

EXTRACTIVES OF LEONOTIS  
AND EURYOPS SPECIES

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## CONTENTS

	<u>Page No.</u>
SUMMARY	1
1. <u>INTRODUCTION</u>	2
1.1 The stereochemistry of marrubiin	2
1.2 Stereochemistry of the ring junction	2
1.3 Stereochemistry of the lactone	6
1.4 Stereochemistry at C-8 and C-9	21
2. <u>EXPERIMENTAL</u>	32
2.1 Extraction of <u>Leonotis dubia</u>	32
2.2 Attempted dehydrogenation of dubiin	34
2.3 Attempted acetylation of dubiin	35
2.4 Hydrogenation of dubiin	36
2.5 Pyrolysis of dubiin	36
2.6 Oxidation of dubiin with chromium trioxide in acetic acid	37
2.7 Attempted hydrolysis of dubiin in acid medium	38
2.8 Attempted sodium borohydride reduction of dubiin	38
2.9 Saponification of dubiin	39
2.10 Attempted aminolysis of dubiin	40
2.11 Attempted tosylation of saponified dubiin	40
2.12 Acetylation of saponified dubiin	
(a) Using acetyl chloride	41
(b) Using acetic anhydride	41
2.13 Attempted determination of the configuration of the secondary hydroxy-group in saponified dubiin by Horeau's method	42
2.14 Attempted aminolysis of saponified dubiin	43
2.15 Treatment of saponified dubiin with sodamide	43
2.16 L-alinate hydrobromide derivative of saponified dubiin	44
2.17 Attempted preparation of the methyl ester of saponified dubiin	45

	<u>Page No.</u>
2.18 Treatment of saponified dubiin with lead tetra- acetate	45
2.19 Oxidation of saponified dubiin	
(a) Using Jones reagent	46
(b) Using Sarett reagent	47
(c) Using Collins reagent	47
(d) Using chromic acid in ether	48
2.20 Deuteration of the ketone (85)	49
2.21 Attempted preparation of the 2,4-dinitrophenyl- hydrazone of the ketone (85)	49
2.22 Attempted preparation of the oxime of the ketone (85)	50
2.23 Attempted preparation of the thioketal of the ketone (85)	50
2.24 Attempted phosphorus trichloride dehydration of the ketone (85)	50
2.25 Attempted Wolff-Kishner reduction of the ketone (85)	51
2.26 Attempted Clemmensen reduction of the ketone (85)	53
2.27 Attempted transesterification of the ketone (85)	
(a) Using boron trifluoride diethyl etherate	53
(b) Using diazomethane	54
(c) Using R <sub>2</sub> ONa	54
2.28 Reduction of dubiin with lithium aluminium hydride	55
2.29 Attempted partial reduction of dubiin and saponified dubiin with lithium aluminium hydride	56
2.30 Periodate oxidation of the tetraol (88)	58
2.31 Attempted dehydrogenation of the tetraol (88)	58
2.32 Acetylation of the tetraol (88)	
(a) Using acetyl chloride	59
(b) Using acetic anhydride	59
2.33 Attempted partial hydrolysis of the triacetate (89)	60
2.34 Silver carbonate-Celite oxidation of the tetraol (88)	60
2.35 Iodination of the tetraol (88) with triphenyl phosphite methiodide	62
2.36 Oxidation of the tetraol (88) with Collins reagent	63

