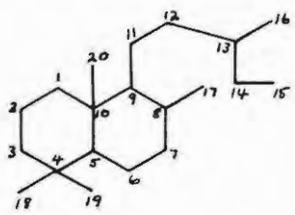
The di-, tri-, tetra and pentacarbocyclic diterpenoids are all derivable in principle from geranyl-geraniol(1) or geranyl-linalool(2) via the bicyclic alcohol(3) of the labdane type. It can be noted immediately that in all authenticated cases the relative stereochemistry at carbon atoms 5, 9 and 10 is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

This review is confined to diterpenoids with a normal labdane skeleton.

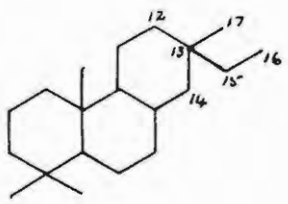
The skeleton of the common labdane diterpenoids can be divided into two partial structures (A→Y) and a side chain unit which can either contain an asymmetric carbon (a→g) or have no such symmetry (Chart 2).

Chart 1

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



LABDANE



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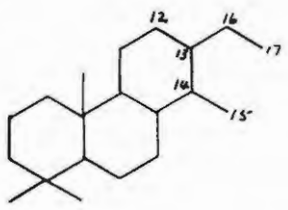

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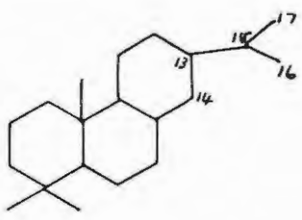

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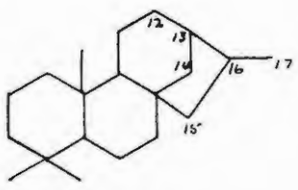
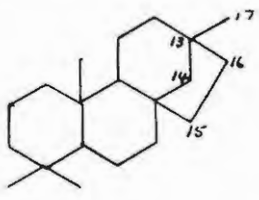
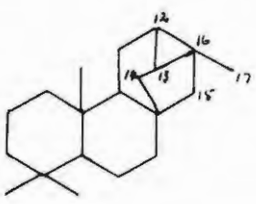


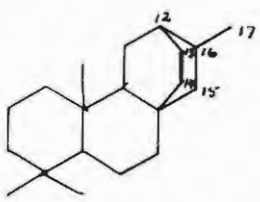
KAURANE



BEYERANE

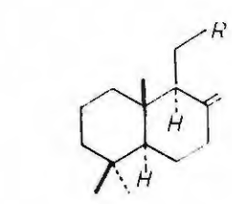


TRACHYLOBANE



ATISANE

Chart 2 — Bicyclic Systems



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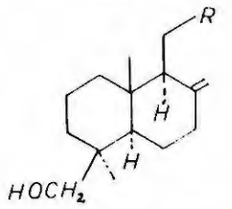

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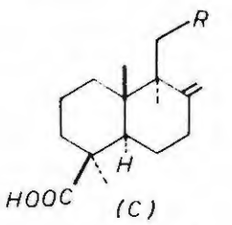

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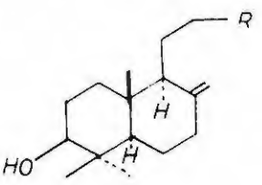

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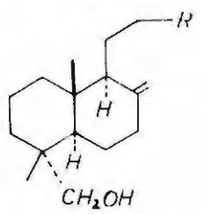

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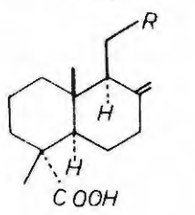

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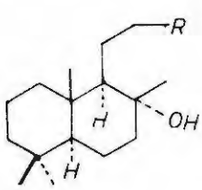

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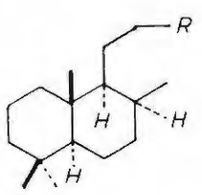

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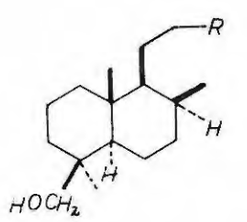

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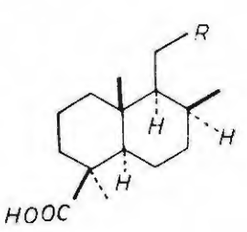

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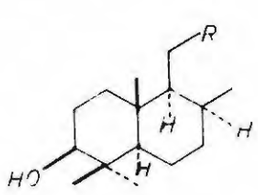

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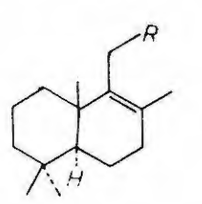

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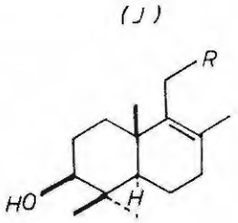

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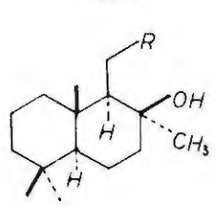

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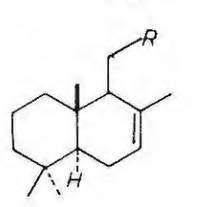

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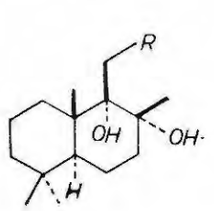

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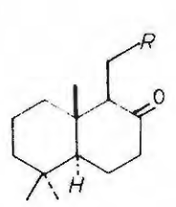

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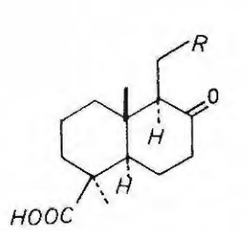

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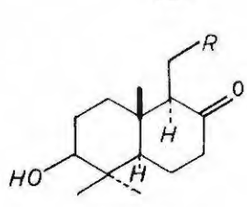

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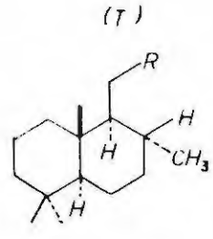

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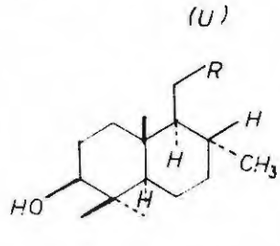
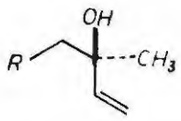


(Y)

Chart 2

Side-chain Units



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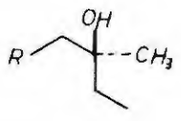

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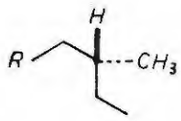

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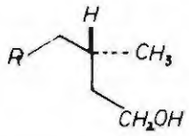

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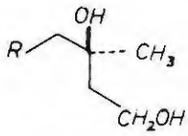

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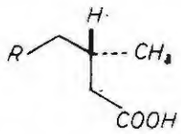

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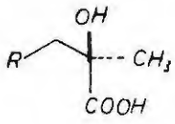

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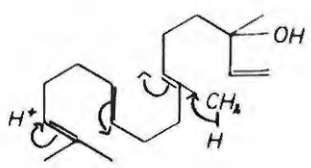

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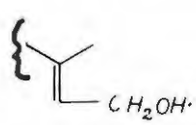

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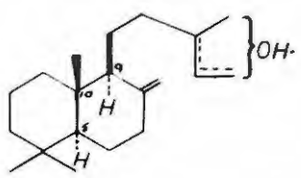

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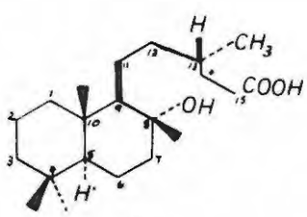

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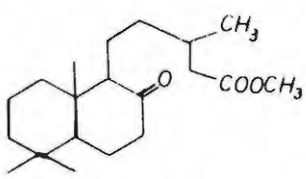

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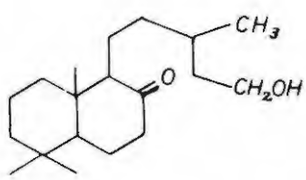

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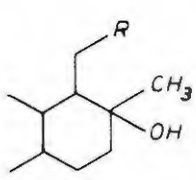

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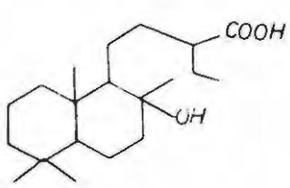

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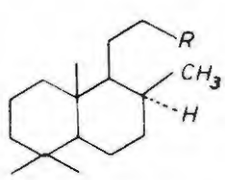


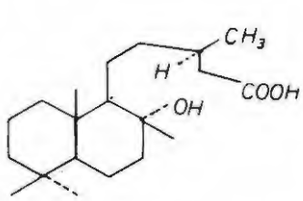
(9)

(9) R = $CH(CH_3)CH_2COOCH_3$

(10) R = $CH(CH_3)COOH$

(11) R = $COCH_3$

(12) R = COOH



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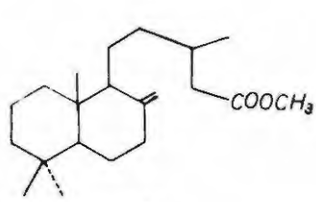

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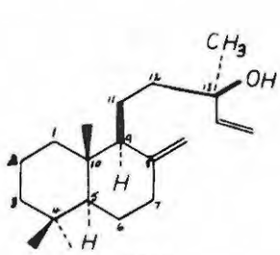

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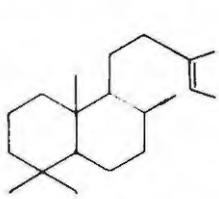

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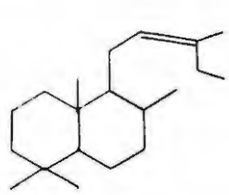

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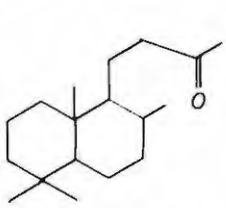

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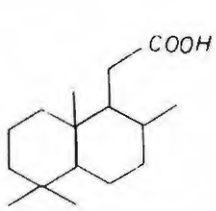

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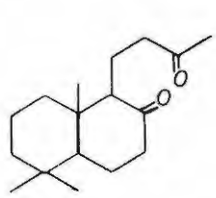


(20)

1.1. Labdanolic acid.

Labdanolic acid(4), $C_{20}H_{36}O_3$, a diterpene acid, was first isolated from the gum labdanum by Cocker and Bowers.[3] The infrared spectrum showed the presence of a carboxyl and a hydroxyl group, but gave no indication as to the presence of a double bond. The equivalent weight confirmed the formula $C_{20}H_{36}O_3$, which after allowance for the carbonyl group indicated that labdanolic acid was bicyclic.

Labdanolic acid was obtained easily by mild hydrolysis of its methyl ester, showing that the carboxyl group was unhindered and not situated in the sterically hindered 4 position as in the majority of diterpene acids. The hydroxyl group was found to be tertiary.

Dehydration of methyl labdanolate with phosphorus trichloride gave a homogeneous product, the infrared absorption of which indicated the presence of a vinylidene group, which was confirmed by ozonolysis, formaldehyde and a keto ester(5) being obtained. Reduction of the carbomethoxyl group in the dehydration product gave the alcohol(6). The keto group in (5) was shown to be somewhat hindered. Methyl labdanolate must contain the grouping(7) with the hydroxyl group in the equatorial conformation to account for the formation of an exocyclic rather than an endocyclic double bond on dehydration.

Dehydrogenation of the alcohol(6) yielded 1:2:5 trimethylnaphthalene and this suggested that labdanolic acid was related to the group of bicyclic diterpenes typified by manoöl and sclareol and that its structure was (4) or (8). That the correct formula was (4) rather than (8) was proved by degradation of the hydrogenation product (9) by the Barbier-Wieland technique to the acid(10) and thence to the methyl ketone(11). Hypoiodite oxidation of (11) gave an acid which was shown to be identical (mixed m.p. and identical infrared) with the acid(12) obtained from marrubiin[4] and ambrein.

The identity of this C_{17}-acid (12) proved that the rings in (4) were trans-fused and that the absolute configuration at C_{10} was the same as in ambrein and in the di- and tri-terpenoids. In turn it followed that the 8-hydroxyl group which had been shown to be equatorial was in the α- and the 8-methyl group in the β-configuration. This was confirmed by comparisons with sclareol and manoöl using molecular rotation differences.

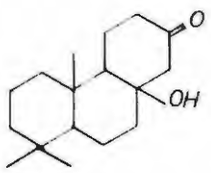
The β-configuration of the side chain was confirmed by the fact that (9) is identical with methyl dihydrocativate, whose structure has been rigorously proved.[5] Cativic acid was related through a common degradation product to manoöl which had been shown to have the side chain β at C_9 and therefore the configuration of labdanolic acid at C_9 is β.

Labdanolic acid has been assigned the S-configuration[6] at C_{13} by analysis of molecular rotation data[7], the reliability of which is increased by the fact that neither methyl labdanolate nor methyl 13-epi-labdanolate(13) exhibit intramolecular hydrogen bonding.[8] This absolute configuration has been shown to be correct.[9]

It should be noted that (4) is closely related to eperuic acid since dehydration of methyl labdanolate with phosgene and pyridine yielded an ester(14) which is a stereoisomer of methyl eperuate.

1.2. Manoöl

Manoöl(15), $C_{20}H_{34}O$, a diterpene alcohol was isolated by Hosking and Brandt[10] from the wood oil of the yellow pine, Dacrydium biforme. It contains two ethylenic linkages since catalytic hydrogenation afforded in two stages dihydromanoöl, $C_{20}H_{36}O$, and tetrahydromanoöl, $C_{20}H_{38}O$. Hydrogen chloride gave with tetrahydromanoöl a hydrochloride which on digestion with aniline yielded a hydrocarbon, tetrahydromanoene. This latter compound was found to be a mixture of (16) and (17) since on ozonolysis it gave a ketone(18) $C_{18}H_{32}O$ and an acid(19),



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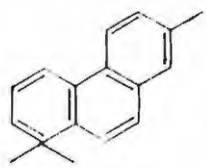

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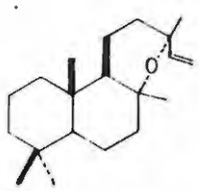

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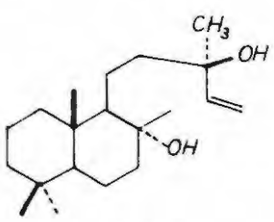

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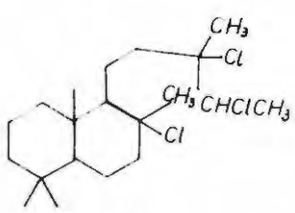

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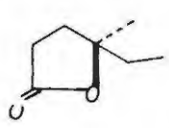

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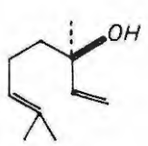

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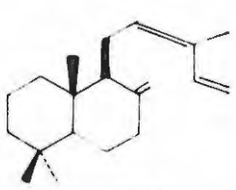

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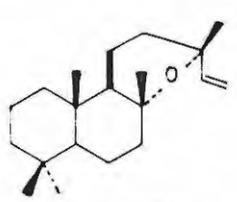

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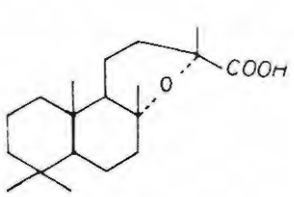

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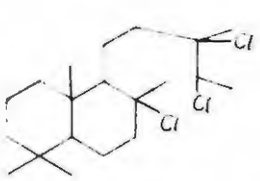

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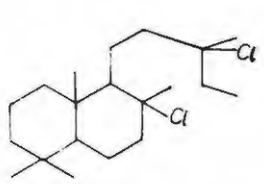

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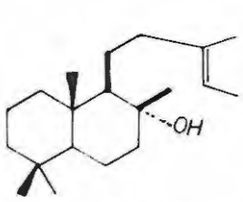

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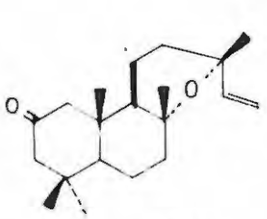

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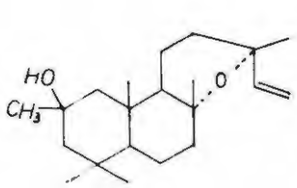

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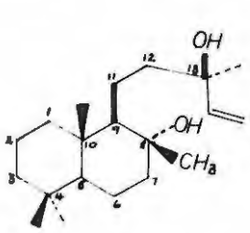

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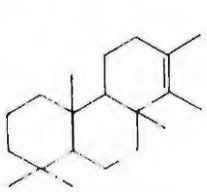

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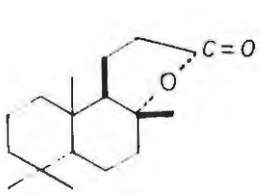


(38)

$C_{16}H_{28}O_2$. The formation of (18) and (19) provided conclusive proof that the hydroxyl group in manoöl must be in the 13-position. The acid(19) has also been obtained by Ruzicka et al[11] from the triterpenoid ambrein and by Burn and Rigby[12] from marrubiin. The rings in manoöl are therefore trans-fused and the configuration at C_{10} is the same as in ambrein (see labdanolic acid).

Direct proof as to the positions of the two ethylenic linkages in manoöl was obtained by its oxidation with ozone and potassium permanganate. Hosking showed that on ozonolysis manoöl gave a diketone(20), in which the carbonyl groups were in the 1:5-position, and which underwent ready internal aldolization with alkali to yield a crystalline saturated hydroxyketone(21). This was converted by treatment with methyl magnesium iodide followed by distillation to a tricyclic hydrocarbon, $C_{18}H_{28}$, which on selenium dehydrogenation yielded pimanthrene(22).

The close relationship between manoöl(15), manoyl oxide(23) and sclareol(24) is shown by the fact that all three give with dry hydrogen chloride in ether the same trichloro-derivative (25). Furthermore, manoöl and abietic acid have been interrelated[13] and hence the trans-fusion of rings A and B in manoöl, manoyl oxide and sclareol is established.

According to Scott et al[2] the C-C bond at C_9 is β.

The absolute stereochemistry of manoöl at C_{13}, based on indirect methods of configurational correlation, has been the subject of protracted discussion and repeated revision.[8,14] However, direct correlation has been achieved[15] by conversion of sclareol into the lactone of γ-hydroxy-γ-methyl-hexanoic acid(26), also obtained from R-linaloöl(27) of known configuration.[16] This proves the 13-R-configuration in both manoöl and sclareol.

(A further relationship between manoöl and sclareol has

been described by P.F. Vlad et al[17] who have synthesised 13-epi-manoöl from 13-epi-sclareol).

1.3. Biformene.

Biformene(28), $C_{20}H_{32}$, a diterpene hydrocarbon was isolated from the heartwood extractives of Dacrydrium biforme.[18] From its ultra-violet and infra-red absorption spectra it contains two conjugated double bonds, an exocyclic methylene group, a vinyl group and a gem-dimethyl group. On hydrogenation biformene gave a saturated liquid hydrocarbon, $C_{20}H_{38}$, and therefore has two rings. Dehydrogenation gave as one of the products 1:2:5 trimethylnaphthalene, and it was suggested that biformene was related to manoöl and possessed the structure(28). This was proved by dehydration of manoöl in acetic acid to biformene and also by the fact that both formed the same trichloride. The ready dehydration of manoöl to (28) led to the proposal that biformene is not present in the wood but is an artefact.

1.4. Manoyl Oxide.

Manoyl oxide(29), $C_{20}H_{34}O$, was isolated from the wood oil of Dacrydium colensoi by Hosking and Brandt.[19] This was the first reported occurrence in nature of a diterpene oxide. Manoyl oxide was shown by catalytic hydrogenation to dihydromanoyl oxide to contain one ethylenic linkage. The completely inert nature of the oxygen suggested that it was probably present as part of an oxide ring. Evidence for the carbon skeleton was obtained by dehydrogenation to a mixture of 1:5:6-trimethylnaphthalene and 1:7:8-trimethylphenanthrene. The presence of the exocyclic methylene group was proved by ozonolysis to formaldehyde and oxidation with potassium permanganate to an acid(30).

Valuable confirmatory evidence that (29) and its dihydroproduct had been correctly formulated was obtained by reaction with hydrogen chloride to manoene trihydrochloride(31) and

dihydro-manoene dihydrochloride(32) respectively. These two derivatives were identical with those prepared by the action of hydrogen chloride on sclareol; dihydrosclareol and manoöl; dihydromanoöl. Accordingly manoöl, sclareol and manoyl oxide are chemically related and their stereochemistry at C_4, C_5, C_9 and C_{10} are identical. The stereochemistry of manoyl oxide at C_8 is based on its hydrogenolysis to 8-α-hydroxy-labd-13-ene (33),[20,21] and at C_{13} by comparison of the cracking patterns in the mass spectra of manoyl oxide and its 13-epi compound. This has been substantiated by J.A. Giles et al.[22]

1.5. 2-Ketomanoyl Oxide.

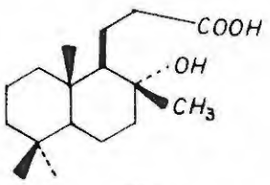
2-Ketomanoyl oxide(34) $C_{20}H_{30}O_2$, was isolated by Hosking and Brandt[19] from the wood oil of the silver pine, Dacrydium colensoi. The close relationship to manoyl oxide(29) was shown by the preparation of this compound from (34) by a Wolff-Kishner reaction. The location of the keto-group was shown by the conversion of (34) to the carbinol (35) by treatment with methyl magnesium iodide, which was dehydrogenated to 1:2:5:7-tetramethyl naphthalene.

Further indirect evidence as to the position of the keto-group in the A ring was obtained by Klyne[23] who showed that by analogy with steroids and triterpenoids it would be expected that diterpenoids containing keto-groups in the A and B rings would have positive and negative Δ C=O values respectively. This is borne out by 2-ketomanoyl oxide which has a value of $+ 76^{\circ}$.

(It should be noted that 3-keto-manoyl oxide also occurs in nature.[24]).

1.6. Sclareol.

The bicyclic, ditertiary diterpenoid alcohol, sclareol(36) $C_{20}H_{36}O_2$, was first isolated in 1924 from the leaves of Salvia sclarea.[25] Catalytic reduction to the saturated dihydrosclareol showed that (36) must be bicyclic and contain



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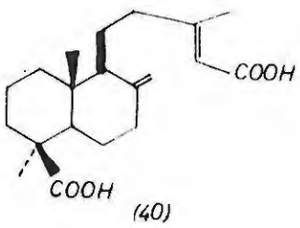

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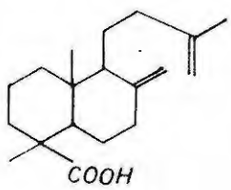

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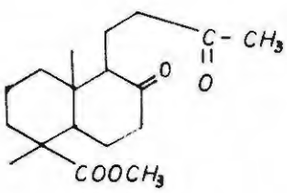

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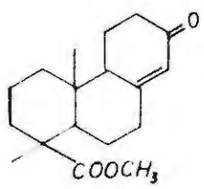

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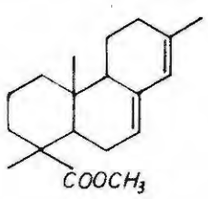

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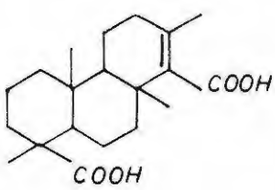

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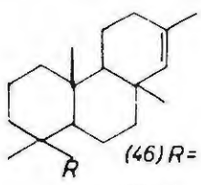
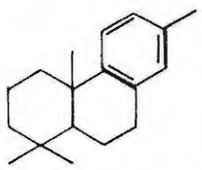


(46) R = COOH

(47) R = CH_2OH



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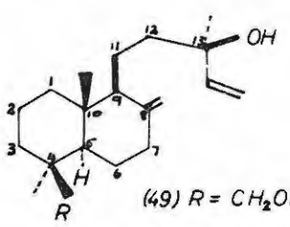
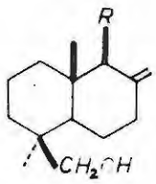


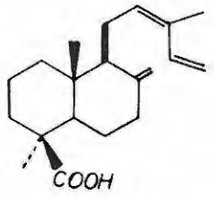
(49) R = CH_2OH

(50) R = CHO



(51) R = $-CH_2-CH_2-C(=CH_2)-CH=CH_2$

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



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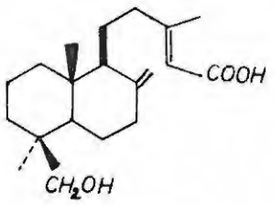

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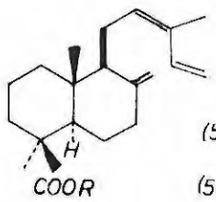
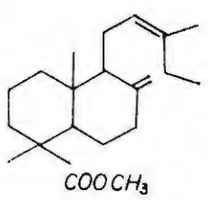


(55) R = H

(56) R = CH_3



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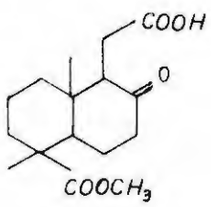

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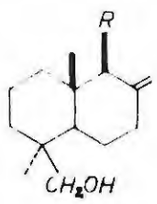


(59)

(59) R = $-CH_2-CH=C(CH_3)-CH=CH_2$

(60) R = $-CH_2-CH_2-C(CH_3)=CH-CH_3$

one ethylenic linkage, while dehydrogenation gave 1:5:6-trimethylnaphthalene, thus partially characterising the carbon skeleton.

Dihydrosclareol was dehydrated with potassium bisulphate to dihydrocyclosclarene(37), $C_{20}H_{34}$, which on dehydrogenation with selenium yielded as a major product 1:7:8-trimethylphenanthrene, suggesting that sclareol possesses the same skeleton as agathenedicarboxylic acid and manoyl oxide. The presence of a methylene side chain was shown by ozonolysis to formaldehyde. Oxidation with chromic acid yielded a lactone (38), identical with a degradation product from the triterpenoid ambrein.[26]

The two uncharacterised oxygen atoms must be present as tertiary alcohols and must be formulated as shown in order best to account for the formation of (37) on dehydration. Treatment of sclareol with hydrogen chloride afforded a trihydrochloride identical with that obtained from manoöl and manoyl oxide. This constitutes further indirect proof as to the correctness of the formula (36) and at the same time defines the stereochemistry at positions 5, 9 and 10. The R-configuration at C_{13} was shown by conversion of sclareol into the lactone of γ-hydroxy-γ-methylhexanoic acid[15] of known absolute stereochemistry (see manoöl). In addition sclareol has been synthesised from (+)-ambreinolide(39)[27] thus confirming the stereochemistry at $C_{5,\ 8,\ 9}$ and $_{10}$ as shown.

1.7. Agathenedicarboxylic Acid (Agathic Acid).

Agathenedicarboxylic acid(40), $C_{20}H_{30}O_4$, a bicyclic resin acid was first isolated by L. Ruzicka and J.R. Hosking[28] from Kauri copal and from the soft and hard grades of Manila copal. The structure(40) assigned to agathenedicarboxylic acid is due entirely to the experiments of Ruzicka and his collaborators,[29] and is based on the following evidence.

The carbon skeleton was indicated by dehydrogenation with sulphur or selenium to 1:5:6-trimethylnaphthalene and pimanthrene (1:7-dimethylphenanthrene) Agathenedicarboxylic acid contains two ethylenic linkages, one of which must be in the αβ-position with one of the carboxyls. This is shown by the u.v. absorption spectrum and by the ease with which carbon dioxide is split out on pyrolysis to give noragathenemonocarboxylic acid(41). The position of these olefinic linkages was proved by a study of the ozonolysis products of the dimethyl ester, the most important product was a 1:5 diketone(42), which readily underwent intramolecular dehydration with alkali to give a tricyclic αβ-unsaturated ketone(43). The latter on reaction with methyl magnesium iodide afforded a conjugated diene (44), which was dehydrogenated with selenium to pimanthrene. This experiment also indicated the position of the carboxyl group which is easily eliminated on pyrolysis.

The second carboxyl group was placed by cyclizing agathenedicarboxylic acid with formic acid to a tricyclic acid, isoagathenedicarboxylic acid(45), which also possessed an αβ-unsaturated carboxyl readily eliminated by heat to isonoragathenemonocarboxylic acid(46). The methyl ester of (46) was reduced to agathenol(47), which on dehydrogenation afforded 1-ethyl-7-methylphenanthrene, identical with the hydrocarbon prepared in a similar way from dextro-pimaric acid.