	<u>Page No.</u>
2.37	Wolff-Kishner reduction of the Collins oxidation product of the tetraol (88) 64
2.38	Attempted tosylation of the tetraol (88) by initial preparation of the toluenesulphinate ester 65
2.39	Tosylation of the tetraol (88) 66
2.40	Reduction of this tosylate with lithium aluminium hydride 67
2.41	N.m.r. experiments using the paramagnetic shift reagent tris (dipivaloylmethanato) europium III, $\text{Eu(dpm)}_3$ 68
2.42	Extraction of <u>Leonotis leonitis</u> 69
2.43	Saponification of leonitin 70
2.44	Acetylation of saponified leonitin 71
2.45	Attempted partial hydrolysis of leonitin 71
2.46	Sodium borohydride reduction of saponified leonitin 73
2.47	Tosylation of the hemi-acetal (112) 73
2.48	Attempted acetoxylation of leonitin 74
2.49	Lithium aluminium hydride reduction of leonitin 74
2.50	Tosylation of the pentaol (113) 75
2.51	Lithium aluminium hydride reduction of the tosylate of the pentaol (113) 75
2.52	Acetylation of the pentaol (113) 76
2.53	Extraction of <u>Leonotis microphylla</u> 76
2.54	Extraction of <u>Euryops tenuissimus</u> 77
2.55	Extraction of <u>Euryops floribundus</u> 78
2.56	Oxidation of euryopsol with Sarett reagent 79
2.57	Manganese dioxide oxidation of euryopsol 80
2.58	Periodate oxidation of euryopsol 80
3.	<u>DISCUSSION</u> 82
3.1	Dubiin 82
3.2	Leonitin 105
3.3	Euryopsol 116
	<u>BIBLIOGRAPHY</u> 125

SUMMARY

The isolation and structure determination of dubiin and leonitin, two new diterpenoid acetates from Leonotis dubia and Leonotis leonitis respectively, are discussed. The compounds are diterpenoids of the labdane type and are closely related to marrubiin.

The proposed structures are based on chemical and spectral evidence. Dubiin,  $C_{22}H_{30}O_6$ , contains a tertiary hydroxy-group, a furan ring and a  $\delta$ -lactone while leonitin,  $C_{22}H_{30}O_7$ , is a  $\gamma$ -dilactone. Both compounds are unusual in being oxygenated at C-20.

The extraction of three Euryops species and the isolation of euryopsol,  $C_{15}H_{22}O_4$ , are also described. A furanoeremophilane structure containing three hydroxy-groups, one of which is at a bridgehead position, is proposed. Euryopsol is the first furanoeremophilanoid with a substituent attached at C-10.

## 1. INTRODUCTION

### 1. 1 The Stereochemistry of Marrubiin

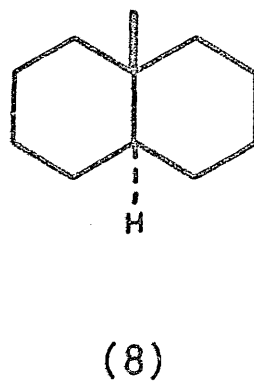
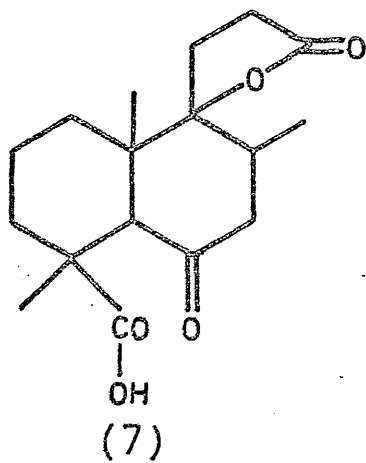
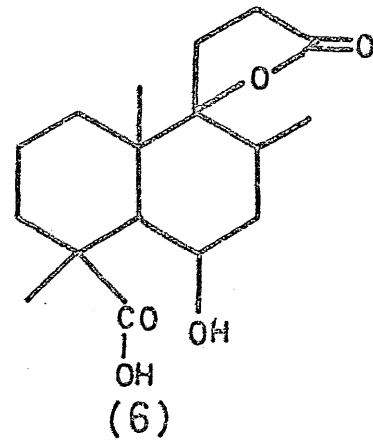
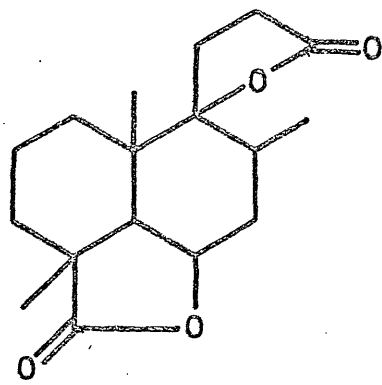
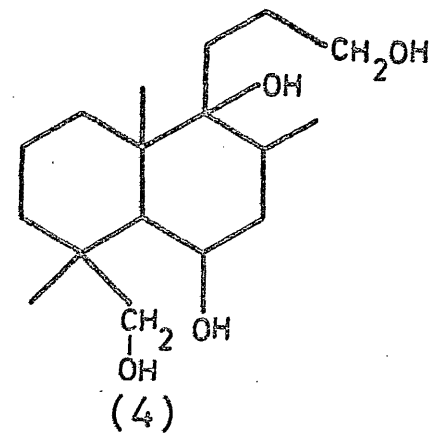
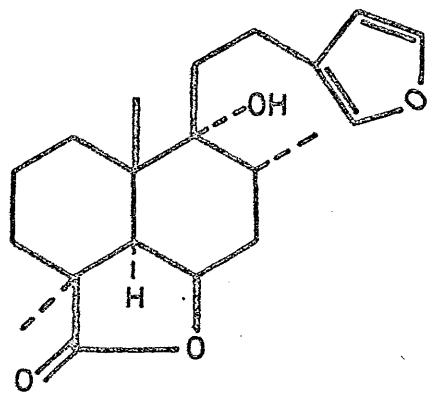
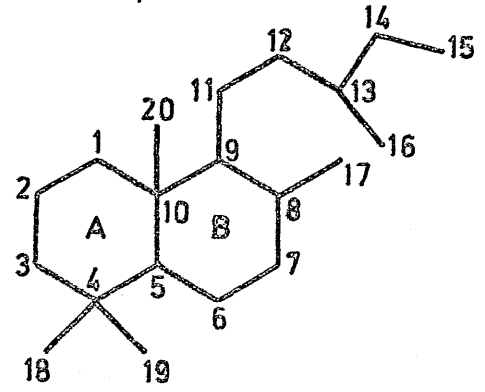
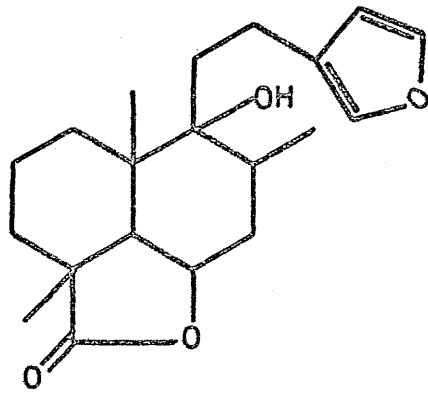
The diterpenoid, marrubiin,  $C_{20}H_{28}O_4$ , (1) was first obtained from horehound (Marrubium vulgare) as early as 1842.<sup>1</sup> It has since also been obtained from Leonotis leonuris.<sup>2</sup> Since the chemistry of the two diterpenoids, dubiin and leonitin, studied in this thesis, is so closely related to that of marrubiin, a thorough knowledge of the latter is essential and will be considered in detail.

The carbon skeleton (2) of marrubiin was first proposed by Lawson and Eustice;<sup>3</sup> the numbering system is that suggested by Overton and McCrindle<sup>4</sup> and will be used throughout this discussion. Although the structure of marrubiin has been known for some time,<sup>5,6</sup> its absolute stereochemistry has been the subject of considerable discussion. However, it is now fixed as shown in (3), as a result of studies of infrared and nuclear magnetic resonance spectra of degradative and synthetic products. Throughout this thesis, chemical shift values of n. m. r. spectra will be given on the delta,  $\delta$ , scale, this now being the preferred scale.<sup>7</sup> In all cases, the internal reference is taken as tetramethylsilane, which has a chemical shift of 0.00.

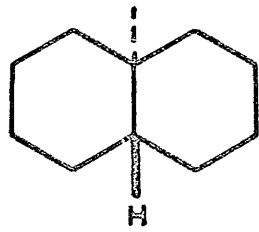
There are six asymmetric centres in marrubiin and these will now be considered in turn.