Rings A and B in agathenedicarboxylic acid are fused as in abietic acid because its derivative (44) could be converted to the partially aromatic hydrocarbon(48), identical with that obtained from manoöl.[30] Since manoöl had already been related to abietic acid,[31] which has the A/B ring fusion trans, it follows that the ring fusion in (40) must also be trans. Further correlation of agathenedicarboxylic acid with manoöl via torulosol proves that the side chain is β-orienated in (40)[32]. The tertiary-carboxyl group of agathenedicarboxylic acid

was shown to be in the 4 β-position[33] and it thus possesses the absolute stereochemistry shown in (40).

1.3. Torulosol and Torulosal.

The diterpenes torulosol(49), $C_{20}H_{34}O_2$, and torulosal(50), $C_{20}H_{32}O_2$, were first isolated from the heartwood of Cupressus torulosa by Enzell.[32] Their structures are based upon the following evidence:-

Oxidation of (49) with chromium trioxide in pyridine gave (50). The fact that the aldehyde group of (50) did not react with silver oxide or Schiff's reagent was attributed to steric hindrance. The infrared and mass spectra of manoöl and torulosol were very similar differing only because of the presence of an additional hydroxyl group in the latter. Torulosol was converted by Huang-Minlon reduction into manoöl of known absolute configuration, thus establishing the structure and stereochemistry at C_5, C_9, C_{10} and C_{13} in (49) and (50).

The position and configuration of the extra oxygen atom was determined by subjecting (49) to an allylic rearrangement to give the triene alcohol(51) and the diol(52) (later shown to be identical with agathadiol and contortodiol). The latter was identical with a specimen obtained from the dimethyl ester of agathenedicarboxylic acid by lithium aluminium hydride reduction. Since the tertiary carboxyl group in agathene-dicarboxylic acid had been shown to be in the 4 β-position[33] it followed that torulosol and torulosal are oxygenated at C_{19}. The triene alcohol(51) and its dihydroproduct obtained on reduction with sodium and alcohol were used to establish the structure of communic acid(53).[34] The correlation of agathene-dicarboxylic acid with manoöl via torulosol proved that the side chain in agathenedicarboxylic acid is β-orientated at C_9. The n.m.r. spectrum of torulosol supported the structure shown and provided additional evidence for the β-orientation of the primary hydroxyl group.

1.9. Agatholic acid.

Agatholic acid(54), $C_{20}H_{32}O_3$, was isolated from Manila copal by Enzell.[32] The infrared, ultraviolet and mass spectra of this acid indicated it was αβ-unsaturated and that it differed from torulosol(50) only with respect to the side chain at C_9. Esterification with diazomethane followed by reduction with lithium aluminium hydride yielded an alcohol, shown to be identical with agathadiol and contortodiol(52). This correlation together with the spectral data established the structure and absolute configuration as in (54). Agathadiol was converted by manganese oxide to a hydroxy-aldehyde which on further oxidation with silver oxide gave agatholic acid.

1.10. Communic acid.

Communic acid(55), $C_{20}H_{32}O_2$, was isolated by V.P. Arya et al[34,41] as its sodium salt from the bark of Juniperus communis. The stable methyl ester(56) gave a maleic anhydride adduct, and on ozonolysis furnished formaldehyde in an amount indicative of two terminal methylene groups. Methyl dihydrocommunate(57), obtained on catalytic hydrogenation of (56), gave on ozonolysis one mole of formaldehyde and a keto-acid(58). Methyl communate(56) was reduced with lithium aluminium hydride to communol(59) which on further reduction in propanol with sodium gave isodihydrocommunol(60), shown to be identical with a degradation product of torulosol,[32] which in turn had been correlated with manoöl and agathic acid. The identification of (60) and the formation of the C_{16} keto-acid (found later to be identical to a keto acid obtained from methyl sciadopate[39,40]), showed that communic acid possesses the absolute structure shown.

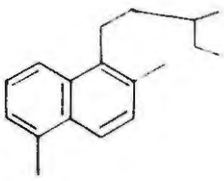
Joye, Lawrence et al[35] consider that elliotinoic acid[36] is in fact identical with communic acid. (The mixed esters had an undepressed melting point, showed a single peak on gas chromatography and in addition the ultraviolet and infrared

spectra were identical). The original structure proposed for elliotinoic acid[37] is incorrect since elliotin, one of the degradation products of elliotinol (identical with communol(59)) was shown to be 1:6-dimethyl-5(3 methyl-pentyl) naphthalene(61) and not 1:6-dimethyl-5-isohexyl naphthalene(62) as originally thought. With the revision of this structure of elliotinoic acid all the known dicyclic diterpenoids conform to the phytol rule[38] and there is no divergence in this series from the manoöl skeletal structure. It can be noted that communic and laevopimaric acid occur in the same plant[42] and this was the first reported case of α- and β-C_4-acids occurring together. Carman suggests that a common carbonium ion exists as an intermediate. Other examples of co-occurrence of α- and β-acids have since been reported.[43]

1.11. Contortolal and Contortodiol.

Contortolal(63), $C_{20}H_{32}O_2$, and contortodiol(64), $C_{20}H_{34}O_2$, are minor components of hodgepole bark, Pinus contorta.[44] The proton count coupled with the analytical and spectral data showed that contortolal was a dicyclic diterpene, containing an exocyclic methylene group, two tertiary methyl groups, a tertiary aldehyde group and a trans $-C(CH_3)=CH-CH_2OH$ grouping. These facts strongly suggested a labdane type of skeleton in which the side chain had been allylically rearranged from the vinyl carbinol in epimanoöl(65) which is the major constituent of the bark.

The structure of contortodiol, isolated via its di-p-phenylazobenzoate, was suggested via its infrared spectrum, n.m.r. spectrum, proton count and analytical data to be (64). It also possesses the allylically rearranged side chain postulated for (63). Rowe and Scroggins[45] originally considered contortodiol to be the C_4-epimer of agathadiol, but their physical properties, mixed melting point, infrared spectra and behaviour



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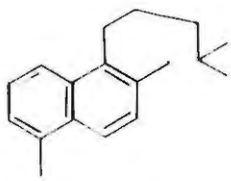

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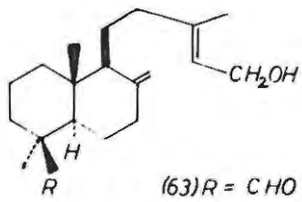
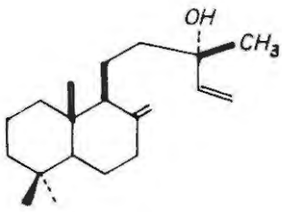


(63) R = CHO

(64) R = CH_2OH



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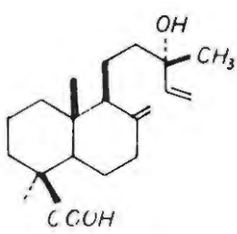

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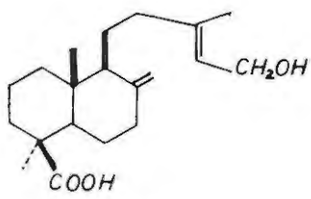

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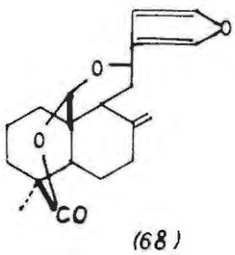

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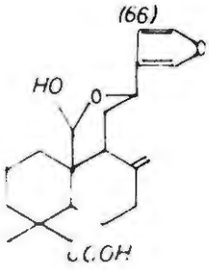

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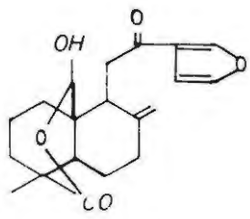

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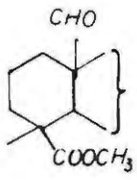

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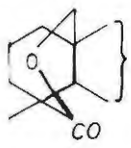

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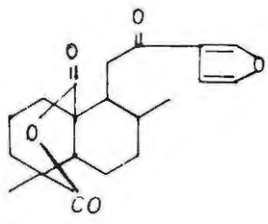

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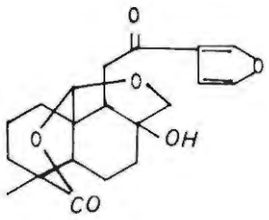

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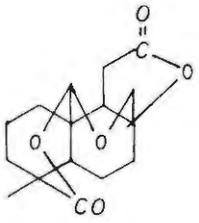

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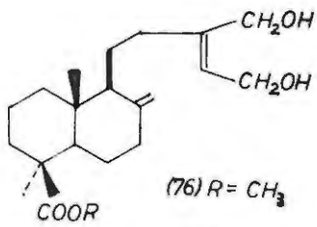
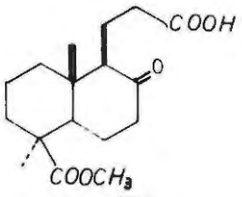


(76) R = CH_3

(77) R = H



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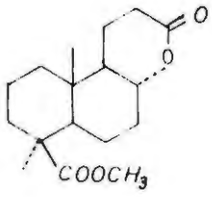

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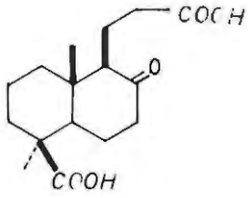


(80)

on thin layer chromatograms showed that they are identical. This identity had previously been suggested by Gough[43] from the fact that axial and equatorial alcohols show a band at 9.80 and 9.60μ respectively.

It should be noted that contortodiol can be obtained from torulosol by allylic rearrangement.[32]

1.12. Cupressic and Iso-cupressic acids.

Cupressic acid(66), $C_{20}H_{30}O_3$, and iso-cupressic acid(67) were isolated together with communic acid from the oleoresin of Cupressis sempervirens,[88,89] and were purified via their methyl esters.

The carbomethoxy group of the methyl ester of cupressic acid is sterically hindered since it resists hydrolysis on prolonged treatment with alcoholic potassium hydroxide. It contains two unconjugated double bonds and the hydroxyl group is tertiary since it resists acylation. The methyl ester of (66) on treatment with lithium aluminium hydride gave torulosol(49) thus establishing the structure and stereochemistry of cupressic acid. The methyl ester of (67) also contains a sterically hindered carbomethoxy group, two double bonds and an alcoholic group which is easily acylated and on treatment with lithium aluminium hydride yielded contortodiol(64), thus also establishing the structure and stereochemistry of iso-cupressic acid as shown in (67).

1.13. Sciadin.

Sciadin(68), $C_{20}H_{24}O_4$, a diterpenoid lactone isolated by Sumimoto from the heartwood of Sciadopitys verticillata[46] was shown to contain a β-substituted furan ring, a vinylidene group, a lactone group and one further oxygen atom in an ether function. Sciadinic acid(69), obtained from sciadin with ethanolic potassium hydroxide afforded on oxidation the keto-lactol(70) which was a β-furyl-ketone. On treatment

with diazomethane (70) yielded the aldehyde ester(71) and with sodium borohydride the keto-lactone(72).

Dihydrosciadinic acid was oxidized to a ketoanhydride(73), found from its infrared spectrum to be a derivative of glutaric anhydride. Alkaline permanganate oxidation of (69) gave the hydroxy-lactone ether(74) which was also a β-keto-furan, and this on ozonolysis gave the dilactone(75), which was shown to be both a δ- and γ-lactone.

These facts when considered in the light of the structures of polyalthic[47] and daniellic[48] acids suggests that sciadin has structure (68).

1.14. Methyl Sciadopate.

Methyl sciadopate(76), $C_{21}H_{34}O_4$, occurs in the heartwood of Sciadopitys verticillata together with sciadin and is considered to be its precursor.[49] Methyl sciadopate resisted hydrolysis in hot ethanolic potash but was saponified by boiling with diethylene glycolic potash to afford an oily acid (77), which after treatment with diazomethane regenerated the original ester. The difficulty of hydrolysis suggested that the ester group was attached to a quaternary carbon in the axial configuration.

The presence of two hydroxyl groups in the molecule was supported by an active hydrogen determination. Methyl sciadopate gave an intense yellow coloration with tetranitromethane, and absorbed two moles of hydrogen, indicating the presence of two double bonds in the molecule, one of which was shown to be a vinylidene group. On oxidation with Jones reagent methyl sciadopate furnished a dicarboxylic acid $C_{21}H_{30}O_6$, the infrared and ultraviolet spectra of which suggested that it was a derivative of maleic acid. This dicarboxylic acid on treatment with acetic anhydride gave an anhydride in quantitative yield indicating that the two hydroxymethyl groups

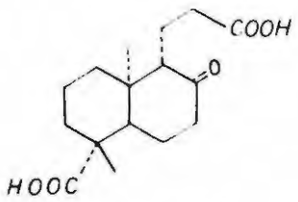
in (76) are cis related.

Ozonolysis of (76) gave a small amount of an acidic oil(78), which on reduction with sodium borohydride yielded the lactonic ester(79) having an infrared absorption at 1727 cm^{-1} indicative of a δ-lactone, which in turn suggested the relationship between the double bonds as shown in (76). Ozonolysis of (77) followed by hydrogen peroxide oxidation gave a keto-acid(80), $C_{16}H_{24}O_5$, the rotatory dispersion curve of which showed a strong negative Cotton effect. In their study of the stereochemistry of daniellic acid, Ourissen et al,[47] prepared the C_{16} keto-acid(80) from agathic acid and the antipodal acid(81) from daniellic acid. Both these acids (80) and (81) showed identical infrared spectra but exhibited opposite signs in their rotatory dispersion curves. The C_{16} keto-acid obtained from (76) was found to be identical with that obtained from agathic acid by mixed melting point and comparison of infrared absorption spectra.

The keto-acid(80) has also been obtained from communic acid. In addition, reduction of (76) with lithium in liquid ammonia in the presence of ethyl alcohol, gave after separation of the products by chromatography on silica gel, isodihydrocommunol(82) a transformation product of communic acid. The relationship between agathic acid, communic acid and methyl sciadopate can thus clearly be seen.

1.15. Colensenone.

Colensenone(83), $C_{19}H_{30}O_2$, isolated from the heartwood of Dacrydium colensoi by Grant and Carman[51] is an A-nor-diterpenoid ketone having a structure closely related to ketomanoyl oxide. The constitution and configuration of colensenone followed from the partial synthesis of colensanone (obtained from (83) by hydrogenation) from dihydro-2-oxomanoyl oxide(84). Autoxidation afforded the diosphenol(85), which



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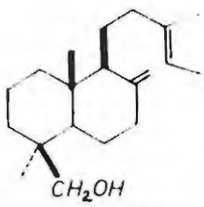

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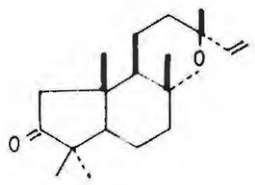

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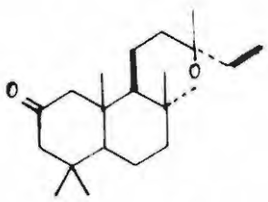

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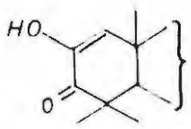

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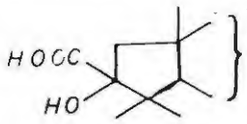

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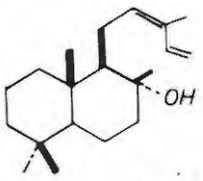

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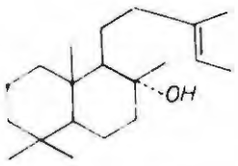

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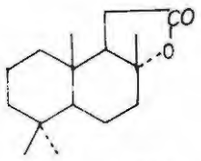

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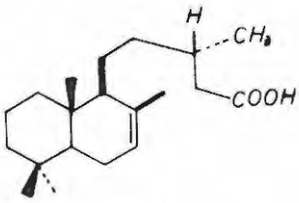

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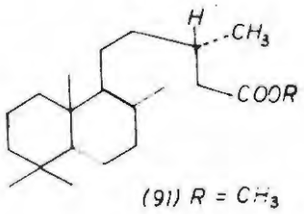
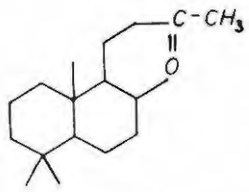


(91) R = CH3

(94) R = H



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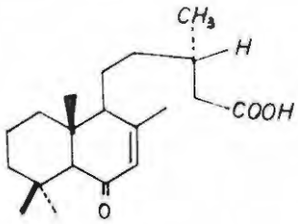
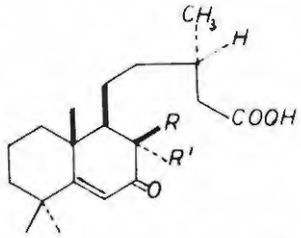
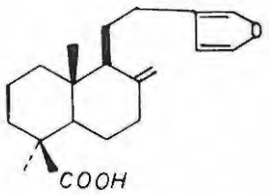


(93)

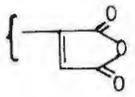


(95) R = CH3 R' = H

(96) R = H R' = CH3



(97)



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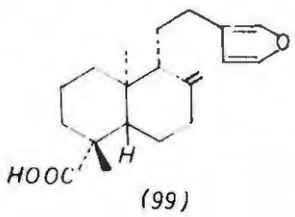
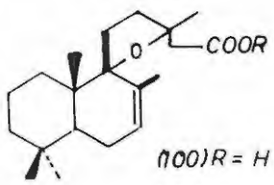


(99)



(100) R = H

(101) R = CH3

underwent a benzilic acid rearrangement to the ring contracted hydroxy-acid(86). Cleavage of the diol obtained with lithium aluminium hydride, followed by treatment with periodic acid gave colensanone.

1.16. Cativic acid.

Cativic acid(90), $C_{20}H_{34}O_2$, was isolated as an oil from the oleo-resin of Prioria capaifera Griseb.[54] Grant and Zeiss[55,56] succeeded in crystallising the acid and in establishing its structure. Cativic acid absorbed one mole of hydrogen and was converted by ozonolysis to a ketone giving a positive iodoform reaction showing that the double bond terminates at a tertiary ring carbon atom bearing a methyl group. The ease of esterification of (90) indicated that the carboxyl group was not situated in the usual sterically hindered 4 position. The location of the carboxyl group and the key to the elucidation of the structure was given by the two step Barbier-Wieland degradation of the dihydro-methyl ester(91) to a methyl ketone, the semicarbazide of which was found to be identical to that of the methyl ketone(92) arising from the degradation of manoöl. This reaction related dihydrocativic acid structurally and stereochemically through manoöl to the di- and triterpenes.

Methyl dihydrocativate(91) has been related[57] to a product obtained from methyl labdanolate by a process which does not affect the configuration at C_{13}, and therefore the absolute configuration is as shown in (90).

1.17. Abienol.

Abienol(87), $C_{20}H_{34}O$, has been isolated from the oleoresin of many Abies species.[52] It was shown to be a bicyclic diterpenoid alcohol possessing a conjugated diene with one of the double bonds present as a vinyl group and the other exocyclic and trisubstituted. Selenium dehydrogenation yielded 1:5:6-

trimethyl naphthalene, which suggested a normal labdane skeleton. The n.m.r. spectrum showed four vinyl protons, a vinylic methyl group and four further methyl groups. In a labdane skeleton these functions could only be accommodated by structure (87).

Hydrogenation of abienol gave a tetrahydro compound; the n.m.r. spectrum of which showed no vinyl protons. The mass spectrum indicated that the hydroxyl group was not located on the side chain. Confirmation of the structure (87) was afforded by reduction with sodium/propanol to isodihydro-abienol(88) whose constants were very close to those of a compound prepared by hydrogenolysis of manoyl oxide.

Carman[53] showed the correctness of the above structure(87) by oxidation of abienol with osmium tetroxide and periodic acid to (+)-norambreinolide(89).

1.18. 6-Oxocativic acid.

6-oxocativic acid(93), $C_{20}H_{32}O_3$, isolated from the gum labdanum by Halsall et al,[58] contains an endocyclic αβ-unsaturated ketone shown by its ultraviolet absorption spectrum. The keto group is exceedingly inert. Hydrogenation yielded a saturated keto acid which on Wolf-Kishner reduction gave dihydrocativic acid(94) showing that the carbon skeleton was the same as that of labdanolic and cativic acids. The two other possible structures for 6-oxocativic acid (95) and (96) are not consistent with the ultraviolet data and also with the fact that the carbonyl group is so inert.

1.19. Lambertianic acid.

Lambertianic acid(97), $C_{20}H_{28}O_3$, isolated from the oleoresin of Pinus lambertiana Dougl.[59] is a bicyclic diterpene acid containing a β-substituted furan ring. (Two such acids, namely polyalthic and daniellic acids, have a similar system but possess the so-called "wrong" configuration, which is the

mirror image of the more common resin acids). The presence of three double bonds was established by hydrogenation and this fact coupled with the elementary composition indicated the presence of three rings. The infrared spectrum showed absorption at 890 cm^{-1} characteristic of an exocyclic methylene group while the absorption at 1480 cm^{-1} and 870 cm^{-1} established that the remaining two double bonds and oxygen atom were present in a furan ring. The n.m.r. spectrum confirmed the presence of two α- and one β-hydrogen on a furan ring (τ 2.78, 2.93, 3.87), two exocyclic vinyl hydrogens (τ 5.15, 5.45) and two quaternary methyl groups (τ 8.90, 9.40). Furthermore since lambertianic acid had a pK_{mcs} value of 8.51 the carboxyl group was considered to have an axial configuration. The position and configuration of the carboxyl group was confirmed by oxidation to the maleic anhydride (98), which was ozonized in glacial acetic acid to the keto acid (80) also obtained from agathic acid.

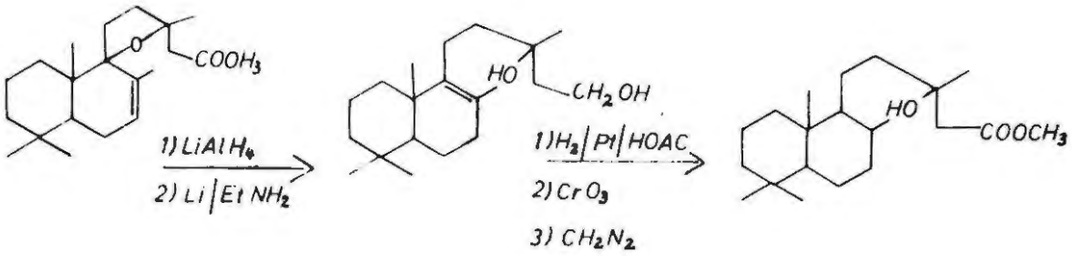
It was suggested that lambertianic acid ($[\alpha]_D$= + 55°) might be the optical antipode of daniellic acid[48] (99) ($[\alpha]_D$= - 58°) and this was substantiated by the n.m.r. spectra.

1.20. Grindelic acid.

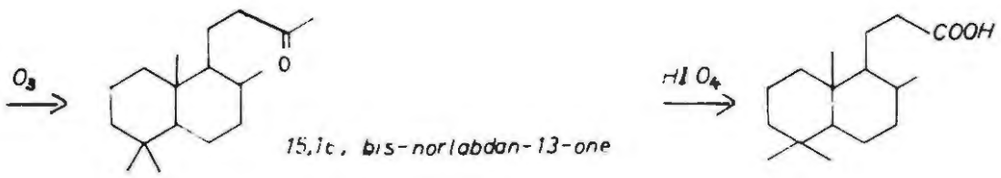
Grindelic acid(100), $C_{20}H_{32}O_3$, was isolated as its methyl ester from Grindelia robusta.[61] Nuclear magnetic resonance studies on methyl grindelate(101) indicated that it contained four methyl groups attached to fully substituted carbon atoms, one of which also carried an oxygen atom. The absence of further absorption, apart from the ester methoxyl group and one vinyl proton below 7.0τ showed that the ether oxygen atom was attached at fully substituted carbon atoms.

From its infrared spectrum methyl grindelate contains an unconjugated ester carbonyl group, a five or six membered ether ring, and a low intensity band at 835 cm^{-1} missing in

Chart 3

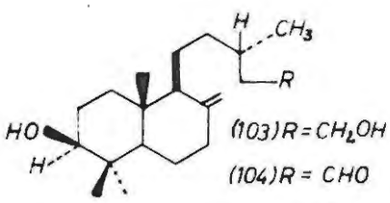


$POCl_3$ | Pyridine → Mixture of unsaturated esters which were separated to yield the α,β-unsaturated esters



15,16 bis-norlabdan-13-one

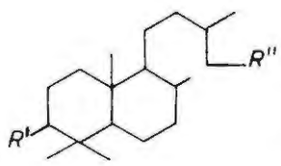
(102)



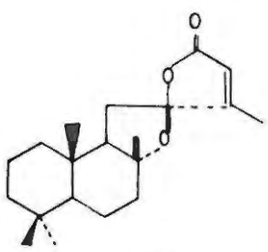
(103) R = CH_2OH

(104) R = CHO

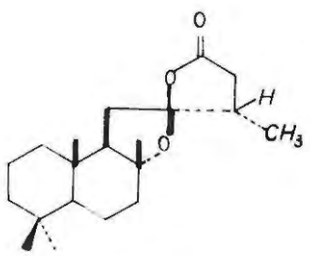
(105) R = COOH



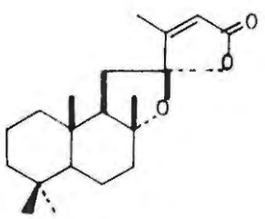
	R'	R''
(106)	OAC	CH_2OH
(107)	OAC	COOH
(108)	OH	COOH
(109)	=O	$COOCH_3$
(110)	H	COOH
(111)	OH	CH_2OAC



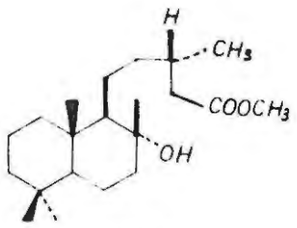
(112)



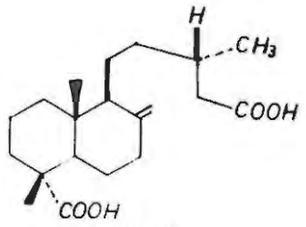
(113)



(114)



(115)



(116)

the dihydro-ester attributed to a trisubstituted double bond. Methyl grindelate was converted (see Chart 3) to 14, 15, 16-trisnorlabdanoic acid(102) thus establishing the stereochemistry at C_4, C_8, C_9 and C_{10}.

1.21. Diterpenes isolated from Araucaria imbricata.

Chandra et al[62] isolated three diterpenes (103), (104), (105) from the bark of Araucaria imbricata differing in the state of oxidation at C_{15}. The infrared spectrum of (103) revealed the presence of a hydroxyl, a vinylidene and a gemdimethyl group, whilst the ultraviolet spectrum indicated the presence of an ethylenic bond. Both oxygen atoms were found to be present as hydroxyl groups. Hydrogenation of the diol (103) gave a saturated dihydrodiol with the uptake of one mole of hydrogen. Ozonolysis afforded formaldehyde which confirmed the presence of an exocyclic methylene group. Acid isomerisation of (103) with sulphuric acid in methanol gave an isomeric diol, the infrared spectrum of which suggested that the original double bond had moved into a tetrasubstituted position typical of bicyclic terpenes containing a 8(17) double bond.[57] Dehydrogenation of (103) yielded 1:2:5-trimethylnaphthalene indicating that it belongs to the group of bicyclic diterpenes typified by labdanolic or eperuic acid. Acetylation and benzoylation gave oily esters, the infrared spectrum of which showed no hydroxyl absorption. Consequently the alcoholic functions in (103) are primary or secondary. Partial hydrolysis of the dihydrodiol diacetate gave a mono-acetate(106), which on oxidation gave an acetoxy-acid(107) without loss of carbon, thus proving the presence of a hydroxyl group. The acetoxy-acid(107) was saponified to a hydroxy-acid(108) which as its methyl ester was oxidized to a keto-ester(109). Reduction of the keto-ester(109) under (Wolff-Kishner conditions gave dihydrocativic acid(110), thus relating the stereochemistry of (103) to the labdane series

and establishing the position of primary hydroxyl at C_{15}.

The secondary hydroxyl group was placed by monoacetylation of the dihydrodiol to the monoacetate(111), which on dehydration with phosphorus oxychloride and subsequent ozonolysis yielded acetone, showing that (111) had undergone the retro-pinacolinic rearrangement associated with 3-β-hydroxy terpenoids having a 4-gemdimethyl group.[63] Accordingly the second hydroxyl group is placed at position 3 in the equatorial (β) configuration. This was confirmed by mass spectral and optical rotary dispersion studies.

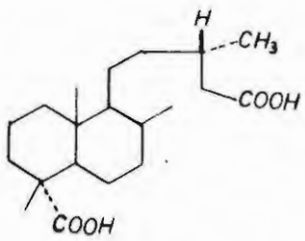
Compounds (104) and (105), also isolated from Araucaria araicana,[64,65] were related to (103) and their structures proved to be as shown.

1.22. The Levantenolides.

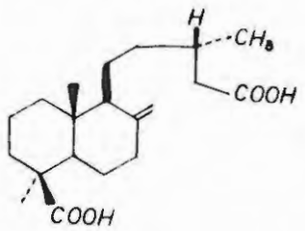
α_1-Levantenolide(112), $C_{20}H_{30}O_3$, α_2-levantenolide(113), $C_{20}H_{32}O_3$ and β-levantenolide(114), $C_{20}H_{30}O_3$, were isolated from Turkish tobacco by J.A. Giles et al[66,67] and shown to be diterpene lactones. α_1- and β-Levantenolide differ only in the fact that they are epimeric at C_{12}. Hydrogenation of α_1-levantenolide gave two epimers, one of which was identical with α_2-levantenolide which was shown to possess the 13-epi configuration and to be closely related to 12α-hydroxy-13-epimanoyl oxide isolated by J.A. Giles et al[68] from Turkish tobacco. α_2-Levantenolide was saponified and then reduced by the Wolff-Kishner method to an acid which gave with diazomethane an ester shown to be identical with methyl-13-epi-labdanolate(115)[69] of known 13S-configuration, proving its stereochemistry to be as shown.

1.23. Pinifolic acid.

Pinifolic acid(116), $C_{20}H_{32}O_4$, a diterpene acid was isolated from the needles of Pinus silvestris L. by Enzell and Theander.[70] Its infrared and ultraviolet spectra indicated



(117)



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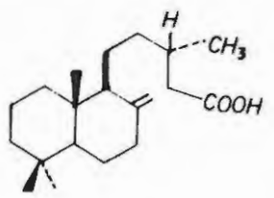

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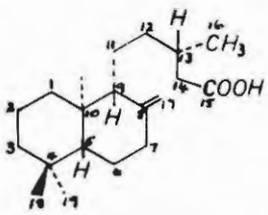

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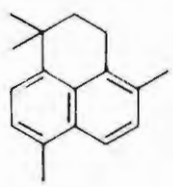

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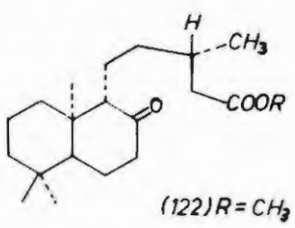
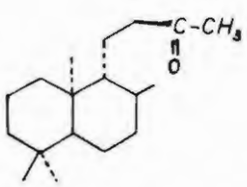


(122) R = CH3

(127) R = H



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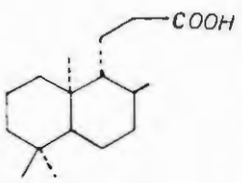
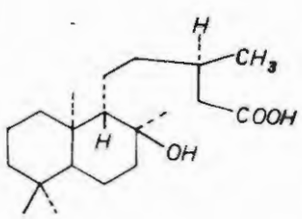


(124)



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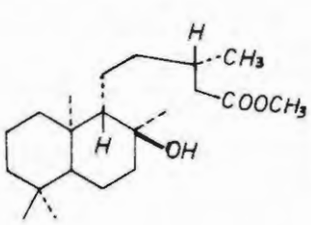
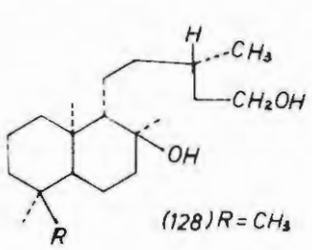
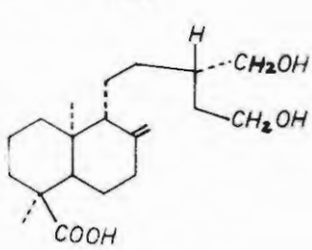


(126)

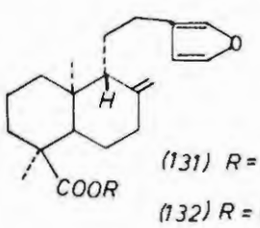


(128) R = CH3

(129) R = CH2OH

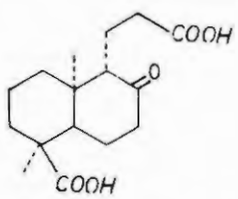


(130)



(131) R = H

(132) R = CH3



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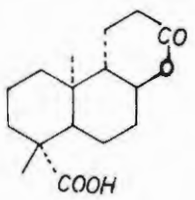

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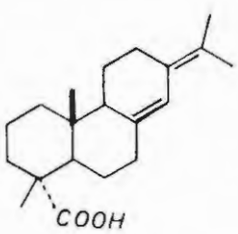

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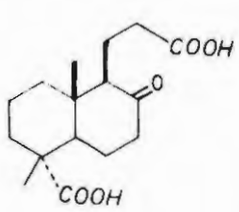

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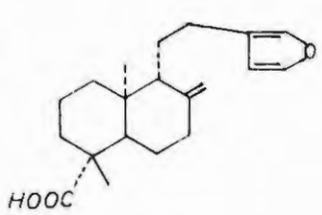


(137)

the presence of an isolated unsymmetrically disubstituted double bond and catalytic hydrogenation over Adams catalyst gave dihydropinifolic acid(117), while dehydrogenation with palladium/charcoal gave 1:2:5-trimethyl naphthalene. These results suggested that pinifolic acid was closely related to dihydroagathic acid(118), which had been prepared from agathic acid by reduction with sodium in butanol.[71] Pinifolic and dihydroagathic acids were found to have similar rotations but somewhat different Rf values. The mass spectra of their dimethyl esters suggested that the two acids were epimeric at C_4.

Pinifolic acid was related to labdanolic acid in the following way: The dimethyl ester of pinifolic acid on treatment with one equivalent of sodium hydroxide/methanol gave the monoester which on reduction with lithium/ammonia gave the hydroxy-acid. The methyl ester of the latter was oxidized to the oxo-ester which on Huang-Minlon reduction gave (119) shown to be identical with labd-8(17)-en-15-oic acid. Ozonolysis of (119) gave a keto-acid which was also identical in all respects with 17-nor-8oxo-labdan-15-oic acid, the absolute configuration of which had been proved.

The stereochemistry of pinifolic acid at C_4 followed from the above findings and at C_5, C_{10}, C_9 and C_{13} from its correlation with labdanolic acid.

1.24. Eperuic acid.

Eperuic acid(120), $C_{20}H_{34}O_2$, the chief constituent of the Wallaba tree, Eperua falcata and other Eperua species,[72] is closely related to cativic acid. Selenium dehydrogenation of the methyl ester yielded 1:2:5-trimethylnaphthalene and 1:1:4:7-tetramethylphenalan(121) thus characterising the carbon skeleton. Since eperuic acid absorbed one mole of hydrogen but showed no absorption at 210mμ the ethylenic bond and carboxyl group are not conjugated. Chemical proof of the position of

the double bond was obtained by ozonolysis and degradation experiments. Ozonolysis of methyl eperuate gave a keto-ester(122), $C_{20}H_{34}O_3$, together with formaldehyde and formic acid. The double bond must therefore be present as a vinylidene group.

The keto-ester(122) yielded pimanthrene(22) on dehydrogenation. A relation to the manoöl-agathic acid group of diterpenes is suggested and this was substantiated by a two step Barbier-Wieland degradation of the dihydro-ester to a methyl ketone(123), $C_{18}H_{32}O$. This was oxidized with sodium hypoiodite to a C_{17}-acid(124), shown to be antipodal to an acid isolated from ambrein and marrubiin.[4] This degradation proved rigidly the correctness of the bicyclic structure of eperuic acid and also the configuration at C_5, C_9 and C_{10}.

The configuration of the side chain follows from the work of Henrick and Jeffries[73] on a diterpene acid(125) isolated from Dononaea labulata, the ester of which is the antipode of methyl labdanolate. The physical constants of the crystalline hydroxy ester of (125) corresponded accurately with those expected for the antipode of methyl labdanolate. Methyl enantio-13-epi-labdanolate(126) was dehydrated to the exocyclic olefin which on ozonolysis and saponification gave the keto-acid(127). The physical constants of the oxime of (127) agreed closely with those of the corresponding oxime of eperuic acid, suggesting that eperuic acid has a carbon skeleton which is antipodal with lab-8(17)-ene 15-oic acid at all points excepting C_{13}. This postulate has been supported by Graham and Overton.[74]

Three new diterpenes have been isolated from Ricinocarpus muricatus by Henrick and Jeffries[73] and their structures have been established as eperuane-8β-15 diol(128), eperuane-8β,15, 18-triol.(129) and 15,16-dihydroxy-eperu-8(17)-ene 18-oic acid(130). Oxidation of (128) with Jones reagent gave an

acid, the methyl ester of which had an infrared spectrum identical with that of methyl 13-epi-labdanolate. However their optical rotations were $[\alpha]_D$ + 2° and $[\alpha]_D$ - 3° respectively, indicating an enantiomeric relationship.

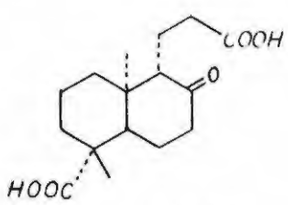
The isolation of the 15, 16 oxygenated eperuanes provides a biosynthetic link with the furano-diterpene polyalthic acid from R. stylosus and Polyalthia fragrans.

1.25. Polyalthic acid.

Polyalthic acid (131), $C_{20}H_{28}O_3$, isolated from Polyalthia fragrans[47] was dehydrogenated to 1:2:5-trimethyl naphthalene showing that it belonged to the group of bicyclic diterpenes typified by labdanolic or eperuic acid. Kuhn-Roth determination showed the presence of two methyl groups and Zerewitinoff analysis indicated the presence of only one active hydrogen. The presence of three double bonds was established by reduction to hexahydropolyalthic acid. Since methyl polyalthate (132) was fairly resistant to alkaline hydrolysis the carboxyl group in polyalthic acid is probably tertiary. The presence of a β-substituted furan ring was indicated by a positive Ehrlich colour reaction, ultraviolet, infrared and n.m.r. spectra. This was confirmed by the application of the Aldier-Rickert degradation which yielded furan 3:4-dicarboxylic acid.

Ozonolysis of polyalthic acid yielded formaldehyde and a six-membered ketone (infrared), showing that it possesses a methylene group attached to a six-membered ring. The relative locations of the methylene group and the β-monosubstituted furan followed from ozonolysis and oxidation of the derived keto-acid to the keto-dicarboxylic acid(133), which was reduced with sodium borohydride to the lactonic acid(134) showing bands in the infrared corresponding to these required for a δ-lactone.

The structure of polyalthic acid was confirmed and its stereochemistry settled by correlation with neo-abietic acid (135),[75] which on drastic ozonolysis, followed by oxidation



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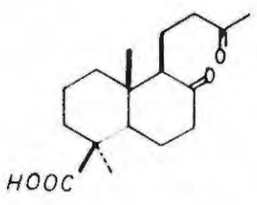

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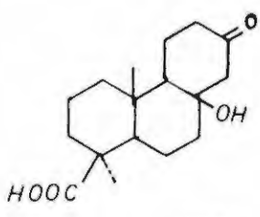

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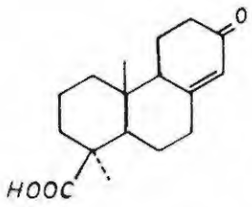

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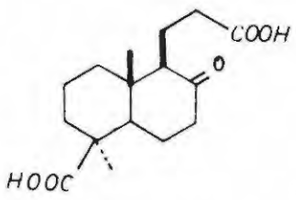

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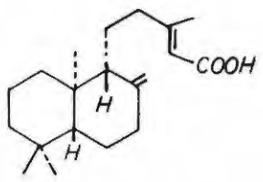

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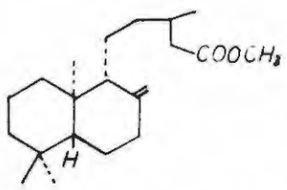

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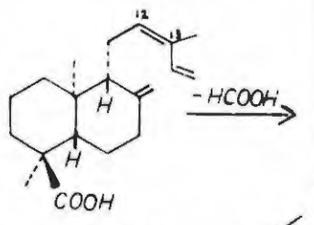

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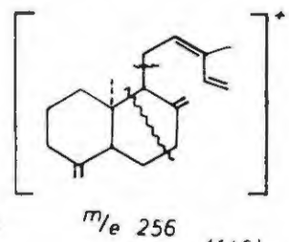
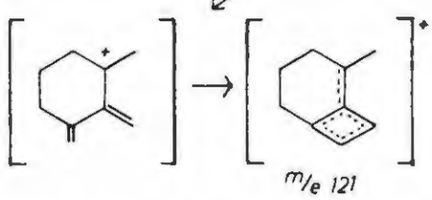


m/e 256 (146)



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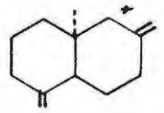

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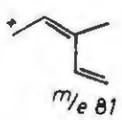

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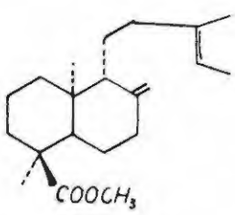

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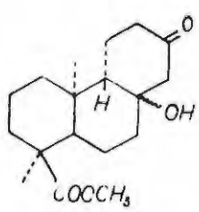

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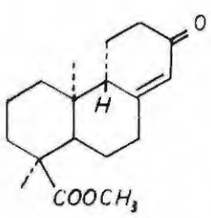

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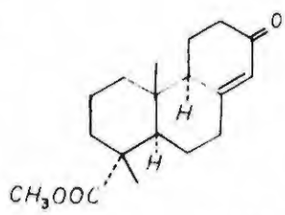

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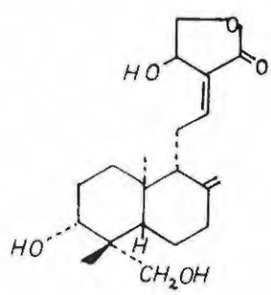


(154)

with alkaline hydrogen peroxide was converted to a keto-dicarboxylic acid(136). This acid possessed the same infrared and X-ray powder patterns as (133). The specific rotations were approximately equal, but opposite in sign showing that they are antipodes.

1.26. Daniellic acid.

Daniellic acid(137), $C_{20}H_{28}O_3$, occurring in Daniellic oliveri[48] is identical with polyalthic acid(131) excepting for the stereochemistry at C_4. Its structure and stereochemistry was proved by correlation with agathic acid of established structure and configuration.

Ozonolysis of daniellic acid yielded a keto-dicarboxylic acid(138), characterised by its p-phenylphenacyl ester. Agathic acid(40) was similarly converted to a diketo-acid(139), which with alkali gave a hydroxy-ketone(140) and an αβ-unsaturated ketone(141) (see mano81). The latter compound on ozonization yielded a keto-dicarboxylic acid(142), shown to have the same melting point and infrared spectrum as compound(138). Their specific rotations were of the same magnitude, but opposite in sign, indicating an antipodal relationship. The absolute stereochemistry of daniellic acid must therefore be as in (137). It can be again noted that lambertianic acid(97) was shown to be the optical antipode of daniellic acid.[59]

1.27. Copalic acid.

Copalic acid(143), $C_{20}H_{32}O_2$, was isolated from the "Brazil Copal", Hymenaea couvbaril L. by Nakano and Djerassi.[71] It is considered to be a mixture consisting of (143) and its double bond isomers. Hydrogenation resulted in the uptake of two equivalents of hydrogen, which indicated that it possessed two double bonds, one of which was shown to be present as an exocyclic methylene function, confirmed by ozonolysis and isolation of formaldehyde. The second double bond in methyl

copalate was shown to be conjugated with the ester function. Saponification of the methyl ester proceeded readily in contrast to the members of the abietic class of diterpenes to give copalic acid.

Selenium dehydrogenation yielded 1:2:5-trimethylnaphthalene and 1:1:4:7-tetramethylphenalan(121), a characteristic degradation product of agathic and cativic acids. Consequently structure(143) was proposed for copalic acid. As far as the absolute configuration is concerned the optical rotary dispersion results of the ketone derived from methyl dihydrocopalate(144) left no doubt that the configuration at C_5 and C_{10} correspond to that in eperuic acid rather than to those in cativic and labdanolic acids.

1.28. Ozic acid.

Ozic Acid(145), $C_{20}H_{30}O_2$, isolated from the wood of Daniella agea[76] contains, according to its n.m.r. spectrum, two tertiary methyl groups, one methyl group, one unsaturated carbon atom bearing no hydrogen and six vinyl hydrogens. The ultraviolet and infrared spectra showed the presence of a conjugated diene. On hydrogenation three moles of hydrogen were absorbed indicating the presence of three double bonds and hence two rings. These results were consistent with structure(145). The mass spectrum of ozic acid was also in agreement with the structure postulated and showed principal peaks at m/e 256, 175, 121 and 81 which correspond to the molecular ion and ion fragments (146), (147), (148) and (149) respectively. N.M.R. and infrared spectra of derivatives of ozic acid suggested that the carboxyl group at C_4 is equatorial. Reduction with sodium in propanol gave an isodihydro compound which on treatment with diazomethane gave a methyl ester(150). The infrared spectrum of (150) as well as that of methyl oziate showed only one strong band at 1240 cm^{-1} consistent with the

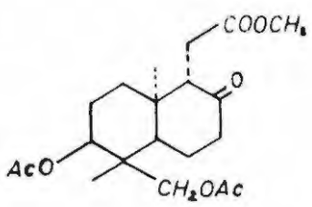
presence of an equatorial methoxy-carbonyl group.[77]

The stereochemistry at C_5, C_9 and C_{10} were inferred by analogy with daniellic acid and confirmed by correlation with neo-abietic acid. Ozonolysis of (150) gave a diketo compound, cyclized with alkali to the ketol(151) which on dehydration with sodium methoxide gave the αβ-unsaturated ketone(152). The infrared and n.m.r. spectra of (152) were identical with those of the αβ-unsaturated ketone from neo-abietic acid (153); the optical rotation was of the same magnitude but of opposite sign. The two compounds are therefore optical antipodes and this confirmed the configuration of ozic acid as shown in (145) leaving only the relative stereochemistry about the C_{12}-C_{13} double bond uncertain.

Ozic acid is similar to communic acid(55) except that it belongs to the less common stereochemical series (with respect to the steroids) and its carboxyl group is equatorial. If axial, it would be the optical antipode of communic acid.

1.29. Andrographolide.

Andrographolide(154), $C_{20}H_{30}O_5$, was isolated by Cava et al[78] from A. Paniculata. It was shown to contain three hydroxyl groups, a vinylidene group and an αβ-unsaturated lactone. Formation of 1:2:5:6-tetramethylnaphthalene on dehydrogenation suggested that it was a bicyclic diterpenoid with a hydroxyl at C_3. Oxidation of triacetylandrographolide with ozone or potassium permanganate followed by methylation gave a keto-ester diacetate(155). Reduction of the derived acid with sodium borohydride yielded a γ-lactone. Tritylation of andrographolide in pyridine afforded the anhydrotrityl ether(156) and this on oxidation and acid hydrolysis gave the nor-ketone(157) and formaldehyde by a retro-aldol cleavage characteristic of the diol system of ring A. The location of the third hydroxyl group and the nature of the αβ-unsaturated



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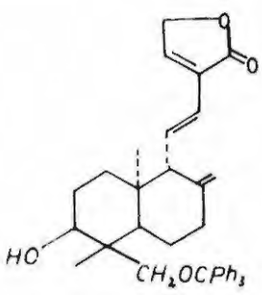

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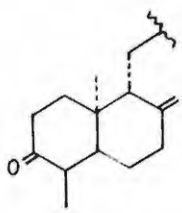

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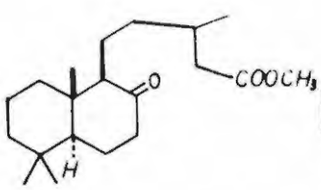

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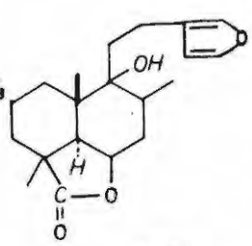

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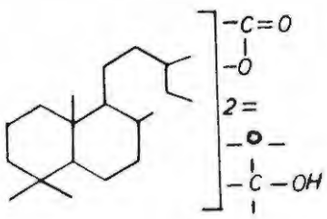

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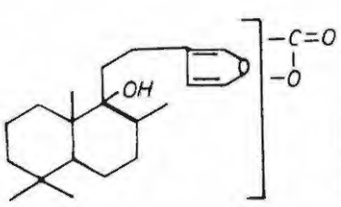

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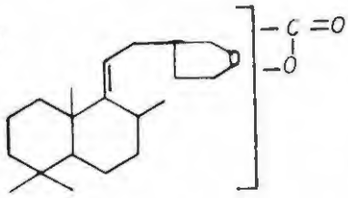

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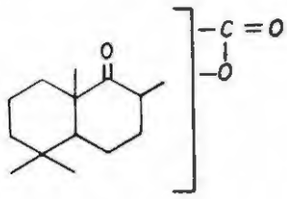

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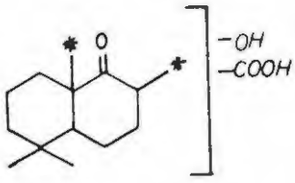

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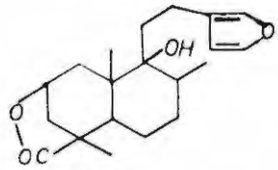

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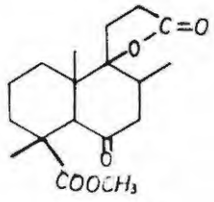

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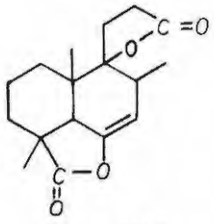

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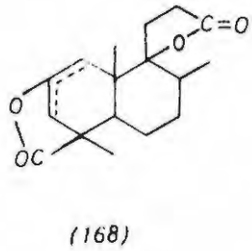

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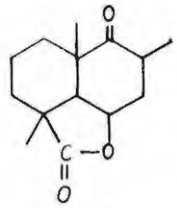

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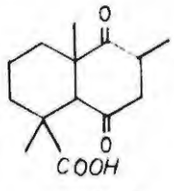

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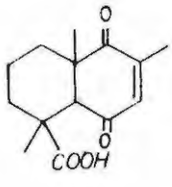

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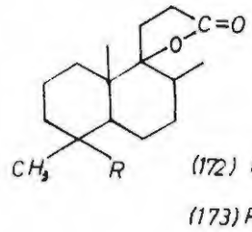


(172) R=COOH

(173) R=CHO

butenolide system are based upon the n.m.r. spectrum of triacetylandrographolide.[79]

The optical rotary disperion studies on the keto-ester (155) showed a strong positive effect, similar to that of a 2 keto-5α-steroid and enantiomeric with that of the keto-ester (158) from labdanolic acid. This indicated the antipodal stereochemistry for (154) as shown.

1.30. Marrubiin.

Marrubiin(159), $C_{20}H_{38}O_4$, the bitter principle of horehound was isolated in 1842. It is a lactone which undergoes ready hydrolysis to marrubic acid, $C_{20}H_{30}O_5$.[80,81,82]. Hydrogenation gave tetrahydromarrubiin indicating the presence of two double bonds in the molecule. One of the remaining oxygen atoms was presumed to be a tertiary hydroxyl group since marrubiin could be dehydrated with phosphorus trichloride or thionyl chloride to give anhydromarrubiin. The remaining oxygen atom present was unreactive and was presumed to be present in an oxide ring. Dehydrogenation with selenium gave 1:2:5-trimethylnaphthalene and this suggested a relationship to manoöl and agathic acid. However marrubiin differed from manoyl oxide and related compounds in that it did not form tricyclic derivatives. These results may be represented by the partial formula(160).

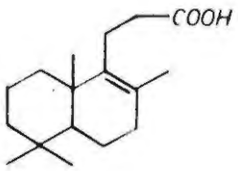
Oxidation of marrubiin with chromic acid in acetic acid gave a product $C_{17}H_{24}O_4$[83,84] (i.e. three carbon atoms were lost together with the inert oxygen). Since both double bonds were destroyed these results were best accommodated by a furan ring, which was confirmed by a study of the light absorption characteristics and colour reactions of marrubiin. The oxidation product was shown to be a di-γ-lactone[85] from which the partial structure(161) for marrubiin follows.

Support for the placing of the tertiary hydroxyl group was

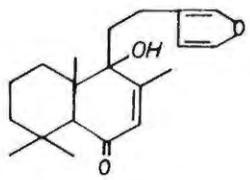
obtained by ozonolysis of anhydrotetrahydromarrubiin(162) to the keto-lactone(163), $C_{14}H_{20}O_3$. In this reaction the entire side chain was removed and the infrared spectrum of the product showed the presence of the original γ-lactone and a band at 1706 cm^{-1}, attributed to a carbonyl group in a six-membered ring. Hydrolysis of (163) gave a hydroxy-acid(164) which did not lose carbon dioxide readily and therefore was presumed not to be a β-keto-acid. Therefore the carbon atoms marked with an asterisk could not be carboxyl since both are β to the carbonyl group. Only the geminal positions remained with the two possible structures (159) and (165) for marrubiin. Evidence for structure (159) was obtained by Cocker et al[84] as follows Methyl marrubate was oxidized with chromic acid to the keto-lactonic ester(166), the acid of which was cyclised to an enol-lactone(167). This product was hydrolysed and reduced to a saturated acid in keeping with an enol-lactone formulation. Since the structure(168) derived from (165) would have been an infringement of Bredt's rule this alternative structure for marrubiin was rejected. The same conclusion and unambigious placing of the hydroxyl group was achieved by Hardy and Rigby.[85] Oxidation of anhydromarrubiin yielded a compound (169) ≡ (163) which on hydrolysis and oxidation gave the diketo-acid (170). This was then oxidized with selenium dioxide to the conjugated ene-dione(171), the ultraviolet spectrum of which (λ_{max} 242mμ) showing it to be in a cisoid arrangement.

Further proof of the structure of marrubiin was obtained by hydrogenolysis of the enol-ketone(167) to the acid(172), which was reduced by the Rosemund procedure to the aldehyde(173) Wolff-Kishner reduction (Huang Minlon procedure) of the aldehyde yielded as one of the products as unsaturated acid (174) found to be identical with a product of known structure and stereochemistry derived from ambrein.[87]

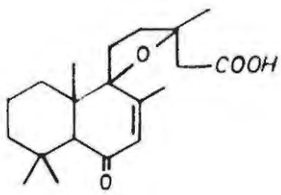
This combined evidence therefore establishes the structure



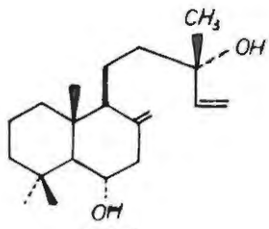
(174)



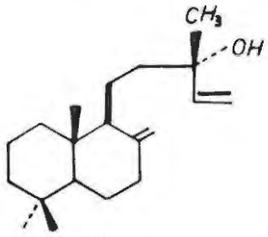
(174a)



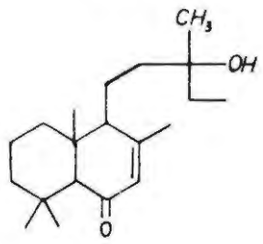
(174b)



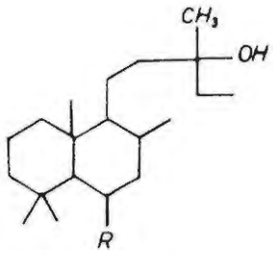
(175)



(176)



(177)



(177a) R = ····OH

(177b) R = O

(177c) R = — OH

of marrubiin unequivocally as (159) in which the stereochemistry at centres 8 and 9 is undefined.

1.31. Solidagenone.

Solidagenone, $C_{20}H_{28}O_3$, isolated from certain Solidago species[50,60] has very recently been shown to have structure (174a) on the basis of its molecular formula, spectroscopic and chemical evidence. The presence of a β-substituted furan and an α,β-unsaturated ketone was shown by spectral data. The ultraviolet spectrum is also consistent with the structure proposed. The presence of a hydroxyl group in solidagenone in close proximity to the furan ring is suggested by the infrared absorption and confirmed by n.m.r. data. Furthermore this hydroxyl group is tertiary since there is no resonance in the n.m.r. attributable to a proton of the type H-C-OH. The n.m.r. also discloses three quaternary methyls (3H singlets at τ 8.99, 8.85 and 8.81) and a singlet at τ 7.28 (IH) is attributed to the hydrogen at C_5. The failure to form derivatives of the tertiary hydroxyl and the sterically hindered ketone is expected for the structure proposed, as is the formation of a saturated ketone on lithium aluminium hydride reduction.

It may be noted that structure (174a) for solidagenone is biogenetically plausible since 6-oxogrindelic acid (174b) is found to be present in Grindelia species which are closely related to Solidaga both belonging to the Compositae.