### 1. 2 Stereochemistry of the ring junction

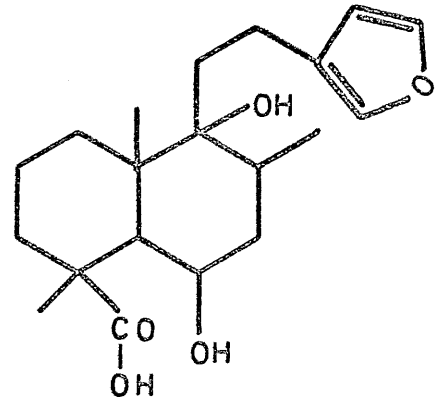
The following series of reactions established<sup>8</sup> that rings A and B are trans-fused. Marrubiin was converted to the tetraol (4) by



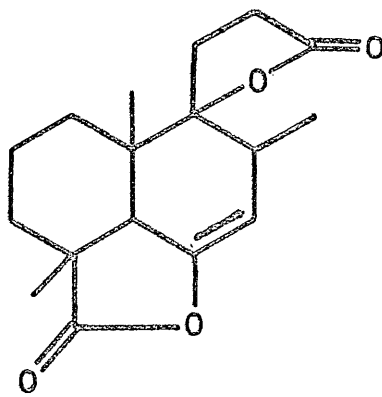
the sequence (1) → (5) → (6) → (4) under conditions which could not have affected the stereochemistry of the A/B ring fusion. The hydroxy-acid (6) was oxidised to the keto-acid (7) which was not isomerised by alkali<sup>6</sup> and therefore, must have rings A and B trans-fused. The reduction of compound (7) with lithium aluminium hydride to the same tetraol (4) showed that the latter, and hence marrubiin, must also have a trans-decalin system. Consequently, marrubiin must have the partial skeleton (8) or (9). In two later communications, Burn and Rigby<sup>9, 10</sup> supported the proposed trans-fused structure for marrubiin. An attempt was made<sup>11</sup> to correlate marrubiin with a compound of known structure. The sodium salt of marrubic acid (10), formed by saponification of marrubiin, was oxidised with permanganate to the acid (6) which, on oxidation with chromic acid, gave the corresponding keto-acid (7). This was converted to an enol-lactone which was shown to be (11) and not (12), since it afforded the aldehyde (13) on ozonolysis.<sup>12</sup> Hydrogenolysis of the enol-lactone gave the lactonic acid (14) as the major (70%) product, but contrary to the findings of Cocker et al.<sup>6</sup> the minor product (20%) was the dilactone (5) and not unchanged enol-lactone. The acid chloride, obtained by thionyl chloride treatment of the lactonic acid (14), was reduced by the Rosenmund procedure to the aldehyde (15), which, on Wolff-Kishner (Huang-Minlon) reduction, gave the expected tetramethyl derivative (16) as the minor (25%) product. This was identical with one of the isoambreinolides prepared<sup>13</sup> by the action of 70% sulphuric acid on ambreinolide (17). However, the major (62%) product of the Wolff-Kishner reduction was an acid which gave a yellow colour with tetranitromethane. Its structure would be