1.32. Larixol.

Larixol (175), $C_{20}H_{33}O_2$, a diterpene alcohol, was isolated from the resin of the European larch, Larix europaea[90,91,92] and also from Larix sibirica.[93] The n.m.r. spectrum of larixol indicated that it was a manoöl derivative possessing an additional secondary hydroxyl group. Since 13-epimanoöl (176) had been found to be one of the main constituents of the oleoresin of L. europaea it was believed that larixol was of the

13-epimanoöl type. Treatment of larixol p-toluene sulphonate with excess lithium aluminium hydride yielded a product identified as 13-epimanoöl (176). This reaction settled the structure of larixol except for the position and relative configuration of the secondary hydroxyl group.

Hydrogenation of larixol using Raney nickel yielded 14-15-dihydrolarixol which on Jones oxidation afforded an oily α,β-unsaturated hydroxy-ketone (177), the rotary dispersion curve of which was similar to 6 oxo-cativic acid. The infrared spectrum of (177) showed no band due to an exocyclic double bond, but the ultraviolet had a maximum at 240 mμ. It was thus apparent that the 8(17)-double bond had isomerised during the oxidation into the 7:8 position. N.m.r. spectral data provided evidence for the 6 α-hydroxyl grouping. This was confirmed by hydrogenation (platinum catalyst) of larixol to tetrahydrolarixol (177a), m.p. 123-4°. Mild chromic acid oxidation gave the ketone (177b) which on reduction with lithium aluminium hydride afforded the alcohol (177c), m.p. 89-90°, which could be oxidised back to the ketone (177b). The alcohol (177c) must therefore be a 6-epimer of tetrahydrolarixol (177a). Hydride attack from the less hindered α-side of the ketone leads to the formation of the alcohol (177c) which must have the 6-β configuration. Accordingly tetrahydrolarixol has the 6 α-configuration.

Thus the structure and absolute configuration of larixol is as shown in (175).

2. EXPERIMENTAL

2.1. The extraction of Leonotis leonurus.

The plant material was collected at "Stone Crescent" about 5 miles to the west of Grahamstown during the period February 1962 to November 1966 and air-dried for 6-10 weeks. The following experiment is typical.

The dried material, collected on 15.10.1963 (13.2 lbs; 5950g) was steeped in cold acetone (25 gallons) for 3 days and the extract evaporated to approximately 8 litres by flash distillation. This extract was stirred with charcoal (May and Baker; 3 x 200g.), filtered through celite, and evaporated to a gum in a "Rotavapour". This gum gave a positive test for potassium (flame test) and for nitrate with diphenylamine and sulphuric acid indicating the presence of potassium nitrate. The gum was dissolved in benzene (300 ml.) and extracted with water (3 x 50 ml.) to remove the inorganic salts. The benzene extract was dried and evaporated to gum, which was dissolved in ethyl alcohol (400 ml.) and left for four days, whereupon the solution set semi-solid. The crystals were filtered off, washed with ethyl alcohol (100 ml.) and dried in a vacuum desiccator (yield 30.4g; 0.51%). The results of some other extractions are recorded in Table 1.

Table 1.

Date of collection.	Weight of material.	Yield of crystalline material.	Percentage yield.
19.11.62	10,000g.	186.5g.	1.87
24.11.62	4,590g.	85.0g.	1.85
1. 4.65	16,600g.	155.0g.	.97
1.11.65	12,150g.	160.0g.	1.32
2. 2.65	11,020g.	125.0g.	1.14

2.2. Purification of the crystalline solid from Leonotis leonurus.

The crystalline material (50.00g.) (obtained from the plant collected in November 1962) was dissolved in dry benzene. However, a certain portion did not go into solution. The undissolved solid was extracted with chloroform, the chloroform extract washed with water, dried (Na_2SO_4) and evaporated. The resulting gum crystallised from methanol to afford colourless needles (1.34g.), m.p. 232-4°. Repeated crystallisation from the same solvent afforded pure compound X (1.25g.), m.p.234-5°.

The benzene solution was poured onto a column, 4" in diameter, of neutral alumina (1000g) treated with dilute hydrochloric acid and activated at 180-190° for 24 hours). Details of the chromatogram are given in the graph (Figure 1) 300 ml. eluates being collected.

The compositions of the eluates were assessed by thin layer chromatography on silica gel (Merck's Kieselgel.G.) using hexane-ethyl acetate (5:2) as the mobile phase. The plates were sprayed with a 30% solution of chlorosulphonic acid in acetic acid, heated at 100° for 10 minutes and viewed under ultraviolet light to bring out the spots of compound X.

Fractions 6-15 were combined and crystallised from benzene (60 ml) to give prisms (7.0g.), m.p. 156°. Recrystallisation from benzene or ethanol to a constant melting point afforded prisms m.p. 160°, undepressed in admixture with authentic marrubiin. The infrared spectra were also identical.

Fractions 21-25, shown by thin layer chromatograms to consist chiefly of compound X, were combined and crystallised from ethanol to give needles (0.73g.), m.p. 230°.

Fractions 26-45 were combined and crystallised from benzene. Thin layer chromatograms showed the crystals (m.p. 120-125°) to be a mixture of marrubiin contaminated with a little of the

third component, compound Y.

Fractions 46-49 were combined and crystallised from ethyl alcohol to give needles, m.p. 98-100°, shown by thin layer chromatograms to consist mainly of compound Y contaminated with a little marrubiin.

Fractions 50-52 were combined and crystallised from ethyl alcohol to afford colourless needles (7.5g.), m.p. 113-4°, of compound Y, raised to m.p. 116° on recrystallisation from the same solvent.

Fractions 53-54 were combined and crystallised from ethyl alcohol to afford colourless needles (4.7g.) m.p. 112°, of compound Y, raised to m.p. 116° on recrystallisation from the same solvent.

Fractions 55-57 were combined but failed to yield a crystalline product

2.3. Purification of the crystalline solid using preparative thin-layer chromatography.

Two plates (1 mm. thick) were prepared by mixing kiesel gel G. (80g.) and distilled water (160 ml.). The crystalline compound (150 mgm.) was dissolved in chloroform, applied to the plate with a fine pipette and run using hexane-ethyl acetate (5:2) as the mobile phase. The spots were developed, on a small portion on either side of the plate, by spraying with 30% chlorosulphonic acid in glacial acetic acid. The spots corresponding to compounds Y and marrubiin were scraped off, extracted with chloroform and filtered through a celite pad. Removal of the solvent in each case yielded a gum which crystallised from ethyl alcohol to afford marrubiin (45 mgm.), m.p. 158-9°, and compound Y (40 mgm.), m.p. 114°, respectively.

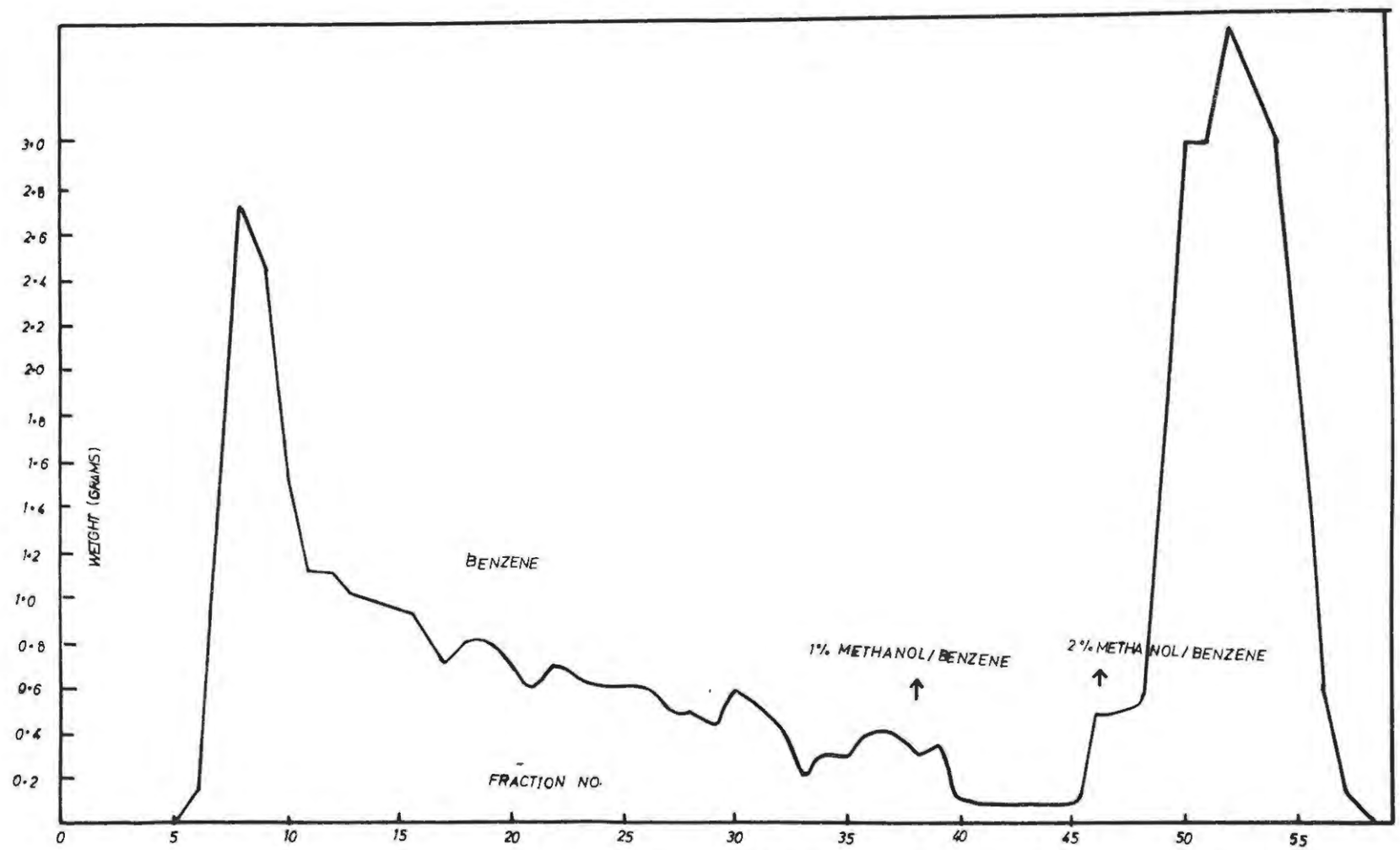


FIG 1. ELUTION CURVE.
CRYSTALLINE SOLID OF L.LEONURUS ON ALUMINA.

2.4 a. Separation of compound "K" using preparative thin-layer chromatography.

Compound "K" together with compounds Y and marrubiin was shown by thin layer chromatography to be present in various fractions obtained from the alumina column (experiment 2.2). Repeated column chromatography with neutral alumina failed to achieve separation. Plates (1 mm. thick) prepared by using silica gel H_{254} were prepared and approx. 90 mgm/plate of the mixture was applied in chloroform using hexane-ethyl acetate (8:2) as the mobile phase. The plates were dried and re-run in the same solvent to afford better separation. The spots corresponding to compound K, viewed under ultraviolet light were scraped off, the silica gel was extracted with 1% methyl alcohol-chloroform, filtered and the solution evaporated to a gum (250 mgm. from 16 plates). This gum failed to crystallise and was rechromatographed on neutral alumina (10g.) using dry benzene as eluant. The gum obtained from the chromatogram failed to crystallise and was distilled at $120^{\circ}/0.05$ mm. to yield a colourless oil which crystallised from dry hexane as prisms (120 mgm.), m.p. 64°, shown by identical infrared spectra and mixed m.p. to be anhydro Y (178).

2.4 b. Pure Compound Y (400 mgm.), was placed on a alumina column (20g.) in benzene and allowed to remain on the column for 7 days. Elution with 2% methanol-benzene afforded a gum which showed the presence of two distinct spots on a thin-layer chromatogram. Separation, by macro-thin-layer chromatography, yielded compound Y and anhydro-Y (50 mgm.), m.p. 68°, undepressed on admixture with an authentic sample of anhydro-Y.

2.5. Colour tests on compounds X, Y and marrubiin.

a) Lieberman Burchard test[84,95]

Compound X, Y and marrubiin (15 mgm.) were separately dissolved in chloroform (2 ml.), acetic anhydride (10 drops)

and concentrated sulphuric acid (2 drops). The results are recorded in Table 2 a.

b) Action of Chromic acid.

Compounds X, Y and marrubiin (25 mgm.) were separately dissolved in acetic acid (1 ml.) and chromic acid (0.01gm.). The results are recorded in Table 2 b.

c) Action of nitromethane.

Compounds X, Y and marrubiin (15 mgm.) were separately dissolved in nitromethane in chloroform. The results are recorded in Table 2 c.

Table 2 a.

Compound	Result	Conclusion
X	No colour change.	No furan ring present.
Y	Green colour produced.	Furan ring present.
Marrubiin	Green colour produced.	Furan ring present.

Table 2 b.

X	No colour change.	No tertiary hydroxyl group.
Y	Green colour produced.	Tertiary hydroxyl group present.
Marrubiin	Green colour produced.	Tertiary hydroxyl group present.

Table 2 c.

X	No colour change.	No unsaturation present
Y	Bright yellow colour produced.	Unsaturation present.
Marrubiin	Bright yellow colour produced.	Unsaturation present.

Compound Y.

Crystallised as colourless shiny needles from ethyl alcohol, m.p. 116°.

Found: C = 75.8, 75.8%

H = 9.1, 8.9%

Molecular Weight (Rast) 330

(Mass spectrogram) 316.

Three double bonds

$[\alpha]_D^{20} = -36^\circ$ (C, 1.2 in chloroform)

$\lambda_{max} = 245\ m\mu$ ($\varepsilon = 13,500$)

$\lambda_{max} = 211\ m\mu$ ($\varepsilon = 6,000$)

Calculated for $C_{20}H_{28}O_3$:

C = 76.0%

H = 8.9%

Molecular Weight = 316.

2.6. Semicarbazone of compound Y.

A solution of compound Y (100 mgm.) in ethyl alcohol (2 ml.) was added to a solution of sodium acetate (150 mgm.) and semicarbazide hydrochloride (100 mgm.) in ethyl alcohol (2 ml.) and water (6 drops) and left overnight. Water was added, the resulting precipitate collected and crystallised several times from ethyl alcohol to afford fine long needles, m.p. 179°.

Found: C = 67.19%

H = 8.56%

Calculated for $C_{21}H_{31}O_3N_3$:

C = 67.59%

H = 8.30%

2.7. Oxime of compound Y.

A solution of compound Y (50 mgm.) in ethyl alcohol (2 ml.) was added to a solution of hydroxylamine hydrochloride (50 mgm.) and sodium acetate (50 mgm.) in ethyl alcohol (1 ml.) and water (5 drops). The mixture was refluxed for two hours and the solvent removed under reduced pressure. The precipitate was dissolved in ethyl alcohol, and filtered to remove sodium

chloride. Crystals (45 mgm.) m.p. 165° deposited on the addition of water. Recrystallisation from ethanol-water afforded prisms (35 mgm.), m.p. 168°.

Found: C = 70.94%

H = 10.32%

N = 3.81%

Calculated for $C_{20}H_{35}O_3N$:

C = 70.99%

H = 10.69%

N = 4.16%

2.8. 2,4-Dinitrophenylhydrazone of compound Y.

A solution of compound Y (50 mgm.) in ethyl alcohol (3 ml.) was added to a hot solution of 2,4-dinitrophenylhydrazone (40 mgm.) in ethyl alcohol (3 ml.) containing one drop of concentrated sulphuric acid. The solution was refluxed for five minutes, the solvent removed and the residue crystallised several times from ethyl alcohol to afford fine bright red needles (α,β-unsaturated ketone), m.p. 152°.

Found: C = 62.71; 62.52%

H = 6.68; 6.47%

N = 10.71%

Calculated for $C_{26}H_{32}N_4O_6$

C = 62.91%

H = 6.05%

N = 11.29%

2.9. Attempted acetylation of compound Y.

Compound Y (55 mgm.) was dissolved in redistilled acetic anhydride (1 ml.) and dry pyridine (1 ml.), the solution heated for 2 minutes, and allowed to stand at room temperature for 48 hours. The solvents were removed under reduced pressure to yield a gum which crystallised from benzene as needles (50 mgm.),

m.p. 114-5° of compound Y, undepressed in admixture with an authentic specimen, m.p. 116°.

2.10 Dehydrogenation of compound Y.

Compound Y (2.00g.) was intimately mixed with 30% palladised charcoal (1.1g.) in a 25 ml. flask fitted with an air condenser 30 cm. in length. The apparatus was flushed with pure nitrogen and then heated at 300-320° for three hours when 250 ml. of gas evolved, chiefly during the first 1 1/2 hours. The reaction product was extracted with hexane (10 x 20 ml.), filtered through a celite pad and dried (Na_2SO_4). The filtered solution was evaporated to about 30 ml., poured onto a column (2 cm. diameter) of neutral alumina (35g.) and eluted with dry hexane. Details of the chromatogram are given in Table 3.

Table 3.

Fraction	Solvent (50 ml. fractions)	Weight.
1	Dry hexane.	10.0 mgm.
2	Dry hexane.	225.0 mgm.
3	Dry hexane.	94 mgm.
4	Dry hexane.	64 mgm.
5	Dry hexane.	15 mgm.
6	Dry hexane.	26 mgm.
7	Dry hexane.	35 mgm.

Fractions 1 and 2 were dissolved in hot ethyl alcohol (5 ml.) and added to a hot solution of trinitrobenzene (50 mgm.) in ethyl alcohol (2 ml.). No colour change resulted.

Fractions 3 and 4 were dissolved in hot alcohol (2 ml.) and added to a hot solution of trinitrobenzene (105 mgm.) in methyl alcohol (2 ml.). Yellow crystals (100 mgm.), m.p. 154°, separated on cooling and were recrystallised from methyl alcohol to give the trinitrobenzene adduct (39 mgm.), m.p. 160°, of

1:2:5-trimethylnaphthalene, undepressed in admixture with an authentic specimen.

Found: C = 59.40%

H = 4.87%

N = 10.44%

Calculated for $C_{19}H_{17}N_3O_6$:

C = 59.53%

H = 4.47%

N = 10.94%

The trinitrobenzene adduct (25 mgm.) was dissolved in hexane (10 ml.) and passed through a column of alumina (3g.). The oil (15 mgm.) remaining on evaporation of the eluate was dissolved in hot ethyl alcohol (1 ml.) and a solution of trinitrotoluene (10 mgm.) in ethyl alcohol (1 ml.) added. Yellow needles (14 mgm.), m.p. 88°, separated on cooling and were recrystallised from methyl alcohol to give the trinitrotoluene adduct (8 mgm.), m.p. 90°, of 1:2:5-trimethylnaphthalene, undepressed in admixture with an authentic specimen.

Found: C = 60.23%

H = 5.29%

Calculated for $C_{20}H_{19}O_6N_3$:

C = 60.45%

H = 4.82%

2.11. Attempted alkaline hydrolysis of compound Y.

a) Compound Y (300 mgm.) was refluxed with 0.4 N ethanolic sodium hydroxide (25 ml.) for 48 hours. The solution was cooled, acidified with dilute hydrochloric acid, water (25 ml.) added and the solution extracted with ether (3 x 50 ml.). The combined ethereal extracts were washed with water (2 x 25 ml.) and dried over Na_2SO_4. Removal of the solvent afforded a gum which crystallised from hexane. Recrystallisation from the

same solvent to a constant melting point gave prisms (178) (65 mgm.), m.p. 68-9° of anhydro-Y.

b) Compound Y (300 mgm.) was refluxed for one hour with potassium hydroxide (1g.) in diethylene glycol (10 ml.). The product was isolated as above to afford prisms, (95 mgm) m.p. 68-9°.

Found: C = 80.48; 80.62%

H = 8.99; 8.68%

λ_{max} (ethyl alcohol) = 250 mμ. (ε = 13,000).

Calculated for $C_{20}H_{26}O_2$:

C = 80.54%

H = 8.73%

2.12 Phosphorous trichloride dehydration of compound Y.

To a boiling solution of compound Y (300 mgm.) in benzene (6 ml.) was added freshly distilled phosphorous trichloride (.048 ml.) in benzene (0.6 ml.). The solution was heated for thirty minutes and poured into water (20 ml.). The aqueous layer was separated and extracted with benzene (3 x 10 ml.). The combined benzene extracts were washed with sodium hydroxide solution (2 x 10 ml.), water (2 x 10 ml.) and dried over Na_2SO_4. Removal of the solvent afforded a gum (150 mgm.) which was passed through a small column of neutral alumina (5g.). Elution with dry hexane afforded a gum which crystallised from hexane (40 mgm.), m.p. 68-9°, undepressed in admixture with anhydro-Y (178). The infrared spectra (chloroform) were also identical.

2.13 Chromic acid oxidation of compound Y.

Compound Y (1.5g.) in acetic acid (7.5 ml.) was cooled to 0°C and treated with chromic acid (3.75g.) in water (6 ml.) and acetic acid (20 ml.) and the solution left at room temperature for four days. The acetic acid was removed under reduced pressure, water (100 ml.) added and the solution extracted with

ether (4 x 50 ml.). The ethereal extract was washed with water (3 x 40 ml.) and extracted with sodium carbonate solution (3 x 50 ml.).

The ethereal solution was washed with water (2 x 30 ml.) and dried over Na_2SO_4. Removal of the solvent yielded a gum (0.25g) which crystallised from benzene-hexane as prisms (179) (200 mgm.), m.p. 134-5°, raised on recrystallisation from the above solvent to 135°.

Found: C = 73.60%

H = 8.89%

λ_{max} = 243 mμ (ε = 14,220)

Calculated for $C_{17}H_{24}O_3$:

C = 73.90%

H = 8.70%

The sodium carbonate extract was acidified with dilute hydrochloric acid and extracted with ether (3 x 75 ml.). The ethereal solution was washed with water (2 x 50 ml.) and dried over Na_2SO_4. Removal of the solvent yielded a solid which crystallised from aqueous ethanol as needles (180) (600 mgm.), m.p. 200°, raised on recrystallisation from the above solvent to 206-7°.

On attempted relactonisation of the acid with pyridine starting material was recovered.

Found: C = 73.68; 73.52%

H = 8.81; 8.80%

Equivalent weight = 280.

Calculated for $C_{17}H_{24}O_3$:

C = 73.90%

H = 8.70%

2.13 b. Micro hydrogenation of the acid (180).

The acid (14.55 mgm.), 10% palladium hydroxide/barium sulphate (15 mgm.) and ethyl alcohol (10 ml.) were shaken under

hydrogen for three hours when 2.153 mls. of hydrogen at N.T.P. (1.82 moles) were absorbed.

2.14 Lithium-ammonia reduction of compound Y.

A solution of compound Y (1.00g.) in ether-dioxane 1:1 (50 ml.) was added in a steady stream to a solution of lithium (110 mgm.) in liquid ammonia (125 ml.). The solution was stirred for thirty minutes and ammonium chloride (2.0g.) added slowly to destroy the excess lithium and the solution allowed to stand overnight. The residue in water (100 ml.) was extracted with ether (3 x 50 ml.). The ethereal extract was washed with dilute hydrochloric acid (2 x 50 ml.), water (3 x 50 ml.) and dried over Na_2SO_4. Removal of the solvent afforded a gum which crystallised from benzene-hexane as needles (181) (0.75g.) m.p. 140-1°, raised on further recrystallisation to 145-6° (0.70g.). $[\alpha]_D^{20}$ - 19.3° (c, 0.96 in chloroform).

Found: C = 75.44%

H = 9.47%

Calculated for $C_{20}H_{30}O_3$:

C = 75.43%

H = 9.50%

2.15 Chromic acid oxidation of lithium-ammonia reduction product of compound Y.

The compound (1.00g.) in acetic acid (5.00 ml.) at 0°C was treated with chromic acid (2.5g.) in water (4 ml.) and acetic acid (14 ml.) and the solution left at room temperature for four days. The acetic acid was removed under reduced pressure, water (60 ml.) added and the solution extracted with ether (3 x 50 ml.). The ethereal extract was washed with water (3 x 20 ml.) and extracted with sodium carbonate solution (3 x 30 ml.).

The ethereal solution was washed with water (2 x 20 ml.) and dried over Na_2SO_4. Removal of the solvent gave a gum

which crystallised from benzene-hexane as needles (182), m.p. 160°, raised on recrystallisation from the above solvent to 164-5°. $[\alpha]_D^{20}$ - 37.9° (c, 0.96 in chloroform).

Found: C = 77.32%

H = 10.48%

Calculated for $C_{17}H_{28}O_2$:

C = 77.22%

H = 10.67%

The sodium carbonate extract was acidified with dilute hydrochloric acid and extracted with ether (2 x 50 ml.). The ethereal solution was washed with water (2 z 20 ml.) and dried over Na_2SO_4. Removal of the solvent yielded a gum which could not be induced to crystallise.

2.16. Attempted Wolff-Kishner (Huang-Minlon modification) reduction of lactone (182).

To a solution of the compound (280 mgm.) in diethylene glycol (10 ml.) was added potassium hydroxide (310 mgm, 5.5 m.moles) in alcohol (2 ml.), 100% hydrazine hydrate (1.5 ml.) and the solution refluxed at 120° for one hour. The condensor was removed, the temperature slowly raised to 180-200°C, the condensor replaced and the solution refluxed for a further 4 hours. The solution was cooled, diluted with water and extracted with ether (3 x 40 ml.). The combined ethereal extracts were washed with water (2 x 20 ml.) and dried (Na_2SO_4). Removal of the solvent yielded a gum (87 mgm.) which failed to crystallise. The aqueous solution was acidified with dilute hydrochloric acid and extracted with ether (3 x 40 ml.). The combined ethereal extracts were washed with water (2 x 20 ml.) and dried (Na_2SO_4). Removal of the solvent yielded a gum (94 mgm.) which failed to crystallise.

After 24 hours the remaining aqueous solution deposited crystals (105 mgm.), m.p. 166-7°. This compound was an acid

containing an α,β-unsaturated keto-group (λ_{max} 249 mμ).

2.17 Sodium borohydride reduction of ketone (181).

To a stirred solution of compound (181) (800mgm.) in methanol (50 ml.) and benzene (5 ml.) at 0° was added an excess of sodium borohydride. The solvent was removed under reduced pressure and water (100 ml.) added. The solution was made faintly acid with dilute hydrochloric acid and the resulting suspension extracted with ether (3 x 75 ml.). The ethereal solution was washed with water (2 x 25 ml.) and dried over Na_2SO_4. Removal of the solvent afforded a gum (700 mgm.), which failed to crystallise but showed the presence of two spots on a thin layer chromatogram. Chromatography of the above gum on neutral alumina (25g.) yielded on elution with benzene a small amount of gum which crystallised from methanol as rhombs, m.p. 190°. Elution with 1% methanol-benzene yielded a gum (500 mgm.), which crystallised from benzene-hexane as rhombs, (183) m.p. 106-7°, raised on further recrystallisation from the above solvent to 108-9°.

Found: C = 74.96%

H = 10.01%

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

C = 74.96%

H = 10.06%

2.18 Chromic acid oxidation of the alcohol (183) (Jones method)

The compound (183) (150 mgm.) in acetone (15 ml.) was treated with 8N chromic acid in sulphuric acid (0.1 ml. - 1 m.m). After ten minutes, during which time the colour of the solution changed from an orange to green, water (60 ml.) was added and the solution left at 5° for one hour. The precipitate was filtered off, dried in a vacuum desiccator and crystallised from benzene-hexane to afford needles (90 mgm.), m.p. 142°, shown to be identical (mixed melting point and infrared spectra)

to the lithium-ammonia reduction product of compound Y (181).

2.19. Preparation of thioketal of lactone (182).

Ethanedithiol (0.3 ml.) and borontrifluoride-diethyl etherate (0.3 ml.) were added to a solution of the lactone (200 mgm.) in acetic acid (1 ml.) and the mixture left at room temperature for 48 hours. The crystalline precipitate (120mgm.) m.p. 237°, was filtered off and the filtrate evaporated to a gum which afforded a precipitate upon addition of methanol. This precipitate was dissolved in benzene (15 ml.), the solution washed with 5N sodium hydroxide solution (2 x 10 ml.), water (2 x 10 ml.) and dried over Na_2SO_4. Removal of the solvent gave a gum which crystallised from benzene-hexane as needles (70 mgm.), m.p. 237°. Recrystallisation of the combined product from benzene-hexane to a constant melting point afforded the thioketal (184), m.p. 240°.

2.20 Raney Nickel desulphurisation of thioketal (184).

A solution of the thioketal (250 mgm, m.p. 240°) in methyl alcohol-acetone (40 ml., 1:1) was heated under reflux for 15 hours with freshly prepared Raney nickel (3g.). The solution was filtered through a celite pad and the solvent removed under reduced pressure. The crystalline precipitate was dissolved in benzene and chromatographed on neutral alumina (8g.), using benzene-hexane (1:1) as eluant. Crystallisation from aqueous methanol afforded needles (175 mgm.) m.p. 93.5°-94°, raised on further recrystallisation from the same solvent to yield the lactone (185), m.p. 94°-94.5°, undepressed in admixture with authentic β-(1-hydroxy-2:5:5:9-tetramethyl-1-decalyl) propionic lactone supplied by Dr. Rigby. The infrared spectra were also identical.

Quantitative alkaline hydrolysis Experiments.

Name of Compd.	Weight of Compound.	Normality of Hydrochloric acid.	Normality of KOH.	Volume of hydrochloric acid required for back titration.	Volume of Potassium Hydroxide.	Volume of acid in blank.	Moles of alkali consumed /molc of compd.
Lactone (185)	79.5 mgm.	0.1015	.0959	13.4 ml.	20 ml.	16.3	.9780
Lactone of compound Y	69.7 mgm.	0.1015	.0959	13.8 ml.	20 ml.	16.3	1.034
Marrubiin	102.8 mgm.	0.0848	.1800	17.4 ml.	10 ml.	21.2 ml.	1.070
Compound X	148.6 mgm.	.1230 N	.320 N	18.25 ml.	10 ml.	25.7	2.15

Table 4.

2.31 Quantitative alkaline hydrolysis experiments.

The results are tabulated in Table 4.

a) Lactone of compound Y (179).

The compound (179) (69.7 mgm.) was refluxed with an ethanolic solution of potassium hydroxide (20 ml.) of known normality, for eight hours. The hot solution was neutralised with hydrochloric acid, of known normality, using phenolpthalein. The alcohol was removed under reduced presure, an excess of acid was added to the cold solution and the mixture allowed to stand at 0^{o} for one hour. The precipitate was filtered off and recrystallised from aqueous methanol to yield an acid (180), m.p. 202-3^{o}, shown by a mixed m.p. and infrared spectrum to be identical to the unsaturated acid (180) obtained on oxidation of compound Y.

b) Lactone (185).

An identical procedure was carried out as above. The precipitate which formed on acidification was recrystallised from aqueous methanol to afford needles, m.p. 93-4^{o}, undepressed on admixture with starting material.

c) Marrubiin.

An identical procedure was carried out as above. The product crystallised from aqueous methanol to afford needles of marrubic acid, m.p. 198^{o}(dec).

d) Compound X.

An identical procedure was carried out as above. The product obtained on acidification however failed to give a sharp melting point.

2.22 Hydrogenation of compound Y.

A mixture of compound Y (3.66g.), 10% palladium hydroxide-barium sulphate (4.0g.) and ethyl alcohol (200 ml.) was shaken under hydrogen for three hours when 773 ml. of hydrogen at N.T.P. (2.98 moles) were absorbed. The solution was filtered through

a celite pad and evaporated to a clear oil, which crystallised from hexane as needles (3.45g.), m.p. 104-5°. Recrystallisation from hexane to a constant melting point yielded hexahydro Y, m.p. 105°. $[\alpha]_D^{20}$ - 17.3° (c, 0.95 in chloroform).

Thin layer chromatography on silica gel plates, run in 20% ethyl acetate in hexane and developed by spraying with 30% chlorosulphonic acid in acetic acid, showed the presence of a single spot.

Found: C = 74.30%

H = 10.69%

λ_{max} = 279 mμ (ε = < 100)

Calculated for $C_{20}H_{34}O_3$:

C = 74.49%

H = 10.63%

2.23 2,4-Dinitrophenylhydrazone of hexahydro-Y.

A solution of hexahydro-Y (100 mgm.) in ethyl alcohol (3 ml.) was added to a hot solution of 2,4 dinitrophenylhydrazine (65 mgm.) in ethyl alcohol (3 ml.) containing one drop of concentrated sulphuric acid. The solution was refluxed for five minutes when a yellow precipitate formed. This was filtered off and recrystallised from ethyl alcohol to give yellow needles, m.p. 119-120°, raised on further recrystallisation from the above solvent to yield hexahydro-Y 2,4-dinitrophenylhydrazone, m.p. 120-1°.

Found: C = 61.31%

H = 7.71%

Calculated for $C_{26}H_{38}N_4O_6$:

C = 61.16%

H = 7.57%

2.24 Phosphorus trichloride dehydration of hexahydro-Y.

To a boiling solution of the compound (1.00g.) in dry benzene (20 ml.) was added phosphorus trichloride (0.25 ml.) in benzene (2.5 ml.). The solution was heated for thirty minutes under reflux, cooled and poured into water (50 ml.). The aqueous layer was separated off and extracted with benzene (3 x 20 ml.). The combined benzene extracts were washed with sodium hydroxide solution (2 x 20 ml.), water (2 x 20 ml.) and dried (Na_2SO_4). Removal of the solvent afforded a gum (0.85g.) which could not be induced to crystallise.

The gum (0.5g.) was distilled at 135°/0.5 mm to clear oil. The oil showed a λ_{max} at 250 mμ (ε = 8035), which indicated a mixture of endo- and exocyclic double bonds.

2.25 Hydrogenation of dehydrated hexahydro Y to compound (186).

A micro-hydrogenation was carried out by shaking a mixture of the compound (30.61 mgm.), 10% palladium hydroxide/$BaSO_4$ (30 mgm.) and ethyl alcohol (10 ml.) under hydrogen for 70 minutes when 2.035 ml. of hydrogen at N.T.P. (0.94 mols.) were absorbed. The solution was filtered through a celite pad and evaporated to a clear oil. (λ_{max} 279 mμ), which could not be induced to crystallise.

2.26 2,4-Dinitrophenylhydrazone of compound (186).

A solution of the above oil (25 mgm.) in ethyl alcohol (2 ml.) was added to a hot solution of 2,4-dinitrophenylhydrazine (20 mgm.) in ethyl alcohol (2 ml.) containing one drop of concentrated sulphuric acid. The solution was refluxed for five minutes when a yellow precipitate formed. This was filtered off and recrystallised from ethyl alcohol to give yellow needles (20 mgm.), m.p. 159°, raised on further recrystallisation to 160-1°.

Found: C = 64.06%

H = 7.92%

Calculated for $C_{20}H_{38}N_4O_5$:

C = 64.00%

H = 7.82%

2.27 Action of methyl magnesium iodide on hexahydro-Y.

A solution of the compound (3.2g., 10 m.moles) in dry tetrahydrofuran (20 ml.) and dry ether (100 ml.) was added dropwise to the grignard reagent, prepared by reacting magnesium (1.0g., 40 m.moles) and methyl iodide (2.5 ml., 40 m. moles), and stirred for 90 minutes at room temperature . After standing for 24 hours the solution was poured into ice-cold water (100 ml.) and ammonium chloride (5.0g.) was cautiously added. The aqueous layer was separated and extracted with ether (3x100 ml.). The combined ethereal extracts were washed with water (2 x 50 ml.) and dried over Na_2SO_4. Removal of the solvent afforded a solid which crystallised from ethyl acetate as prisms (187) (2.6g.), m.p. 162-3°, raised on recrystallisation from the above solvent to 167-8°. $[\alpha]_D^{20}$ - 17.3° (c, 0.95 in chloroform).

Found: C = 74.08%

H = 11.00%

Calculated for $C_{21}H_{38}O_3$:

C = 74.51%

H = 11.31%

2.28 Dehydrogenation of alcohol (187).

The compound (187) (2.02g.) was intimately mixed with 30% palladised charcoal (1.1g.) in a 25 ml. flask fitted with an air condenser 30 cm. in length. The apparatus was flushed with pure nitrogen and then heated at 300-320° for two hours when 340 ml. of gas was evolved. The reaction product was extracted with hexane (10 x 25 ml.), filtered through a celite pad and dried (Na_2SO_4). The filtered solution was evaporated to approx. 30 ml., poured into a column (2 cm. diameter) of

neutral alumina and eluted with dry hexane, collecting 50 ml. fractions.

Fraction 3 yielded an oil (480 mgm.) which was dissolved in hot ethyl alcohol (8 ml.) and added to a hot solution of trinitrobenzene (120 mgm.) in ethyl alcohol (4 ml.). Yellow needles (120 mgm.), m.p. 169-171°, separated on cooling and were recrystallised from ethyl alcohol to give the trinitrobenzene adduct (45 mgm.), m.p. 177-8°, of 1:2:3:5-tetramethylnaphthalene. Admixture with an authentic specimen, m.p. 180°,[96] gave a mixed m.p. 173-80°.

Compound X

Crystallised as fine needles from ethyl alcohol, m.p. 234°.

Found: C = 69.50, 68.9, 63.96, 68.63%

H = 8.3, 8.0, 8.19, 8.24%

Molecular weight (Rast) 345

(Mass spectrogram) 348

No double bonds

$[\alpha]_D^{20}$ = + 10.6° (c, 1.4 in chloroform).

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

C = 69.00%

H = 8.1%

Molecular Weight = 348.

2.29 Lithium aluminium hydride reduction of compound X.

A solution of compound X (1.5g.) in 200 ml. of tetrahydrofuran (dried over sodium and distilled over lithium aluminium hydride) was refluxed with lithium aluminium hydride (3.0g.) for 24 hours. The solution was cooled, the excess hydride decomposed by adding wet ether (100 ml.), followed by dilute sulphuric acid (100 ml.). The aqueous layer was extracted with ether (3 x 100 ml.), the combined ethereal extracts were washed with sodium bicarbonate solution (2 x 50 ml.), water (2 x 50 ml).

and dried (Na_2SO_4). Removal of the solvent yielded a precipitate (1.1g.) which crystallised from aqueous methanol to yield prisms (188), (1.1g.), m.p. 205°, raised on recrystallisation from the same solvent to 207-8°.

Found: C = 66.94, 67.19%

H = 9.65, 10.04%

Calculated for $C_{20}H_{36}O_5$:

C = 67.38%

H = 10.18%

2.30 Dehydrogenation of compound X.

Compound X (1.00g.) was intimately mixed with 30% palladised charcoal (540 mgm.) in a 25 ml. flask fitted with an air condenser 30 cm. in length. The apparatus was flushed with pure nitrogen and then heated at 300-320° for 2 hours when 180 ml. of gas was evolved. The reaction product was extracted with hot hexane (10 x 20 ml.), filtered through a celite pad and dried (Na_2SO_4). The filtered solution was evaporated to 25 ml., poured onto a column (2 cm. diameter) of neutral alumina (30g.) and eluted with dry hexane. Details of the chromatogram are given in Table 5.

Table 5.

Fraction	Solvent (50 ml. fractions)	Weight
1	Dry hexane	-
2	Dry hexane	22 mgm.
3	Dry hexane	96 mgm.
4	Dry hexane	10 mgm.

Fraction 3 was dissolved in hot ethyl alcohol (5 ml.) and added to a hot solution of trinitrobenzene (60 mgm.) in ethyl-alcohol (2 ml.). Yellow crystals (55 mgm.), m.p. 157° separated on cooling and were recrystallised from methyl alcohol to afford needles of the trinitrobenzene adduct (35 mgm.), m.p. 159-60°, of 1:2:5-trimethylnaphthalene; undepressed in admixture

with an authentic specimen.

The trinitrobenzene adduct (30 mgm.) was dissolved in hexane (10 ml.) and passed through a column of alumina (3g.). The oil (12 mgm.) remaining on evaporation of the eluate was dissolved in hot ethyl alcohol (1 ml.) and a solution of trinitrotoluene (14 mgm.) in ethyl alcohol (1 ml.) added. Yellow needles (12 mgm.), m.p. 87°, separated on cooling and were recrystallised from methyl alcohol to give the trinitrotoluene adduct (7.0 mgm.), m.p. 89-90°, of 1:2:5-trimethylnaphthalene, undepressed in admixture with an authentic specimen.

2.31 Alkaline hydrolysis of compound X.

Compound X (500 mgm.) was refluxed with ethyl alcohol (65 ml.) and N/1 sodium hydroxide (20 ml.) for 16 hours, water (50 ml.) added and the alcohol removed under reduced pressure. The aqueous solution was acidified (to pH3) with dilute hydrochloric acid, potassium carbonate added and the alkaline solution digested for 3 hours at 60°. The solution was filtered through hardened filter paper and extracted with ether (2 x 50 ml.) to remove any unchanged material. The aqueous solution was acidified with dilute hydrochloric acid and extracted with ether (4 x 50 ml.), the combined ethereal extracts washed with water (2 x 30 ml.) and dried (Na_2SO_4). Diazomethane in ether and methanol (10 ml.) was added to a cooled solution of the ethereal extract and the solution allowed to stand for 30 minutes. Removal of the solvents yielded a gum which crystallised from benzene-hexane as prisms (189) (139 mgm.), m.p. 167°, raised on recrystallisation from the above solvent to 168-9°.

Found: C = 66.51%

H = 8.84%

Calculated for $C_{21}H_{32}O_6$:

C = 66.3%

H = 8.42%

2.32 Attempts at fission of the ether linkage in:

(a) Compound X.

(1) Action of hydroiodic acid.

Compound X (200 mgm.) was mixed with pure hydroiodic acid (6 ml.) and carbon dioxide was passed through the solution for 25 minutes. The solution was heated for one hour at 150°, cooled, diluted with water and extracted with chloroform (3 x 30 ml.). The combined chloroform extracts were washed with sodium thiosulphate solution (4 x 40 ml.), water (2 x 40 ml.) and dried (Na_2SO_4). Evaporation of the solvent afforded a yellow colloidal gum which failed to yield any recognisable product.

(2) Action of acetic anhydride and p-toluene sulphonic acid.

To a solution of compound X (42 mgm.) in freshly distilled acetic anhydride (2.5 ml.) was added p-toluene sulphonic acid (45 mgm.). The solution was heated for one hour at 120°, cooled and evaporated to approx. 1 ml. Water (3 ml.) was added and the solution allowed to stand for a few hours. Crystals deposited, which were filtered off and recrystallised from methanol to afford needles, (35 mgm.), m.p. 234°, undepresed on admixture with starting material.

(3) Action of ammonia.

Dry ammonia was passed through a solution of compound X (100 mgm.) in chloroform (2 ml.) and ethyl alcohol (5 ml.) for two hours and the solution allowed to stand for 24 hours. Evaporation of the solvent gave a gum which crystallised from ethyl alcohol as needles (90 mgm.), m.p. 234°, undepressed on admixture with starting material.

(4) Action of borontrifluoride-diethyl etherate.[97]

Borontrifluoride-diethyl etherate (5 drops) was added to a solution of compound X (60 mgm.) in dry tetrahydrofuran (4 ml). The solution was heated under reflux for one hour, allowed to

remain at room temperature for 48 hours, water (10 ml.) added and the solution extracted with ether (3 x 20 ml.). The combined ethereal extracts were washed with 10% aqueous sodium bicarbonate (2 x 10 ml.), water (2 x 10 ml.), and dried (Na_2SO_4). Removal of the solvent yielded a solid which crystallised from ethyl alcohol as needles (42 mgm.), m.p. 231°, undepressed in admixture with compound X. The infrared spectra were also identical.

(b) Lithium aluminium hydride reduction product of compound X.

(1) Action of hydrochloric acid.

The compound (188) (20 mgm.) in ethyl alcohol (4 ml.) and conc. hydrochloric acid (1 ml.) was refluxed under nitrogen for 4 hours. Water was added, the alcohol evaporated and the aqueous solution extracted with ether (3 x 20 ml.) The combined ethereal extracts were washed with water (2 x 10 ml.) sodium carbonate solution (2 x 10 ml.), water (2 x 10 ml.) and dried (Na_2SO_4). Removal of the solvent yielded a gum which failed to crystallise. Thin layer chromatography on silica gel plates, run in 15% ethyl acetate in hexane and developed by spraying with 30% chlorosulphonic acid in acetic acid, showed the presence of at least five distinct spots.

This experiment was repeated with varying concentrations of acids, using different temperatures and pressures. However isolation of any recognizable product proved unsuccessful.

(2) Action of borontrifluoride diethyl etherate.[97]

To a solution of compound (188) (42 mgm.) in acetic anhydride (2 ml.) and dry ether (1 ml.) at 0°C. was added freshly distilled borontrifluoride diethyl etherate (0.4 ml.), previously cooled to 0°C, and the solution allowed to stand at 0°C for 24 hours. The mixture was poured into ice-water, allowed to stand for a few hours and extracted with ether (3 x 15 ml.). The combined ethereal extracts were washed with sodium carbonate

solution (2 x 10 ml.), water (2 x 10 ml.) and dried (Na_2SO_4). Removal of the solvent yielded a gum which failed to crystallise. The gum was dissolved in an alcoholic solution of potassium hydroxide (10%), allowed to stand overnight and poured into water. The alcohol was removed and the aqueous solution extracted with ether (3 x 20 ml.). The combined ethereal extracts were washed with dilute hydrochloric acid (2 x 15 ml.), water (2 x 15 ml.) and dried (Na_2SO_4). Removal of the solvent afforded a gum which again failed to crystallise. Thin layer chromatography on silica gel plates, run in 50% ethyl acetate in hexane and developed by spraying with 30% chlorosulphonic acid in acetic acid, showed the presence of a large proportion of starting material.

2.33 N,N'-dicyclohexylcarbodiimide oxidation of tetrol (133).

The compound (460 mgm.) was dissolved in dimethyl-sulfoxide (4 ml.) and benzene (4 ml.) containing pyridine (0.4 ml.) and trifluoracetic acid (0.2 ml.). After addition of NN'-dicyclohexylcarbodiimide (3.4g.) the sealed reaction mixture was left at room temperature for 12 hours. Ether (100 ml.) was added followed by a solution of oxalic acid (1.0g.) in methanol (10 ml.). After gas evolution had ceased, approximately 30 minutes, water (100 ml.) was added and the insoluble dicyclohexylurea removed by filtration. The organic phase was washed with sodium bicarbonate solution (2 x 50 ml.), water (2 x 50 ml.) and dried (Na_2SO_4). Removal of the solvent afforded a gum (400 mgm.) which failed to crystallise. Chromatography of the above gum on neutral alumina (20g.) yielded on elution with benzene a gum which crystallised from aqueous methanol as needles, m.p. 196°, raised on recrystallisation from the above solvent to 199-200°. A mixed melting point with starting material showed a strong depression (m.p. 180°).

Marrubiin.

Crystallised as prisms from benzene, m.p. 160°.

Found: C = 72.4%

H = 8.7%

Molecular weight (Mass spectrogram) = 332

2 double bonds

$[\alpha]_D^{20}$ = + 33.3° (c, 1.0 in chloroform)

Calculated for $C_{20}H_{28}O_4$:

C = 72.2%

H = 8.4%

Molecular weight = 332

2.34 Lithium aluminium hydride reduction of marrubiin.

A solution of marrubiin (1.5g.) in tetrahydrofuran (50 ml.) (dried over sodium and distilled over lithium aluminium hydride) was refluxed with lithium aluminium hydride (1.5g.) for 20 hours. Excess lithium aluminium hydride was added at periodic intervals to ensure excess was present. The solution was cooled, the excess hydride decomposed by adding wet ether (50 ml.) followed by dilute sulphuric acid (50 ml.). The aqueous layer was extracted with ether (5 x 50 ml.), the combined ethereal extracts washed with sodium bicarbonate solution (2 x 50 ml.), water (2 x 50 ml.) and dried (Na_2SO_4). Removal of the solvent yielded a gum which crystallised from aqueous ethanol as needles to afford marrubenol (190) (1.45g.) m.p. 134-5°, raised on recrystallisation from the same solvent to 136-7° (Cocker et al[84] record m.p. 138°).

2.35 Hydrogenation of marrubenol.

A mixture of marrubenol (1.492g.), 10% palladium hydroxide-barium sulphate (1.5g.) and ethyl alcohol (100 ml.), was shaken under hydrogen for three hours when 176 ml. of hydrogen at N.T.P. (1.90 moles) were absorbed. The solution was filtered through a celite pad and evaporated to an oil which crystallised

from aqueous ethyl alcohol as needles of marrubanol, m.p. 140°-1°, raised on recrystallisation to m.p. 142°. $[\alpha]_D^{20}$ + 15.7° (c, 0.96 in chloroform). (Cocker et al[84] record m.p. 174°).

Found: C = 70.84%

H = 10.54%

Calculated for $C_{20}H_{36}O_4$:

C = 70.60%

H = 10.60%

2.36 Mesylation of marrubenol.

An ice-cold solution of methanesulphonyl chloride (1.00 ml, 4 m.moles) in pyridine (2.5 ml.) was added to marrubenol (660 mgm., 2 m.moles) in dry pyridine (5 ml.) at 0°C, and allowed to stand for 12 hours at 0°C. The solution was poured into ice-water and the oily precipitate extracted with ether (3 x 25 ml.). The combined ethereal extract was washed with cold 5% hydrochloric acid (2 x 10 ml.), water (2 x 10 ml.), sodium bicarbonate solution (2 x 10 ml.), water (2 x 10 ml.) and dried (Na_2SO_4). Removal of the solvent yielded a gum which crystallised from dry hexane as needles (191) (300 mgm.), m.p. 119°, raised on recrystallisation from the same solvent to afford needles m.p. 122°, which gave a negative test for the presence of sulphur.

Found: C = 75.16%

H = 9.40%

Calculated for $C_{20}H_{30}O_3$:

C = 75.43%

H = 9.50%

2.37 Tosylation of marrubenol.

An ice-cold solution of p-toluene sulphonyl chloride (1.520g.) in pyridine (3 ml.) was added to marrubenol (660 mgm.) in pyridine (5 ml.) at 0°C and the mixture kept at 0°C for

48 hours. The solution was poured into ice-water and worked up in an identical manner as for the "mesylate". Crystallisation from hexane afforded needles (360 mgm.), m.p. 121-2° undepressed on admixture with compound (191).

2.38 Action of borontrifluoride diethyl etherate on ether (191).

To a solution of the compound (191) (100 mgm.) in dry ether (14 ml.) at 0° was added freshly distilled borontrifluoride diethyl etherate (15 drops), and the solution allowed to stand at 0° for 12 hours. The ethereal solution was washed with water (2 x 5 ml.), sodium carbonate solution (2 x 5 ml.), water (2 x 5 ml.) and dried (Na_2SO_4). Removal of the solvent afforded a gum which crystallised from hexane as needles (90 mgm) m.p. 122°, undepressed on admixture with starting material.

2.39 Phosphorus trichloride dehydration of tetrahydromarrubiin.

To a boiling solution of tetrahydromarrubiin (2.00g.) in benzene (40 ml.) was added freshly distilled phosphorus trichloride (0.5 ml.) in benzene (2 ml.). The solution was heated for thirty minutes and poured into water (100 ml.). The aqueous layer was separated and extracted with benzene (2 x 50 ml.). The combined benzene extracts were washed with 10% sodium hydroxide solution (3 x 50 ml.), water (3 x 50 ml.) and dried (Na_2SO_4). Removal of the solvent yielded a gum which deposited needles (450 mgm.), m.p. 121°, upon lixiviation with dry ether. Recrystallisation from ether afforded needles of anhydro-tetrahydromarrubiin (400 mgm.), m.p. 124°, which were depressed on admixture with starting material.

2.40 Phosphorus oxychloride dehydration of tetrahydromarrubiin.

A solution of tetrahydromarrubiin (1.0g.) in pyridine (20 ml.) was treated with phosphorus-oxychloride (6 ml.) and heated at 90° for two hours. The solution was cooled, poured into water (100 ml.) and extracted with chloroform (2 x 75 ml). The combined chloroform extracts were washed with dilute hydrochloric

acid (2 x 50 ml.), water (2 x 50 ml.) and dried (Na_2SO_4). Removal of the solvent afforded a gum which failed to crystallise. Thin layer chromatography showed the presence of two distinct spots, one of which corresponded to anhydrotetrahydromarrubiin (exp. 2.38). The major dehydration product was purified by alumina chromatography but failed to crystallise.

2.41 Ozonolysis of anhydrotetrahydromarrubiin.

Anhydrotetrahydromarrubiin (280 mgm.) was ozonized in acetic acid (50 ml.), the solvent removed and the residue decomposed with zinc and steamdistilled. The residual crystalline material was filtered off, dissolved in benzene (25 ml.) and washed with potassium hydrogen carbonate solution (2 x 10 ml.), water (2 x 10 ml.) and dried (Na_2SO_4). Removal of the solvent afforded the ketone (192) which crystallised from methanol as prisms (80 mgm.), m.p. 194°. The ketone formed a 2,4-dinitrophenyl hydrazone m.p. 252° (Hardy, Rigby and Moody give m.p. 250-251.5°).

2.42 Reaction of marrubiin with zinc dust and acetic acid.[98]

Acid-washed zinc dust[99] (1.00g.) was added to a solution of marrubiin (100 mgm.) in acetic acid (20 ml.) and the mixture heated at 100° for 84 hours with constant stirring. Fresh zinc dust was added in 400 mgm. portions at 12 hour intervals during the heating period. The mixture was cooled, filtered, the acetic acid removed, water added and extracted with ether. The combined ethereal extracts were washed with water and dried (Na_2SO_4). Removal of the solvent yielded a gum which on a thin layer chromatogram showed the presence of only starting material.

2.43 Alkaline hydrolysis of marrubiin.

Marrubiin (5.0 grams) was refluxed with 10% alcoholic sodium hydroxide (100 ml.) for 16 hours. After removal of the alcohol the acid was liberated with hydrochloric acid and extrac-

ted with ether (3 x 50 ml.). The combined ethereal extracts were washed with water (3 x 30 ml.) and dried (Na_2SO_4). Removal of the solvent afforded a gum which crystallised from aqueous methanol as fine needles of marrubic acid (4.5g.), m.p. 198° (dec.), raised on recrystallization to 203-4° (dec.). (Lawson and Eustice[81] record 205° (dec.)).

2.44 Action of chlor-acetyl chloride on marrubic acid.

Chlor-acetyl chloride (1 ml.) was added to a solution of marrubic acid (250 mgm.) in dry tetrahydrofuran (5 ml.) and the mixture allowed to stand at room temperature for 24 hours. The solution was poured into water (20 ml.), allowed to stand for 30 minutes and extracted with ether (2 x 30 ml.). The combined ethereal extracts were washed with water (2 x 15 ml.) and dried (Na_2SO_4). Removal of the solvent yielded a gum (300 mgm.) which was chromatographed on neutral alumina (15g.) using $^1/_2$% methanol-benzene as eluant. Crystallisation from benzene-hexane afforded prisms (193) (155 mgm.), m.p. 121-6°, raised on recrystallisation from the above solvent m.p. 128°-30°.

2.45. Preparation of iodoacetyl marrubic acid.

The chloracetate (193) (150 mgm.) and finely powdered potassium iodide (1.0 g.) in acetone (25 ml.) was refluxed for 3 hours. The mixture was cooled, poured into water (50 ml.), the acetone removed and the solution extracted with ether (2 x 40 ml.). The combined ethereal extracts were washed with water (2 x 20 ml.) and dried (Na_2SO_4). Removal of the solvent gave a gum which was chromatographed on neutral alumina (15 g.) using 1% methanol-benzene as eluant. Crystallisation from benzene-hexane afforded needles (194) (120 mgm.), m.p. 92°, raised on recrystallisation from the same solvent to 92-3°.

Found: C = 54.36, 54.15%

H = 6.09, 6.23%

I = 21.80, 21.71%

Calculated for $C_{22}H_{31}O_6I$:

C = 50.97%

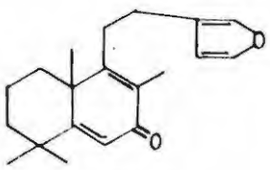
H = 6.03%

I = 24.5%

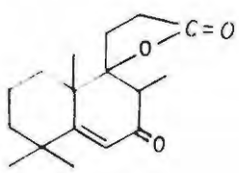
The iodo-compound was dissolved in sodium bicarbonate solution and continuously extracted with ether to remove any possible neutral material. The alkaline solution was acidified and extracted with ether. The ethereal solution was washed with water and dried (Na_2SO_4). Removal of the solvent gave a gum which crystallised from benzene-hexane as needles m.p. 93°.