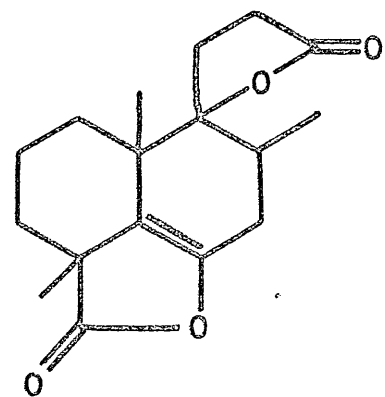
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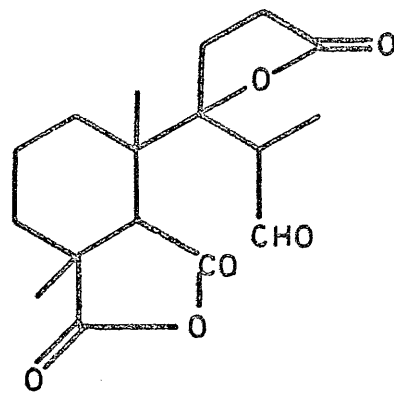
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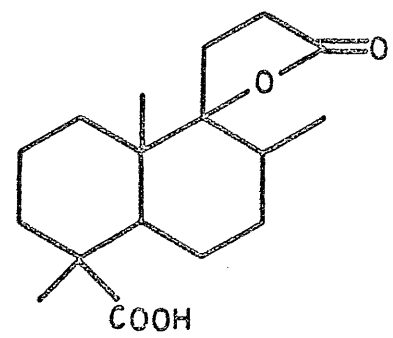
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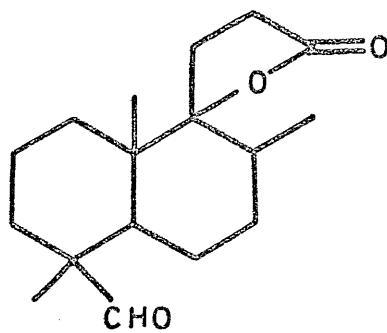
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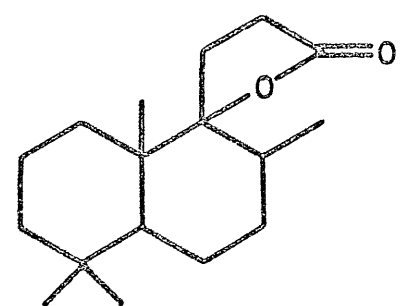
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(14)



(15)



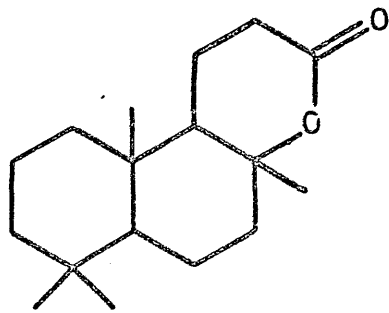
(16)

expected to be either (18) or (19), but since it was not decarboxylated at high temperature, it was not a  $\beta, \gamma$ -unsaturated acid and was therefore assigned the structure (19). This compound was also prepared<sup>14, 15</sup> by the action of sulphuric acid on ambreinolide (17) and was shown to be identical (m. p., mixed m. p. and infrared spectrum) with that from marrubiin, the identity being confirmed by comparison of the hydrogenated acids (20) and by conversion, with osmium tetroxide, of the acid derived from marrubiin to the known lactone (21).

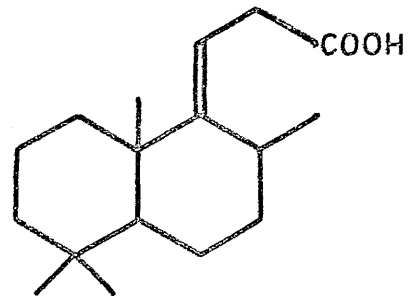
This correlation confirmed the stereochemistry of the ring fusion and the angular methyl group in marrubiin. The asymmetric centre carrying the bridgehead methyl group in these compounds remained unaffected during these transformations; thus the angular methyl group was assigned the  $\beta$ -configuration bearing that stereochemical relationship to the ring system which was found to be general for the resin acids, the triterpenoids and the steroids. Some of the dilactone (5) was recovered in the initial hydrogenation and this showed that the ring junction was also unaffected, giving marrubiin a trans-ring junction. More recently this has been confirmed by ORD studies<sup>16</sup> performed on the ozonolysis product of anhydrotetrahydromarrubiin (see later), which shows a positive Cotton effect.

### 1. 3 Stereochemistry of the lactone

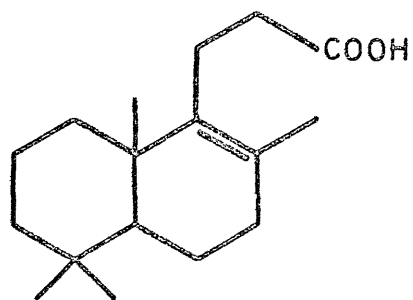
Cocker, Edward and Holley<sup>8</sup> showed that closure of the lactone ring in marrubiin and some of its derivatives produces a