Found: C = 54.26%

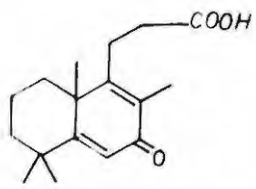
H = 6.31%



(178)



(179)



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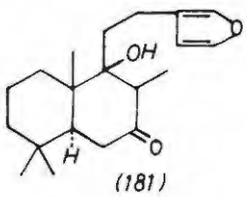

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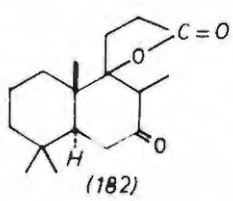

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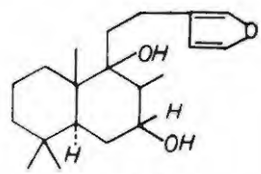

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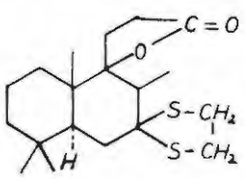

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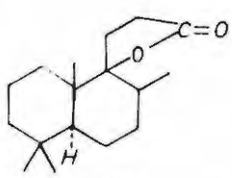

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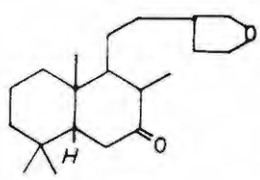

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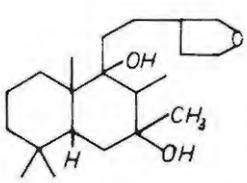

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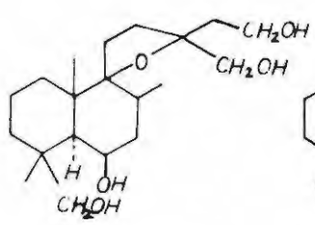

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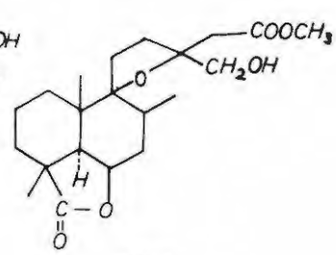

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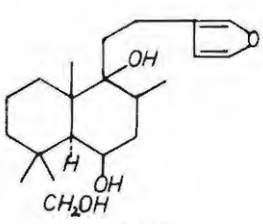

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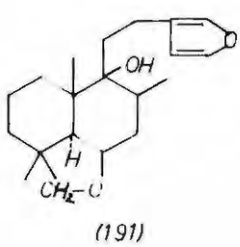

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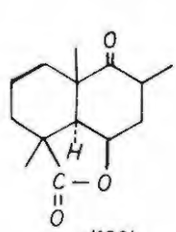

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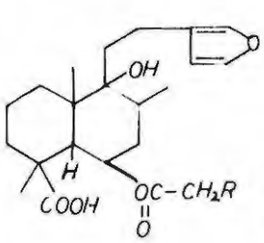


(193) R = Cl

(194) R = I

Extraction of Leonotis leonitis.

The plant material was collected on the farm "Slaai-kraal" about 10 miles to the northwest of Grahamstown during late autumn. The dried material (17 lbs) was steeped in cold acetone for 3 days and the extract evaporated to approximately 5 litres by flash distillation. The extract was stirred with charcoal (3 x 150g.), filtered through celite and evaporated under reduced pressure to a gum which crystallised from ethyl alcohol (.100 ml.) as needles; yield 12.4g. (0.163%).

Purification of the crystalline solid from Leonotis leonitis.

The crystalline solid (12.4g.), shown by thin-layer chromatography to consist predominately of one compound, was dissolved in benzene (50 ml.) and chromatographed on neutral alumina (350g.). Elution with 1% methanol-benzene afforded a gum which crystallised from benzene as needles of compound R, m.p. 117°, raised on recrystallisation from the same solvent to 118-9°. $[\alpha]_D^{20}$ -17.6° (c, 0.97 in chloroform).

Found: C = 68.74, 68.61, 69.05%

H = 7.59, 7.40, 7.73%

Discussion.

Various species of Leonotis found in South Africa are called "dagga". This is a misnomer since the name "dagga" correctly refers to Cannabis sativa. Very little chemical, pharmacological or clinical investigation has been performed on the genus. Included among the various species of Leonotis reported are L. leonurus (Linn.) Ait., L. leonitis R.Br., L. microphylla Skan, L. leonitis var hirtiflora skan, L. napetaefolia R.Br, L. dubia E. Mey, L. mollis Benth, L. latifolia Guerke, and L. dysophylla Benth.

Leonotis species, in particular L. leonurus, have been used for a long time principally as an antidote for snake-bite. They have also been used in cases of whooping cough, influenza, coughs, chest infections and headaches to name but a few.

Leonotis leonurus is widespread and grows up to 8 feet in height. It is referred to as "kop dagga" or "wilde dagga" in order to distinguish it from the other less common species L. leonitis or "klip dagga" which is a stunted bush about one foot in height.

The first chemical study on the leaves of Leonotis leonurus (Labiatae) was performed by Cragg and Little,[112] who isolated two compounds, compound X $C_{20}H_{28}O_5$, m.p. 233-4°, and compound Y $C_{20}H_{28}O_3$, m.p. 114-6°. The plant was reinvestigated by Rivett[111] who isolated in addition a third compound $C_{20}H_{28}O_4$, m.p. 159-60°, shown to be marrubiin which had previously been isolated from Marrubium vulgare L.[81]

The aerial portions of Leonotis leonurus were extracted at various periods during the year, the yield of the crystalline material from the resinous extract varied with the season. A maximum yield was found during autumn and late spring. The leaves were air-dried for 3-6 weeks and extracted by steeping

in cold acetone for 1-2 days. After filtration the extract was concentrated by flash distillation and clarified with decolourising charcoal to yield a light-brown solution. Removal of the solvent afforded a brown sticky gum which was dissolved in alcohol and allowed to stand for 2 weeks. The resulting crystalline-solid, amounting to almost 2% $^{w}/_{v}$ of the leaves, was filtered off and shown by thin-layer chromatography to be a mixture containing at least three compounds.

The crystalline solid was dissolved in benzene. The insoluble residue was extracted with chloroform and recrystallised from ethyl alcohol to afford compound X. The benzene soluble material was chromatographed on alumina and the column eluted with benzene to yield marrubiin. Further elution with benzene yielded a mixture consisting of marrubiin, compound Y and a third compound, previously undedected on thin-layer chromatograms. This latter compound, isolated in a pure state by macro-thin-layer chromatography, was shown to be anhydro-Y (m.p., mixed m.p. and identical infrared spectra). A separate experiment proved that it was an artefact arising from dehydration of compound Y on the alumina column. Elution with 2% methyl alcohol-benzene afforded relatively pure compound Y which was purified by recrystallisation. Further elution with increasing concentrations of methyl alcohol afforded a small amount of compound X.

The course of the chromatogram was followed by thin-layer chromatography on silica-gel plates and by examination of the infrared spectra of the fractions. The latter method is ideal for differentiating between the three compounds. Thus,
Compound Y and marrubiin are substituted furans having a strong band at 875 cm^{-1} (11.45μ).
Compound X and marrubiin are γ-lactones having a strong band at 1750 cm^{-1} (5.7μ).
Compound Y is an α,β-unsaturated ketone having a characteristic

band at 1660 cm^{-1} (6.0μ).

Marrubiin and compound Y, the major constituents are present in approximately equal amounts in the crude crystalline solid whereas compound X is a minor constituent. Depending on the time of collection only 0.5-1.5 g. of compound X was obtained from 50g. of crude crystalline material.

Marrubiin.

Marrubiin, the major constituent of Marrubium vulgare, has recently been found to be present in the leaves of Leonotis leonurus.[111] Although the constitution (195) for marrubiin is generally accepted, its stereochemistry has been the subject of protracted discussion and is by no means secure except at position C_5 and C_{10}.

The correlation of marrubiin with the ambrein-degradation product, isoambreinolide, served to confirm the stereochemistry of the ring fusion and of the C_{10} angular methyl group.[12,23] The asymmetric centre carrying the bridge-head methyl group (C_{10}) remained unaffected during the transformations and therefore the angular methyl group in marrubiin bears the same stereochemical relationship to the ring system which has been found to be general for the resin acids, the triterpenoids and steroids.

Cocker, Edward and Holley[101] proposed the stereochemistry of marrubiin shown in (196). Marrubiin was converted to the tetrol (199) by the sequence (195)→(197)→(198)→(199) under conditions which did not affect the stereochemistry of the A/B ring fusion. The hydroxy-acid (198) was oxidised to the keto-acid (200) which was not isomerised by alkali[84] and therefore must have the trans A/B fusion. The reduction of (200) with lithium aluminium hydride to the same tetrol (199) showed that the latter and hence marrubiin must have the trans-decalin system.

However in a later communication[102] Cocker revised the configuration for the lactone ring. The application of Hudson's rule as developed by Klyne[103] in fact indicated the configuration of marrubiin to be as shown in (201) since the lactone ring makes a positive contribution to the molecular rotation. Cocker deduced that the potential 6-hydroxyl group

must be β and that in consequence the 4-carboxyl group must also be β. However there is no stereochemical reason for omitting from consideration a "skew" lactone bridge 4α→6β or 4β→6α so that configuration at C_4 does not follow from that at C_6 or vice-versa, as has been assumed.[12]

According to Cocker the C_8-methyl group of the keto-lactone (202) must be in the stable equatorial conformation shown because it is not epimerised by boiling in alkali. Since compound (202) was formed from marrubiin under conditions unlikely to epimerise this centre, the C_8 methyl group of marrubiin must be as shown and the C_8 hydrogen must be axial. However Burn and Rigby stated that if the methyl group at position 8 was axial in marrubiin it would be expected that the tri-α-substituted ketone would pass into its C_8 epimer with great ease.

The configuration at C_9 is based on the fact that marrubiin or tetrahydromarrubiin on dehydration gives predominately the product which has the exocyclic double bond. Cocker stated that if the C_9-hydroxyl has the equatorial conformation it is suitably disposed with respect to a C_{11}-hydrogen atom for the trans-elimination of water under the influence of phosphorus trichloride.[104] This necessitates an α-orientation of the side chain of marrubiin at variance with the usual orientation of the 7:8-secopimaranes. However only 30-37% of the exocyclic compound was obtained and in the absence of evidence that exocyclic dehydration is favoured any arguments based on that assumption are speculative.

Castine, Wheeler and Wheeler[105] suggested that the conformation of the lactone ring in marrubiin at C_4 and C_6 was α. The keto-acid (203) was reduced using (a) lithium, ammonia, methanol and (b) sodium borohydride and in each case only tetrahydromarrubic acid was obtained (in 70% yield). The result of the lithium-ammonia-methanol reduction of (203) indica-

ted that the hydroxyl group at C_6 in (204) is equatorial and hence α.[106] It had been shown by Cocker that the keto-group at C_6 is hindered. Application of Bartons generalizations on the stereochemistry of hydride reductions suggest that borohydride reduction of (203) should not yield (204) but its epimer, and in order to explain this Castine et al suggested that the carboxyl group in (203) was equatorial. Previous work showed that a carboxyl group in this position exerted an electrostatic shielding effect which prevented the attack of the borohydride from the α-side of (203) and therefore favours the formation of (204).

Fulke and McCrindle[107] in a recent communication have presented further evidence which allows the assignment of the stereochemistry at C_4, C_6 and C_8 together with a brief comment at C_9 and give the stereochemistry of marrubiin as (205).

Confirmation that the lactone ring is cis-fused and β-orientated was deduced from the n.m.r. spectra of marrubiin and its derivatives.

The stereochemistry at C_8 was resolved as follows:- In the case of an axial (β) C_8-methyl group one would expect that a change from the hydroxy-acetate (206) to the keto-acetate (207) would give rise to an upfield shift of about 15-20 c.p.s. for the C_8 and C_{10}-methyl groups.[108] The secondary methyl signal (C_8) in fact shifts downfield by 3.5 c.p.s. and is therefore probably equatorial (α) while the methyl group at C_{10} shifts upfield (22 c.p.s.) as expected. According to Fulke and McCrindle the C_8-proton is therefore axial and it seemed likely that dehydration experiments would demonstrate whether or not the tertiary hydroxyl at C_9 was in the favourable trans-diaxial relationship with respect to this proton. However attempts to dehydrate tetrahydromarrubiin (hot phosphorus oxychloride-pyridine for three hours) resulted in recovery of starting material. With the proviso that the axial proton at

C_8 may not be accessible to base, the compound would therefore appear not to have the trans-diaxial geometry favourable for ionic elimination and therefore the hydroxyl at C_9 is probably equatorial (β).

It should be noted that the C_{10}-methyl group and the C_9-proton are trans arranged in all diterpenes of proven stereochemistry (see page 1). In the case of marrubiin the hydroxyl group at C_9 introduces an unknown factor and it is not known whether the above rule still applies.

Marrubiin has now been found to be present in the leaves of Leonotis leonurus.[111] The work carried out on marrubiin was concerned mainly with its stereochemistry and its correlation with compound Y and compound X. The infrared spectrum of marrubiin is given in Figure 2. The n.m.r. spectrum of marrubiin (in $CDCl_3$) (Figure 3) possessed the following bands.

A τ = 9.04 (J = 6.5 c.p.s.); a doublet, equivalent to three protons, attributed to a secondary methyl group at C_{17}.

B τ = 8.96; a singlet, equivalent to three protons, attributed to a tertiary methyl group at C_{20}.

C τ = 8.72; a singlet, equivalent to three protons, attributed to a tertiary methyl group at C_{18}.

D τ = 5.28; a poorly resolved triplet, equivalent to one proton, attributed to the 6 α-hydrogen atom.

E τ = 3.72; a multiplet, equivalent to one proton, attributed to a β-hydrogen on a furan ring at C_{14}

F τ = 2.76; a multiplet, equivalent to one proton, attributed to an α-hydrogen on a furan ring at C_{16}

G τ = 2.64; a multiplet, equivalent to one proton, attributed to an α-hydrogen on a furan ring at C_{15}.

The 19, 6 β-stereochemistry for the ring A, B lactone follows from the n.m.r. signal at τ = 5.28 arising from an equatorial proton having an eq./eq. and eq./axial coupling

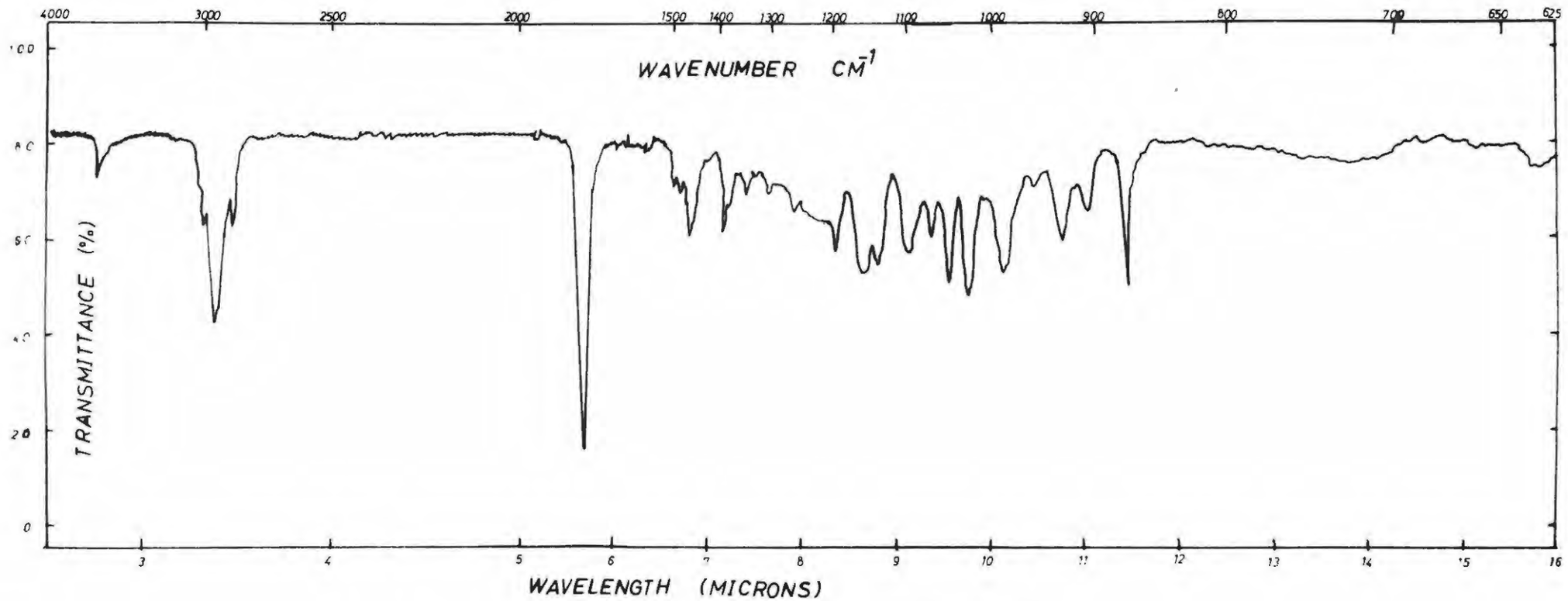


FIG.2 INFRARED SPECTRUM OF MARRUBIIN.

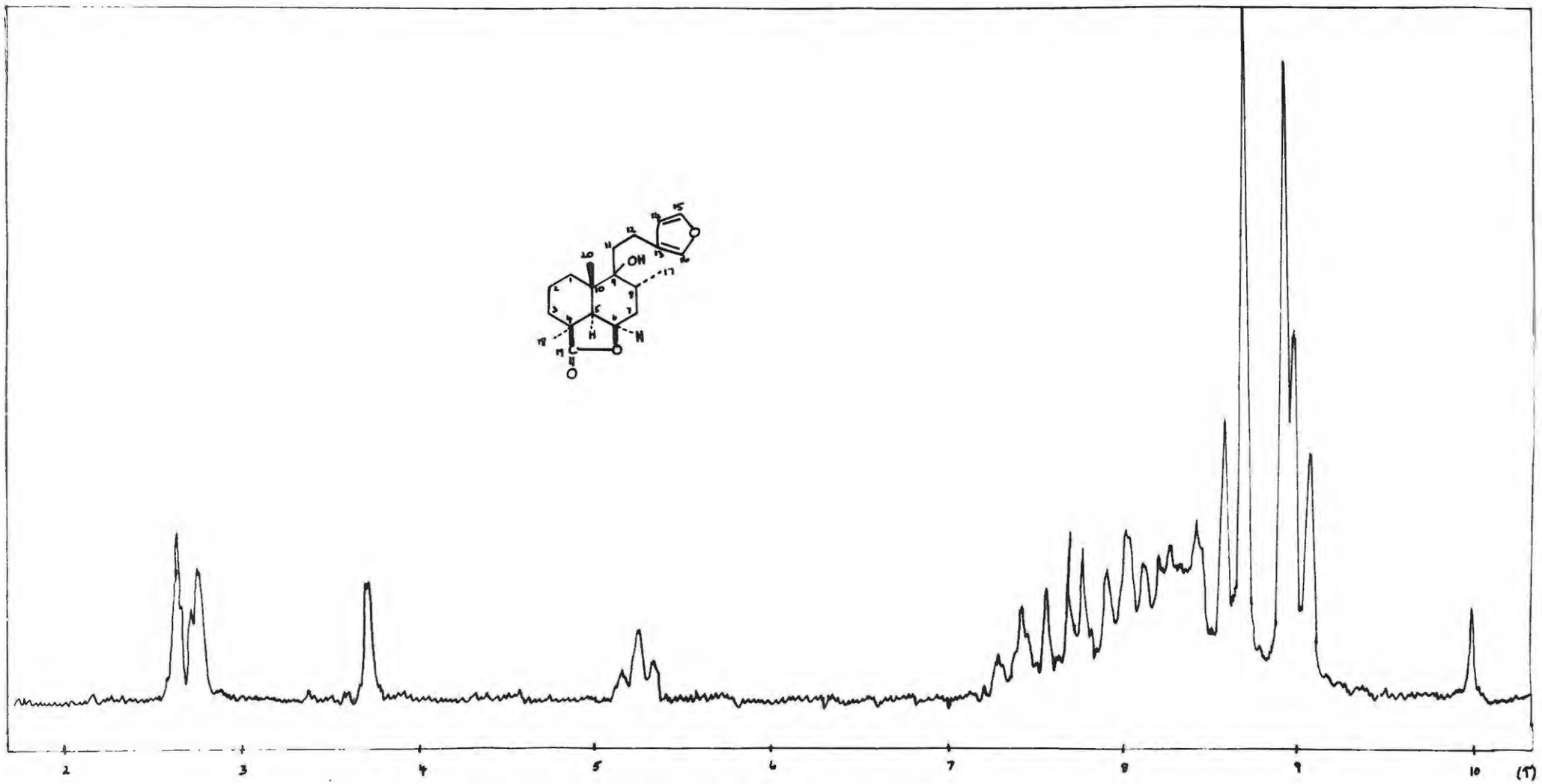
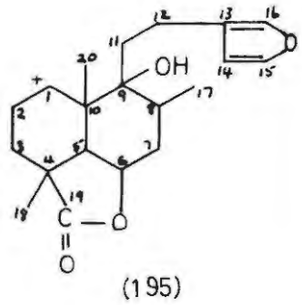


FIG 3. N.M.R. SPECTRUM OF MARRUBIIN.

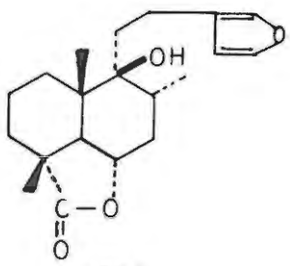
(confirmed by Fulke and McCrindle[107]). The oxygen function at C_6 in marrubiin and its derivatives is therefore β-orientated. This eliminates the stereochemistry proposed by Castine et al[105] as the proton at C_6 should, if their proposal is correct, show a trans-diaxial coupling. (It can be noted at this stage that the n.m.r. spectra of compound X and marrubiin are almost identical with respect to the 19, 6 β-lactone and the methyl groups, which is indicative of identical stereochemistry. The C_8 proton has been shown by Fulke and McCrindle to be in the axial (β) conformation (see page 83). In order to attempt to resolve the uncertainty of the stereochemistry of the tertiary hydroxyl at C_9 the dehydration experiments described by Burn and Rigby and by Fulke and McCrindle on tetrahydromarrubiin were repeated. Dehydration with phosphorus trichloride yielded anhydrotetrahydromarrubiin, m.p. 123°, in agreement with the value reported. That the double bond formed was exocyclic was confirmed by ozonolysis to yield the ketone (202) identified via its 2:4-dinitrophenylhydrazone. Tetrahydromarrubiin on treatment with $POCl_3$-pyridine yielded a mixture of compounds, one of which was identified as anhydrotetrahydromarrubiin. The major product of this reaction, an oil, shown by thin layer chromatography to be a single compound differing from starting material, could unfortunately not be characterised. Fulke and McCrindle's experiments on tetrahydromarrubiin with $POCl_3$-pyridine resulted in recovery of starting material (see page 83)). Accordingly the results from these dehydration experiments were again inconclusive.

Some attempt was made to find a suitable crystalline derivative by means of which the stereochemistry could be settled by X-ray crystallography. Marrubic acid(208) was converted via the chloroacetate (209) to the nicely crystalline iodoacetate (210), which on analysis gave low iodine and high carbon values. The possibility that marrubiin might be a con-

taminant (marrubic acid was absent as shown by T.L.C.) was ruled out by continuous extraction of the alkaline solution of iodoacetyl marrubic acid with ether. The acidic product gave the same analytical values as before. Repeated experiments gave the same result. The reason for these unsatisfactory analyses is not known.



(195)



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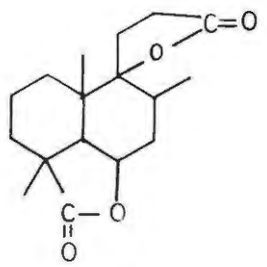

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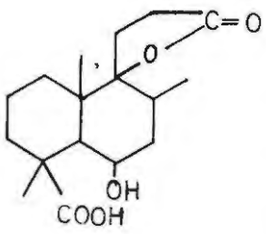

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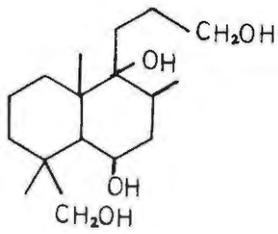

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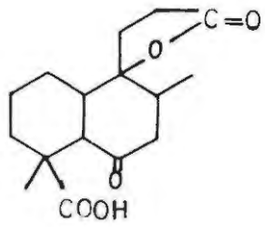

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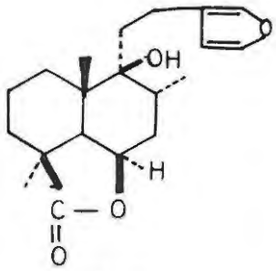

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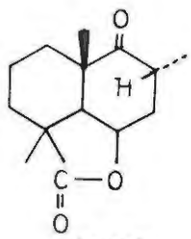

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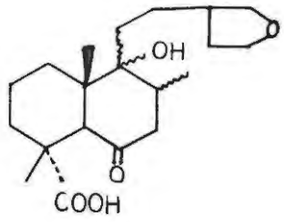

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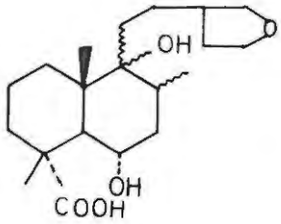

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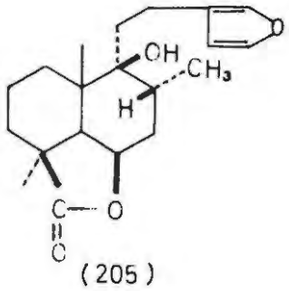

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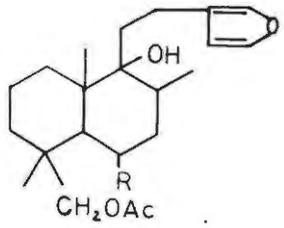
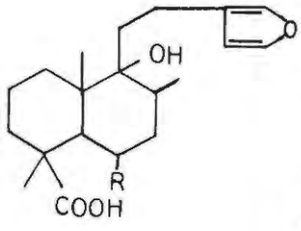


(206) R = OH

(207) R = O



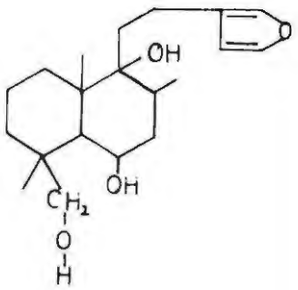
(208) R = OH

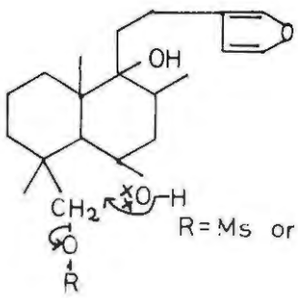
(209) R = O-C-CH_2Cl (O)

(210) R = O-C-CH_2I (O)

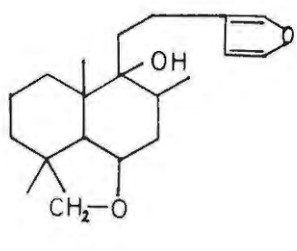
(211) R = H

Scheme 1





R = Ms or Ts



Compound Y

Previous workers[111,112] showed compound Y to have the empirical formula $C_{20}H_{28}O_3$. The mass spectrogram confirmed the molecular weight of 316. Compound Y formed a crystalline 2:4-dinitrophenylhydrazone, a semicarbazone and an oxime. It did not reduce Tollen's reagent and therefore contains a keto-group. It is triply-unsaturated affording a crystalline hexahydroderivative, m.p. 104-5°, and possesses four C-methyl groups.

The infrared spectrum (chloroform)(Figure 4) showed a small peak at 3600 cm^{-1} due to a free hydroxyl group which is presumably tertiary since it resists benzoylation and acetylation. Peaks at 1660 cm^{-1} and 876 cm^{-1} suggested the presence of an α,β-unsaturated ketone and a furan ring respectively It did not possess an infrared absorption band due to an exocyclic methylene group (11.2μ) and the absence of such a function was confirmed by ozonolysis; no formaldehyde was produced. The ultraviolet spectrum of compound Y showed a maximum at 245 mμ (ε = 13,500) indicative of an α,β-unsaturated ketone and an end absorption at 210 mμ (ε = 6000). This latter absorption was of a magnitude comparable with those of other furan derivatives. (Table 7).

Table 7.

Ultraviolet absorption spectra of some substituted furans.

Compound	λ	ε
Marrubiin	212 mμ	5620
Dihydrocolumbin[113]	210 mμ	5700
Limonin[114]	210 mμ	6000
Polyalthic acid[47]	213 mμ	6200

Compound Y gave a purple colour in the Ehrlich test and a green colour in the Liebermann-Burchard test[84], which confirmed the presence of a furan ring in the molecule.

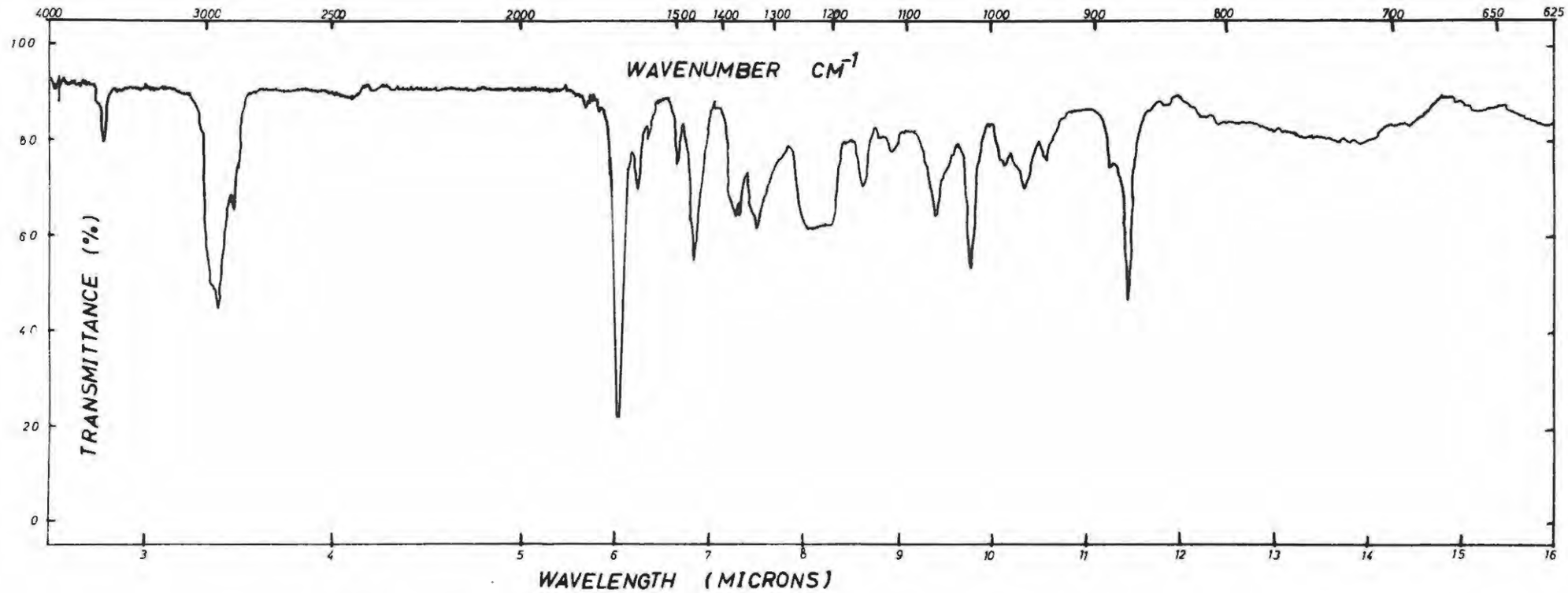


FIG. 4 INFRARED SPECTRUM OF COMPOUND Y.

On treatment of compound Y with alkali Cragg and Little[112] obtained a crystalline compound $C_{17}H_{24}O_2$, m.p. 58-9°. This experiment was repeated and the crystalline compound, m.p.68-9°, obtained analysed for $C_{20}H_{26}O_2$, indicating that a molecule of water had been lost during the reaction. The same compound resulted on treatment with phosphorus trichloride. On catalytic hydrogenation (10% palladium hydroxide - barium sulphate) the anhydro-compound absorbed 4 mols. of hydrogen, one more than compound Y itself. The ultraviolet spectrum of anhydro-Y was practically identical with that of compound Y. The infrared spectrum however showed that the C=C stretching frequency at 1620 cm^{-1} was considerably more intense than that in compound Y. These facts are accommodated by a cross-conjugated dienone system in anhydro-Y (178). The hydroxyl group is thus probably tertiary as supported by the fact that it can be removed forming a tetra-unsaturated compound. It can be noted that anhydro-Y was also isolated during the purification of the crystalline solid from Leonotis leonurus by alumina chromatography and is thus also an artefact arising from dehydration of compound Y on the column.

The third oxygen atom was inert and presumed to be contained in a furan ring as was indicated by colour reactions, ultraviolet, infrared and n.m.r. spectra. The presence of a furan ring may be proved chemically by the application of the Aldier-Rickert degradation to yield furan, 3,4-dicarboxylic acid, depending on the substitution on the furan ring (as was done with polyalthic acid).

The nature of the carbon skeleton of compound Y was shown by palladium-charcoal dehydrogenation which yielded 1:2:5-trimethylnaphthalene, identified by comparison with an authentic specimen (m.p., mixed m.p. of the trinitrobenzene and trinitrotoluene adducts and identical infrared and ultraviolet spectra of the hydrocarbon itself). This indicated a relationship

with the diterpene group and supported the C_{20} formula. In the case of agathic acid, sclareol and manoyl oxide, the presence or introduction of a double bond in the side chain attached to the hydronaphthalene nucleus enables isomerisation to take place yielding a phenanthrene derivative on dehydrogenation. However no phenanthrene derivatives were obtained on dehydrogenation and in this respect it differs from manoyl oxide and other compounds of this class. Since compound Y is also a substituted furan one can at this stage assume with some certainty that it has the same ring system (212) as in marrubiin.

Chromic acid oxidation of compound Y afforded two compounds, a crystalline neutral compound, m.p. 135°, and a crystalline acid, m.p. 206-7°, both of which give the formula $C_{17}H_{24}O_3$ on analysis. The neutral compound (179) exhibited an infrared absorption peak at 1750 cm^{-1} (γ-lactone) and an absorption peak at 1660 cm^{-1} showing that the α,β-unsaturated keto-group was still intact. From the analytical figures it is clear that three carbon atoms, two double bonds and the inert oxygen atom have been lost during the oxidation. Alkaline hydrolysis of the lactone (179) resulted in the formation of the acid (180), the infrared spectrum of which showed absorption bands at 1730 cm^{-1} (carboxyl), 1630 cm^{-1} (α,β-unsaturated ketone) but no hydroxyl peak at 3600 cm^{-1}. The analytical figures of the acid were identical with those of the lactone. This was perplexing at first as one would expect an extra element of water in the acid but was explained when it transpired that the acid contained an extra double bond, shown by hydrogenation, when it absorbed two moles of hydrogen. The partial structure of the acid would thus be expected to be (213) or (214). The acid was not decarboxylated even at high temperatures (250°) and therefore it was not a β,γ- but a γ,δ-unsaturated acid.[12] The formation of the γ-lactone (179)

shows that the hydroxyl group in compound Y is attached to C_9 as in marrubiin.

On treatment with hydrogen in the presence of palladium hydroxide-barium sulphate an alcoholic solution of compound Y absorbed the equivalent of six atoms of hydrogen to yield hexahydro-Y (215), m.p. 104-5°, (λ_{max} 279 mμ, ε = <100) indicating the presence of three double bonds in the molecule. Two molecules of hydrogen were absorbed rapidly (saturation of the furan ring) whereas the third molecule was absorbed more slowly (saturation of the double bond shown by later experiments to be at C_5-C_6).

By application of Woodward's rules as modified by the Fiesers' to its ultraviolet spectrum, two possibilities (216 and 217) exist for the partial structure of compound Y. The λ_{max} calculated value for (216) is 239 mμ and for (217) is 244mμ whereas the observed value for compound Y is 245 mμ.

Phosphorus trichloride dehydration of hexahydro Y yielded an oily α,β-unsaturated ketone (λ_{max} 250 mμ, ε = 8,500) which can only be accommodated by partial structure (217). The formation of the α,β-unsaturated ketone on dehydration of hexahydro Y indicates that the position of attachment of the hydroxyl group must be at C_8 or C_9. That the position of its attachment is at C_9 had previously been indicated by the formation of the γ-lactone (179) on oxidation.

The position of the keto-group at C_7 in compound Y was substantiated by conversion of hexahydro Y with methyl magnesium iodide to the secondary alcohol (187), m.p. 167-8°, which on palladium-charcoal dehydrogenation yielded 1:2:3:5-tetramethylnaphthalene, identified by comparison with an authentic specimen[96] (m.p. and mixed m.p. of the trinitrobenzene adduct).

If the structure thus postulated for compound Y is correct then depending upon the absolute configuration an interconversion should be possible with marrubiin. The simplest correla-

tion appeared to be via one of the "isoambreinolides" (prepared by the action of 70% sulphuric acid on ambreinolide by Colin-Asselineau et al[115]) which had been shown to be identical to one of the degradation products of marrubiin.[12] An initial difficulty lay in the fact that the hydrogen atom at C_5 in isoambreinolide is in an α-configuration trans to the C_{10}-β-methyl group. Saturation of the C_5-C_6 double bond in compound Y by hydrogenation resulted in cis-addition with the hydrogen atom at C_5 in the β-configuration. At the same time hydrogenation resulted in saturation of the furan side chain thus rendering the compound unamenable to oxidative degradation. However this difficulty was overcome by reduction of compound Y with lithium in liquid ammonia,[116] which yielded the ketone (181), m.p. 145-6°, as the major product together with an alcohol (183), m.p. 107-8°. The alcohol on oxidation with the Jones' reagent afforded the ketone (181). The infrared spectrum of the ketone lacked the absorption band due to the α,β-unsaturated ketone (1660 cm^{-1}), but showed a maxima at 1700 cm^{-1} and 870 cm^{-1} attributed to a saturated six-ring ketone and furan ring respectively. This lithium-ammonia reduction is stereo-specific[117] resulting in the formation of an α-(axial) C_5-proton, and at the same time leaving the furan ring intact.

Oxidation of the ketone (181) with chromium trioxide in acetic acid (Ghigis method[118]) afforded two compounds, a crystalline neutral compound (182), m.p. 164-5°, and an oily acid presumably having structure (218). The infrared spectrum of (182) showed strong absorption peaks at 1760 cm^{-1} (γ-lactone) and 1700 cm^{-1} (saturated six-ring ketone) but lacked an absorption peak at 870 cm^{-1} due to the furan ring. The infrared spectrum of the acid (218) lacked the absorption band due to the lactone function but showed a maxima at 1660 cm^{-1} attributed to an α,β-unsaturated keto-group.

Attempted removal of the keto-group in (182) by a Wolff-

Kishner (Huang-Minlon modification) reduction resulted in cleavage of the lactone to yield an α,β-unsaturated keto-acid (λ_{max} 249 mμ ε = 12,500.) However the crystalline thioketal of (182) was readily prepared (no ketone band at 1700 cm^{-1}) and converted to the crystalline deoxo-compound (185), m.p. 93-4°, with Raney nickel. This was shown to be identical (m.p., mixed m.p., infrared spectrum and optical rotation) with an authentic specimen of the γ-lactone of β-(1:2 dihydroxy-2:5:5:9-tetramethyl-1-decalyl) proprionic acid supplied by Dr. Rigby. Alkaline hydrolysis of the lactone indicated that one mole of alkali had been consumed but the product obtained on neutralisation was found to be identical with starting material (m.p. and mixed m.p.) and it was thus apparent that (relactonisation had taken place. No unsaturated acid was formed as might have been expected.

The above correlation of compound Y with marrubiin via the ambrein-degradation product establishes the skeleton (219) in compound Y. The formation of the γ-lactone (185) gives further proof of the tertiary hydroxyl group at C_9. The asymmetric centre bearing the bridge-head methyl group remained unaffected during the transformations and therefore the angular methyl group in compound Y bears the same stereochemical relationship to the ring system which has been found to be general for the resin acids, the triterpenoids and steroids.

The formation of the lactone (185) from both compound Y and marrubiin is indicative of identical stereochemistry at C_9 (the exact configuration of which is at present unresolved). However this is not necessarily true regarding the stereochemistry at C_8 since keto-enol tautomerism could possibly have taken place during the lithium-ammonia reduction resulting in a change of configuration at C_8 (see scheme 2).

These proposals concerning the structure and stereochemistry of compound Y were placed on a firmer basis by n.m.r. and mass

spectral results which verified the structure and stereochemistry of compound Y as shown in (220).

The n.m.r. spectrum of compound Y (in $CDCl_3$) (Figure 5) possessed the following bands:

A τ = 8.82; a singlet, equivalent to three protons, attributed to a tertiary methyl group at C_{18}.

B τ = 8.76; a singlet, equivalent to three protons, attributed to a tertiary methyl group at C_{19}.

C τ = 8.71 (J = 6.5 c.p.s.); a doublet, equivalent to three protons, attributed to the methyl group at C_{17}.

D τ = 8.63; a singlet, equivalent to three protons, attributed to a tertiary methyl group at C_{20}.

E τ = 7.28 (J = 6.5 c.p.s.); a quartet, equivalent to one proton, attributed to the hydrogen at C_8.

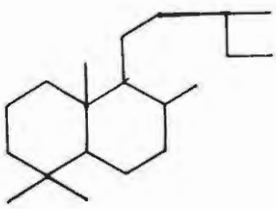
F τ = 3.89; a singlet, equivalent to one proton, attributed to an olefinic proton at C_6.

G τ = 3.71; a multiplet, equivalent to one proton, attributed to a β-hydrogen on a furan ring at C_{14}.

H τ = 2.76; a multiplet, equivalent to one proton, attributed to an α-hydrogen on a furan ring at C_{16}.

I τ = 2.64; a multiplet, equivalent to one proton, attributed to an α-hydrogen on a furan ring at C_{15}.

The n.m.r. spectra of compound Y and marrubiin are identical with respect to the furan ring, and hence this ring is β-substituted in compound Y. The appearance of a one proton quartet centered at τ = 7.23 (J = 6.5 c.p.s.) is due to the C_8-hydrogen which is shifted upfield by 0.32 p.p.m. in benzene. This is indicative of an axial proton, α to a carbonyl group. The C_{17}-methyl doublet at τ = 3.71 (J = 6.5 c.p.s.) is shifted downfield by 0.08 p.p.m. in benzene and thus this group is equatorial. Bhacca and Williams[119] have determined the shifts induced by benzene on methyl groups adjacent to a carbonyl function and



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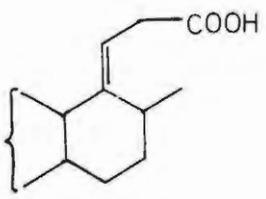

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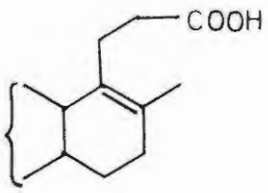

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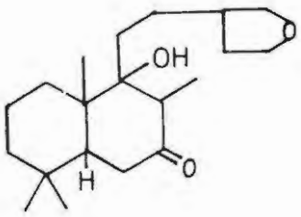

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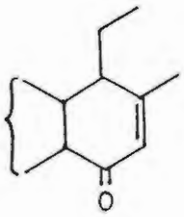

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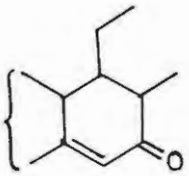
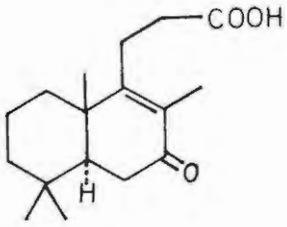
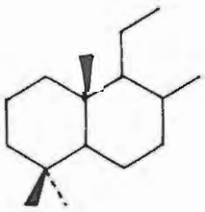


(217)

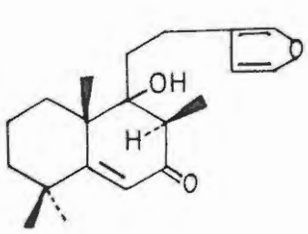


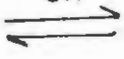
(218)

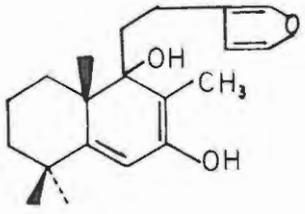


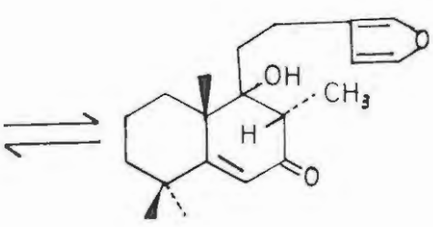
(219)

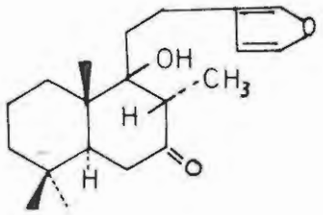
Scheme 2











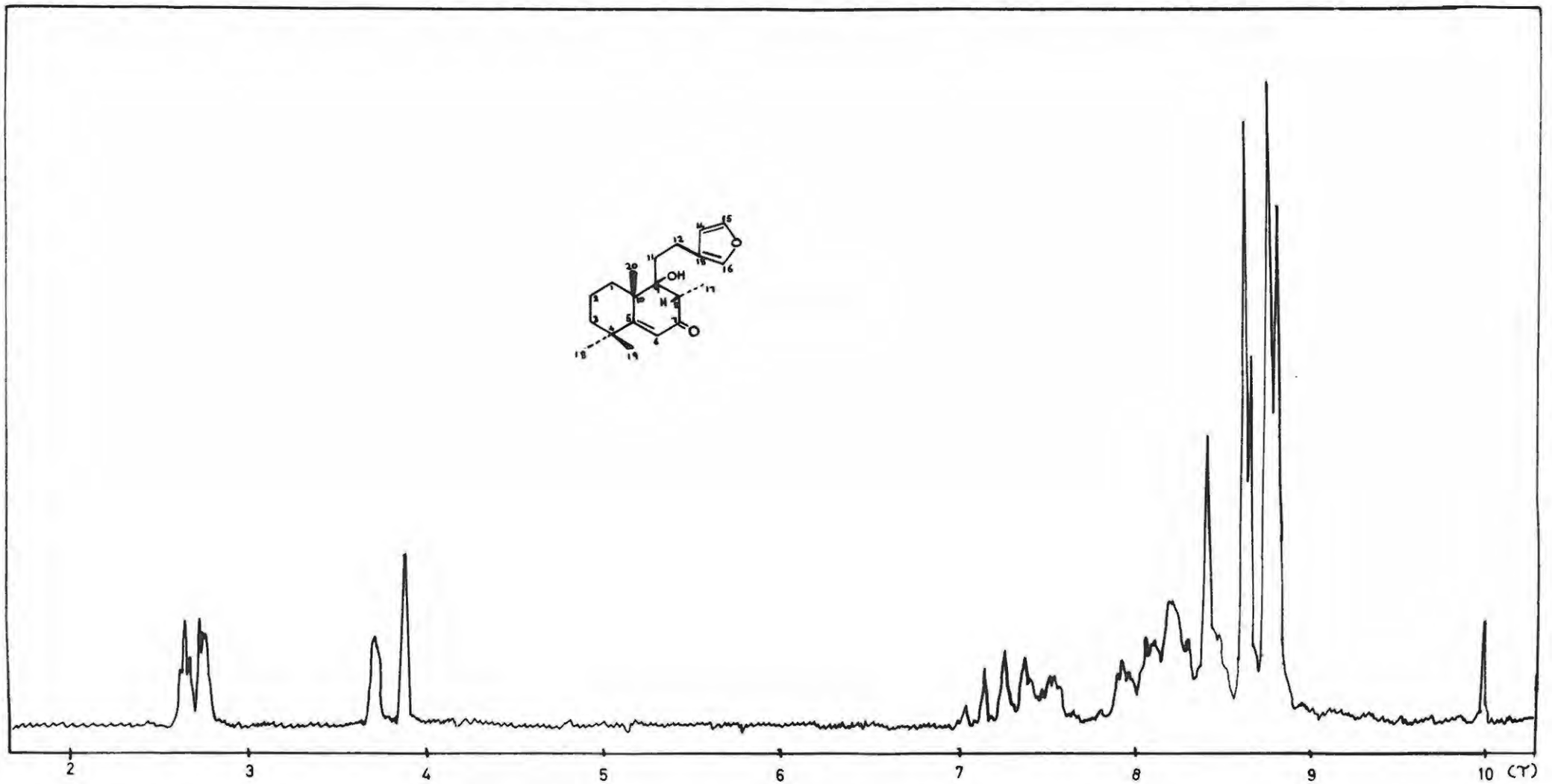


FIG 5. N.M.R. SPECTRUM OF COMPOUND Y.

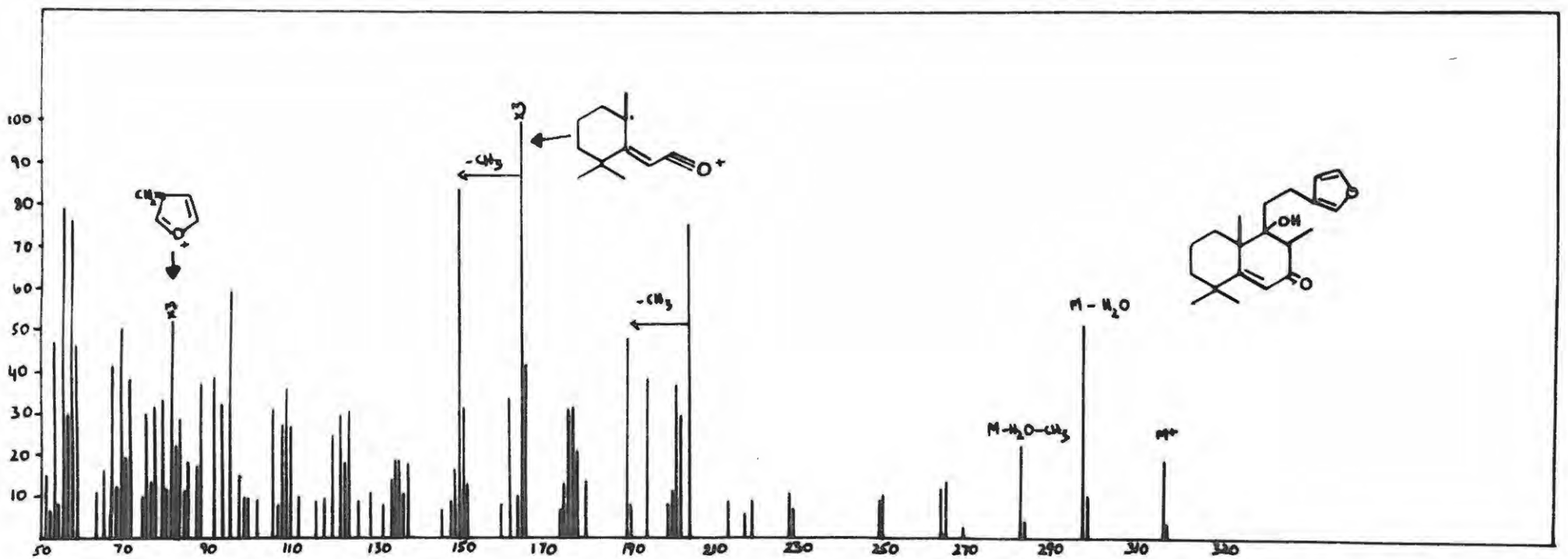
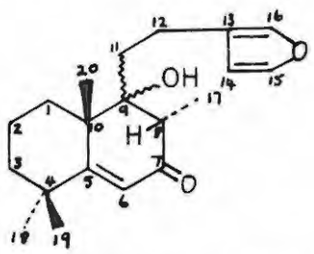
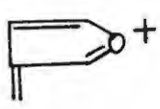


FIG 6. MASS SPECTRUM OF COMPOUND Y.



Compound Y (220)



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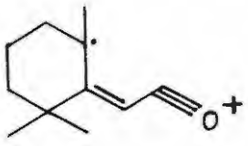
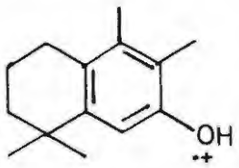
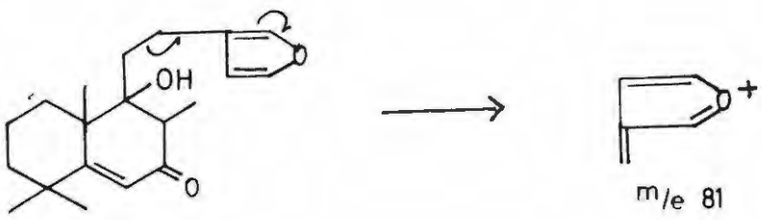


(222)

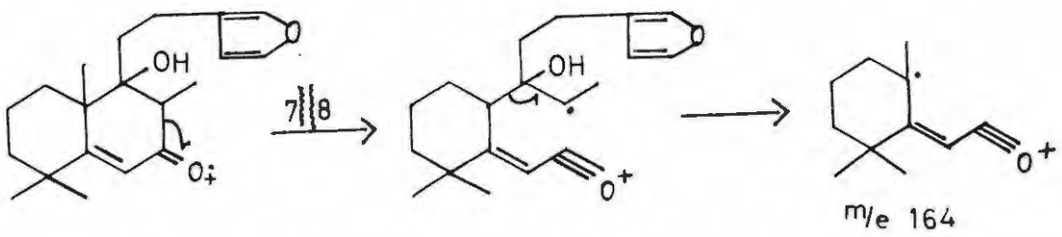


(223)

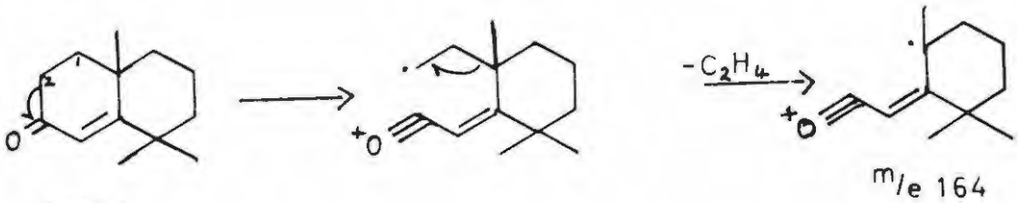
Scheme 3



m/e 81



m/e 164



(224)

m/e 164

find that these shifts are dependent upon the axial or equatorial nature of the methyl group. If $\Delta = \delta^{obs.}_{CDCl_3} - \delta_{C_6H_6}$ on passing from deuterochloroform to benzene solution is positive (i.e. an upfield shift), the methyl group is axial. Equatorial methyl groups adjacent to a carbonyl function suffer a small downfield shift (Δ values of 0.06-0.07 p.p.m.).

From the n.m.r. evidence it thus appears that both compound Y and marrubiin have identical stereochemistry regarding the C_8-hydrogen and the C_{17}-methyl group. The attachment of the alkyl furan side-chain is probably axial (α) as the signal from the C_{20}-methyl group is shifted upfield in benzene by 0.46 p.p.m., which is as expected for a $\Delta^{5,6}$-7-one with no contribution from the tertiary hydroxyl group.[120] This indicates that the hydroxyl group is <u>cis</u> to the C_{20}-methyl group.

The mass spectrum of compound Y (Figure 6) was also in agreement with structure (220) and showed significant peaks at m/e 81, 164 and 204 which corresponded to the molecular ion fragments (221) (222) and (223) respectively. The strong (100%) peak at m/e 81 arises from the cleavage of the bond β to the furan ring.[122] The significant peak 14 mass units higher at m/e 95, indicates that the cleavage of the C_9-C_{11} bond becomes important as might be expected from the presence of the C_9-hydroxyl group; the corresponding ion may be a seven membered ring analogue of ion (221)[123] (The mass spectrum of marrubiin also contains these two peaks at m/e 81 and 95). The very strong peak at m/e 164, due to fragment (222), is analagous to the loss of C_1 and C_2 in the spectrum of the ene-one (224). (See Scheme 3). The peak occuring at m/e 204 may correspond to the fragment (223).

Compound X.

The previously suggested formula,[111,112] $C_{20}H_{28}O_5$ is supported by its mass spectrum which gave a molecular weight of 348. Its infrared spectrum (Figure 7) in chloroform exhibited a strong band at 1760 cm^{-1} (γ-lactone) but no bands due to a hydroxy-group (3600 cm^{-1}) or furan (875 cm^{-1}). The absence of a furan ring in the molecule is supported by the negative Ehrlich and Liebermann-Buchard tests. Compound X is transparent to ultraviolet light; the absence of absorption at 210-220 mμ confirms the absence of a β-furyl moiety. In addition compound X failed to absorb hydrogen on catalytic reduction and did not show a colour change with tetranitromethane.[121] Judging from its infrared spectrum and failure to form derivatives compound X does not possess a keto-group.

The nature of four of the oxygen atoms was shown on alkali treatment when 2.1 equivalents of alkali were consumed indicating the presence of two lactone groups in compound X. The acid obtained failed to give a sharp melting point showing that relactonisation of at least one of the lactone groups was taking place. This difficulty was overcome by initial treatment of the acid with an ethereal solution of diazomethane to yield the lactonic methyl hydroxy-ester $C_{21}H_{32}O_6$ (225). The infrared absorption spectrum of (225) exhibited absorption bands at 1760 cm^{-1}, 1700 cm^{-1} and 3400 cm^{-1} attributed to a γ-lactone, ester carbonyl and bonded hydroxyl group respectively. Relactonisation of one of the acid groups was not surprising as marrubic acid (208) tends to relactonise to marrubiin and also resists esterification, so that diazomethane, even in cold ether, relactonises it to marrubiin.[84]

The nature of the carbon skeleton of compound X was shown by palladium-charcoal dehydrogenation which yielded 1:2:5-trimethylnaphthalene, identified by comparison with an authentic specimen (m.p., mixed m.p. of the trinitrobenzene and trinitro-

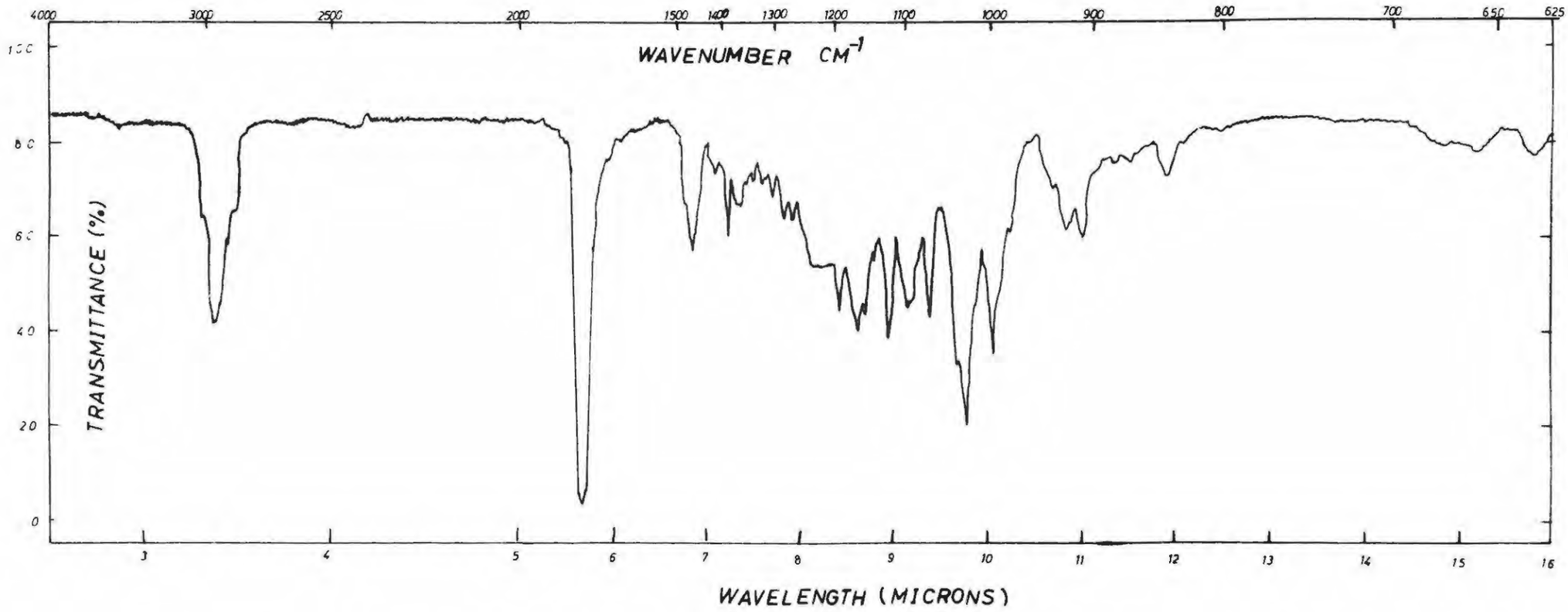


FIG.7 INFRARED SPECTRUM OF COMPOUND X.

toluene adducts and identical infrared and ultraviolet spectra of the hydrocarbon itself). This indicated a relationship with compound Y and marrubiin and supported the C_{20} formula.

Lithium aluminium hydride reduction of compound X afforded a crystalline tetrol (226) $C_{20}H_{36}O_5$, the infrared spectrum (KBr disc) of which exhibited a strong absorption band at 3300 cm^{-1} (hydroxyl) but showed no absorption peak at 1760 cm^{-1} (γ-lactone).

The absence of a hydroxyl function in compound X was further indicated by its resistance to acetylation and benzoylation. Attempted dehydration with phosphorus oxychloride-pyridine and phosphorus trichloride resulted in recovery of starting material. The one oxygen atom was completely inert and is thus presumed to be present as an ether function.

Attempts at fission of the ether link in compound X and compound (226) using a variety of reagents e.g. borontrifluoride diethyletherate, hydroiodic acid, hydrochloric acid and ammonia, resulted in recovery of starting material in each case. According to Lawson and Eustice[81] failure to split the ether link with hydrogen chloride precludes the possibility of the oxygen bridge being situated between the tertiary atoms C_8 and C_{13} as the ether link in manoyl-oxide (29), which contains this system, is readily ruptured by this reagent. However this is not necessarily true since manoyl-oxide contains an allylic ether grouping which is susceptible to this reagent. This is also the case with grindelic acid (100)[61] which has the oxygen bridge between C_9 and C_{13}.

The n.m.r. and mass spectral results suggest structure (227) for compound X and at the same time provided evidence regarding certain aspects of its stereochemistry.

The n.m.r. spectrum of compound X (in $CDCl_3$)(Figure 8) possessed the following bands:-

A τ = 9.16 (J = 6.5. c.p.s.); a doublet, equivalent to three protons, attributed to a secondary methyl group at C_{17}

B τ = 8.96; a singlet, equivalent to three protons, attributed to a tertiary methyl group at C_{20}.

C τ = 8.72; a singlet, equivalent to three protons, attributed to a tertiary methyl group at C_{18}.

D τ = 7.27 (J = 17 c.p.s.), a quartet, equivalent to two protons, attributed to the C_{14}-methylene group.

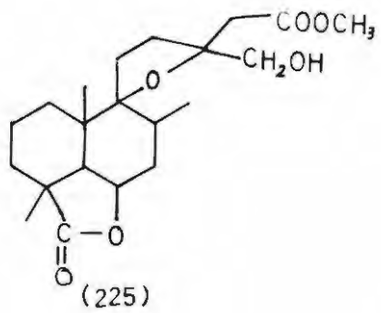
E τ = 5.81 (J = 9 c.p.s.), a quartet, equivalent to two protons, attributed to the C_{16}-methylene group.

F τ = 5.32; a poorly resolved triplet, equivalent to one proton, attributed to the 6α-hydrogen atom.

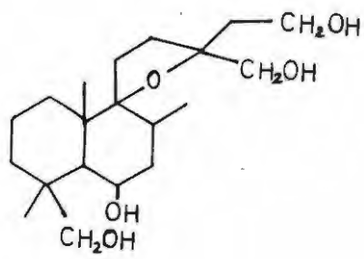
The n.m.r. spectra of compound X and marrubiin (Figure 3) are almost identical with respect to the 19, 6 β-lactone and methyl groups which therefore possess the same stereochemistry. The assignment of the 19, 6β-stereochemistry for the ring A, B lactone follows from the n.m.r. signal at τ = 5.32 arising from an equatorial proton having an eq./eq. and an eq./axial coupling, as in marrubiin. The small difference (regarding the methyl groups) occurs in their benzene spectra where the upfield shift of the C_{17}-methyl group in compound X is much larger (+ 0.49 p.p.m.) than that for the C_{17}-methyl group in marrubiin (+ 0.27 p.p.m.). This is almost certainly due to the second lactone ring in compound X. The very large coupling constant (J = 17 c.p.s.) for the C_{14}-methylene protons is typical of geminal protons in a 5-membered ring next to a carbonyl group, and that for the C_{16}-methylene protons (J = 9 c.p.s.) is normal for the type of system shown.[120]

It would thus appear from their n.m.r. spectra that compound X and marrubiin have identical stereochemistry about the C_8-hydrogen and the C_{17}-methyl group, namely that the C_8-hydrogen is axial (β) and the C_{17}-methyl group is equatorial (α).

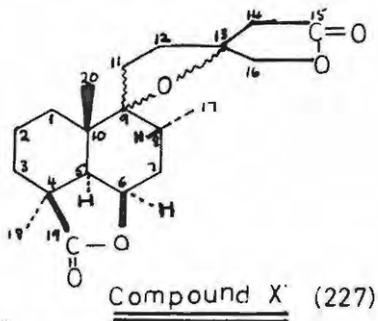
The mass spectrum of compound X (Figure 9) is also in agreement with the structure postulated and showed principal peaks at m/e 289, 211, 181, 162 and 109 which correspond to the molecular ion fragments (228), (229), (230), (231) and (232) respectively. A possible fragmentation mechanism is given in scheme 4. It may be noted that if the structure proposed for compound X is correct, then, depending upon the absolute configuration, an interconversion should be possible to grindelic acid via grindelane (235) (see scheme 5). However, attempted thioketal formation of compound (234) proved abortive, probably due to steric effects (Wolff-Kishner reduction would give a stable six-membered cyclic hydrazide[12]) and thus the precise location of the ether bridge by classical chemical means was unsuccessful.



(225)

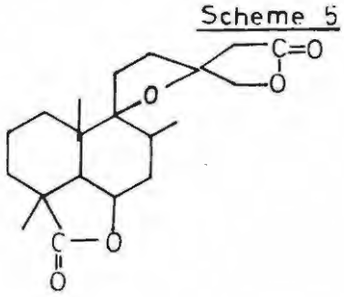


(226)

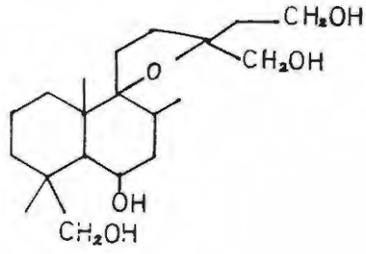


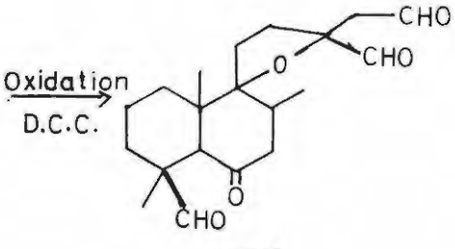
Compound X (227)

Scheme 5



$LiALH_4$

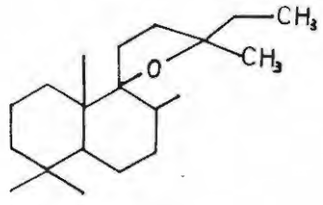




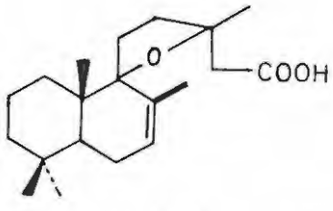
Oxidation
D.C.C.

(234)

1) Ethane-dithiol
2) Raney Nickel



(235)



Grindelic Acid

1) $LiAlH_4$
2) Tosylation
3) $LiAlH_4$
4) Hydrogenation

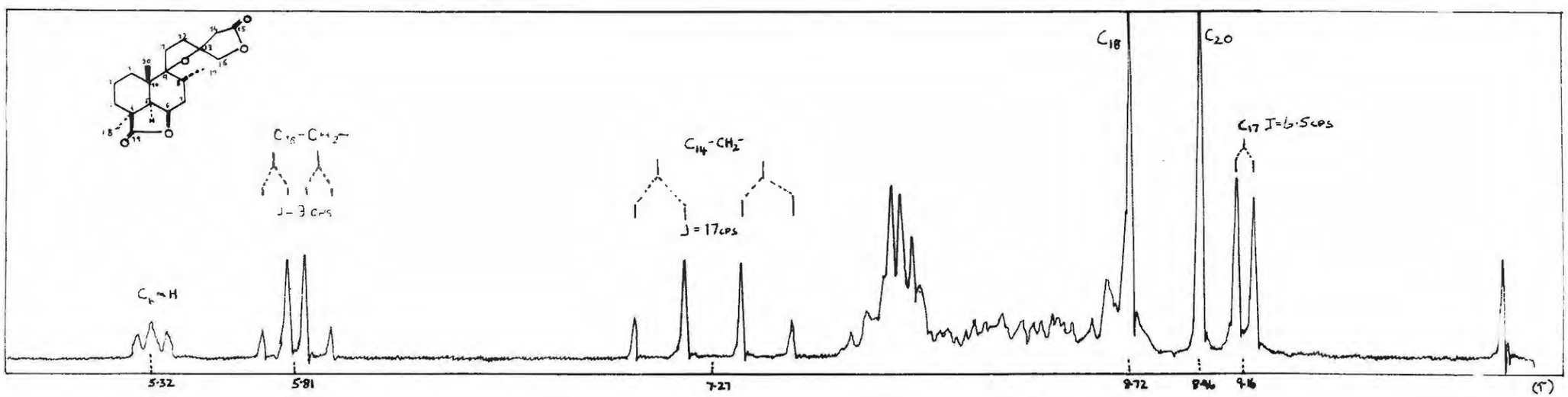


FIG. 8. N.M.R. SPECTRUM OF COMPOUND X.

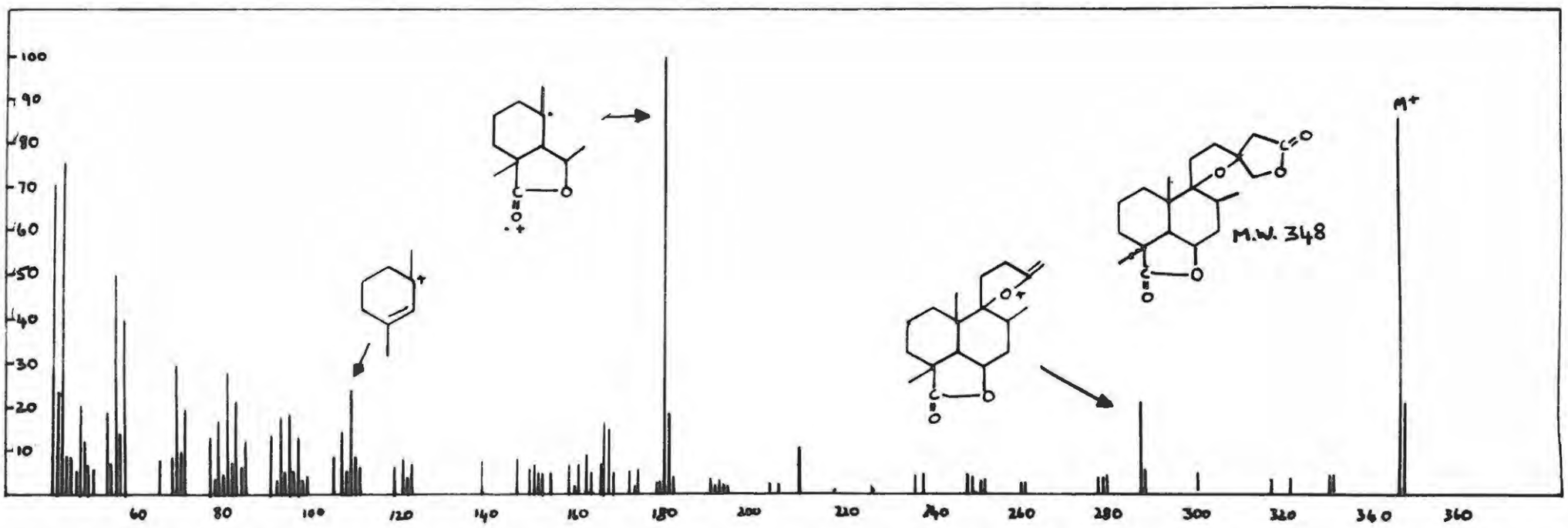
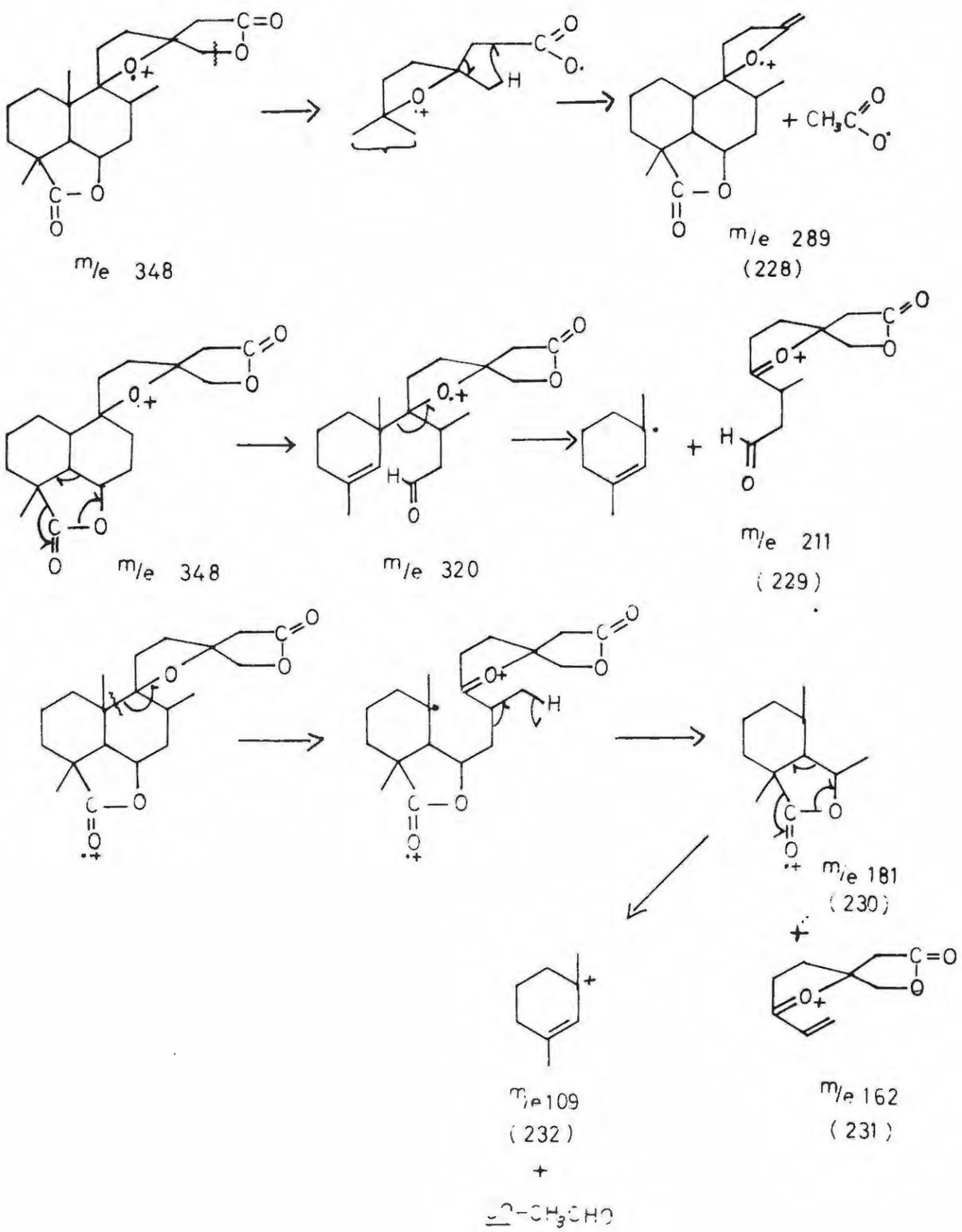


FIG 9. MASS SPECTRUM OF COMPOUND X.

Scheme 4

Possible fragmentation mechanism for the mass spectrum of compound X



Compound R.

The crystalline material obtained from an acetone extract of the leaves of Leonotis leonitis, when chromatographed on alumina, afforded compound R, m.p. 118-9°. Although consistent C, H analyses were obtained the molecular weight from the mass spectrum was indecisive and accordingly a molecular formula cannot be definitely assigned. The following statements regarding its structure are therefore tentative and a more detailed study is at present being undertaken.

A preliminary investigation indicated that compound R was a dilactone (approximately 1.84 moles of alkali were consumed) containing a furan ring and three C-methyl groups. The infrared spectrum (chloroform) (Figure 10) showed absorption bands at 1760 cm^{-1} (γ-lactone), 1740 cm^{-1} (δ-lactone) and 874 cm^{-1} (furan ring). The ultraviolet spectrum was transparent in the region 220-300 mμ but showed a maximum at 216 mμ ($E_{1\ cm}^{1\%}$ = 240).

The n.m.r. spectrum of compound "R" (in $CDCl_3$)(Figure 11) possessed the following bands:

A τ = 9.04 (J = 3.5 c.p.s.); a doublet, equivalent to three protons, attributed to a secondary methyl group.

B τ = 8.74; a singlet, equivalent to three protons, attributed to a tertiary methyl group.

C τ = 8.06; a singlet, equivalent to three protons, attributed to a tertiary methyl group.

D τ = 5.81 (J = 6.5 c.p.s.); a quartet, equivalent to two protons, attributed to a free methylene group adjacent to an oxygen function.

E τ = 5.32; a poorly resolved triplet, equivalent to one proton, attributed to an equatorial proton exhibiting eq./eq. and eq./axial coupling.

F τ = 3.81; a multiplet, equivalent to one proton, attributed

to a β-hydrogen on a furan ring;

G. τ = 2.87; a multiplet, equivalent to one proton, attributed to an α-hydrogen on a furan ring

The n.m.r. spectrum of compound R is similar to that of marrubiin and compound X with respect to the 19, 6β-lactone and the methyl groups. The appearance of the three methyl signals at τ=9.04 (J=3.5 c.p.s.), τ =8.74 and τ = 8.06 indicates a structure similar to that of marrubiin and compound X. The 19, 6β-stereochemistry for the ring A,B lactone follows from the signal at τ = 5.32 arising from an equatorial proton having an eq./eq. and eq./axial coupling (see compound X). The n.m.r. spectrum of the compound also indicated the presence in the molecule of a β-substituted furan ring, the presence of which was further substantiated by the mass spectrum which showed significant peaks at $m/_e$ 81 and $m/_e$ 95.

It may be further noted that dehydrogenation of compound R with palladium/charcoal gave no naphthalene derivatives.

Any structural proposal at this stage would be speculative.

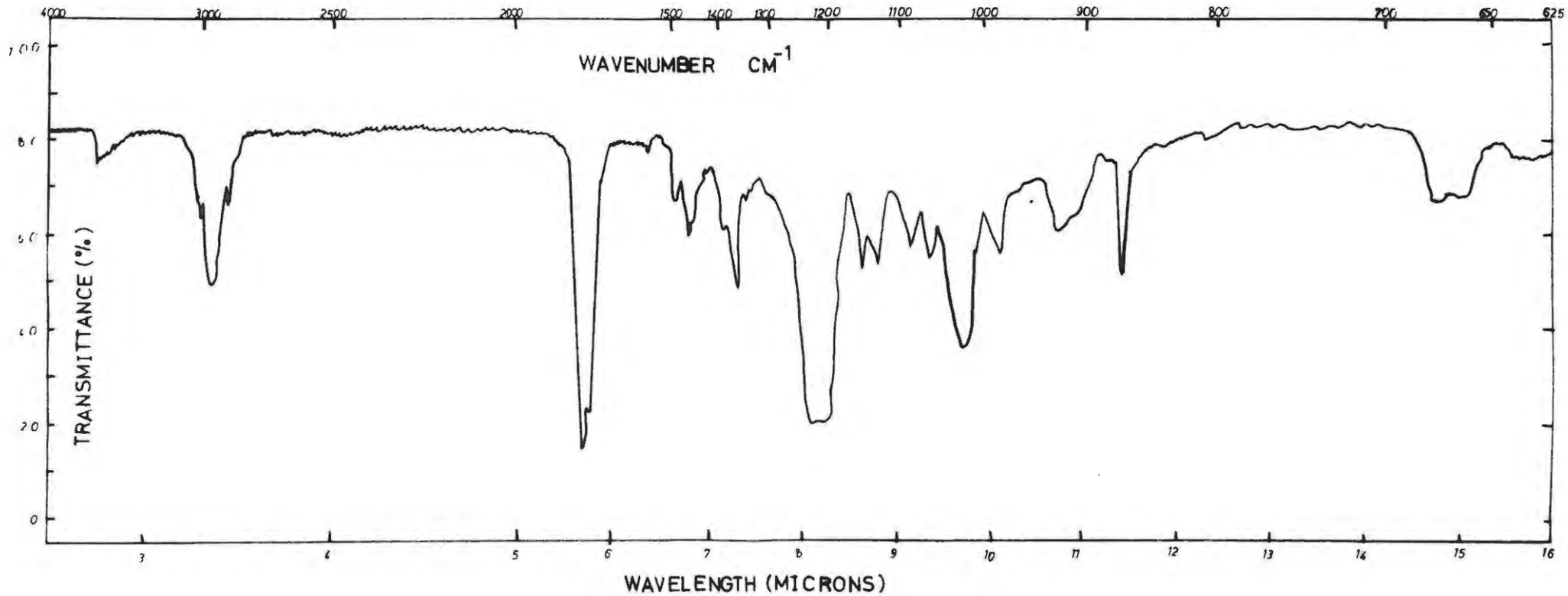


FIG 10. INFRARED SPECTRUM OF COMPOUND R.

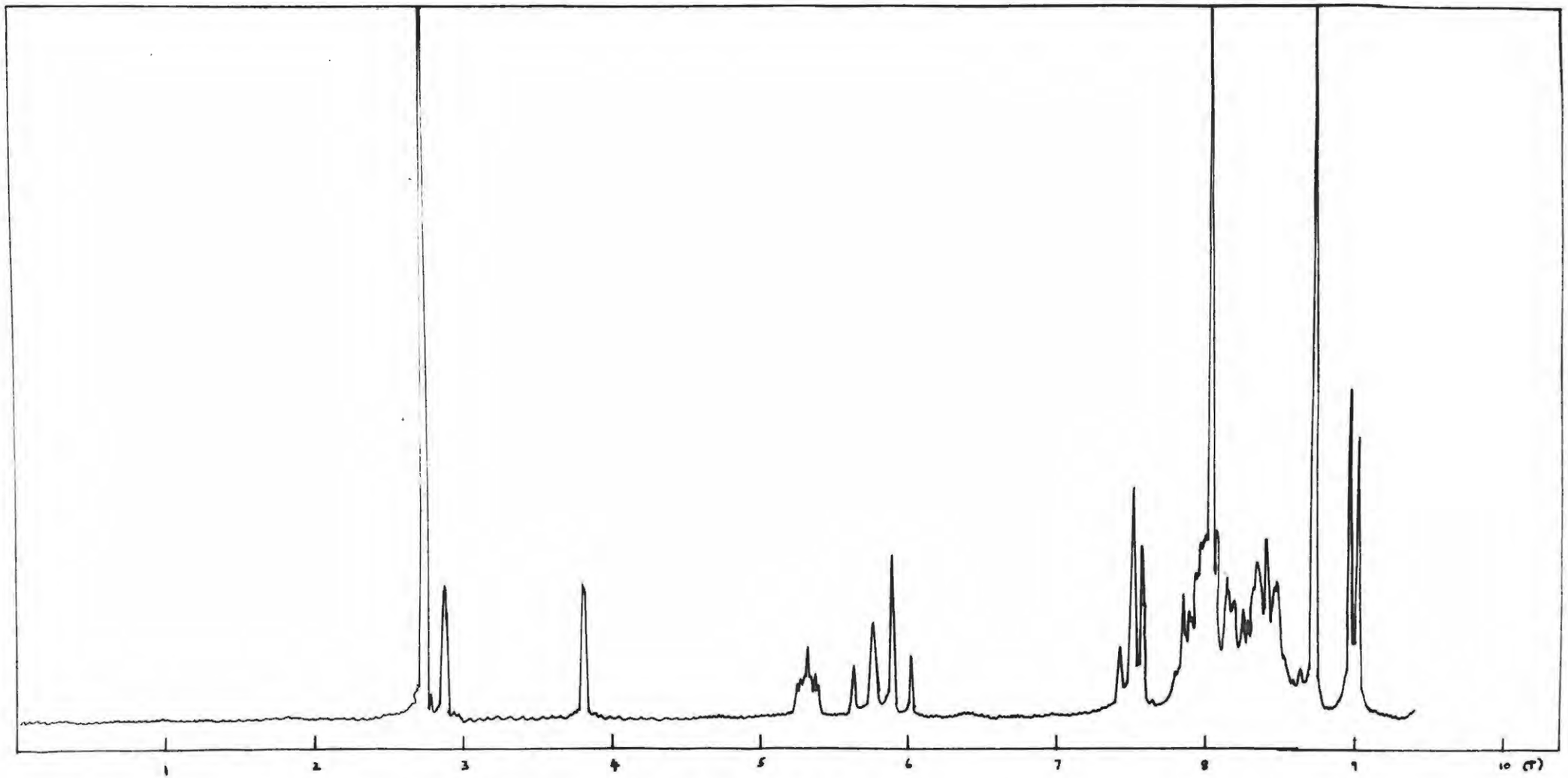


FIG 11. N.M.R. SPECTRUM OF COMPOUND R.

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