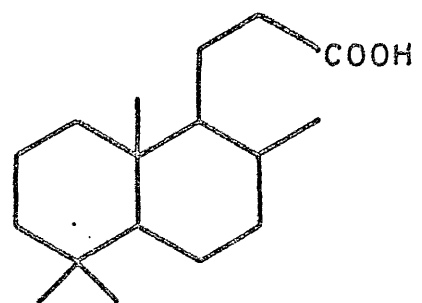
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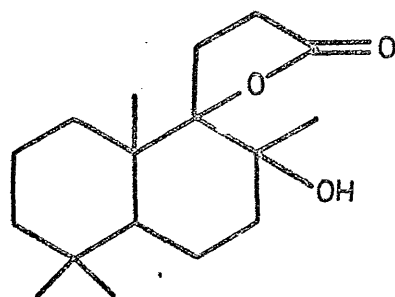
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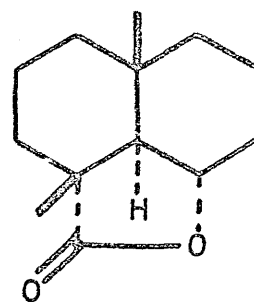
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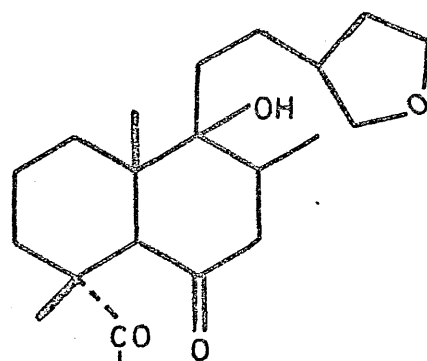
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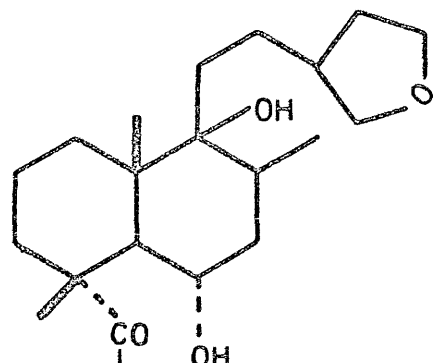
(21)



(22)



(23)



(24)

positive shift in the molecular rotation. Application of Hudson's rule, as developed by Klyne,<sup>17</sup> which states that the stereochemistry of the carbon atom carrying the potential hydroxy-group in  $\gamma$ - or  $\delta$ -sugar-lactones determines the sign of the rotation contribution of the lactone ring, led Cocker *et al.* to propose that the oxygen atom at C-6 is  $\alpha$ . Since a trans-configuration of the lactone ring would involve excessive strain, they suggested that the carboxy-group should be cis, giving rise to an  $\alpha$ -oriented lactone ring. Therefore, since it had already been established that the 20-methyl group was in the  $\beta$ -configuration, the decalin system was considered to have the configuration shown in (22), with the lactone ring joined by 4,6 equatorial-equatorial bonds.

This configuration of the lactone was confirmed by Castine, Wheeler and Wheeler.<sup>18</sup> They reduced the keto-acid (23) using (a) lithium, ammonia and methanol and (b) sodium borohydride. Reduction (a) is known to give equatorial alcohols while (b) gives axial products. However, under both sets of conditions, they obtained the same product, tetrahydromarrubic acid, shown as (24), in better than 70% yield. The result of the lithium-ammonia-methanol reduction of (23) indicated that the hydroxy-group at C-6 in tetrahydromarrubic acid was equatorial and hence  $\alpha$ .<sup>19</sup> However, application of Barton's generalisations<sup>20</sup> concerning the stereochemistry of hydride reductions suggested that the borohydride reduction of compound (23) had proceeded in an anomalous manner. In general, reduction with sodium borohydride affords the equatorial alcohol if the keto-group is not hindered and the axial epimer if it is hindered. Since the keto-group in marrubiin is hindered, it would be expected that the borohydride reduction of compound (23) should yield not (24),

but its C-6 epimer. To explain this unexpected formation of compound (24), the authors suggested that, since the carboxy-group of (23) was equatorial, the formation of compound (24) was favoured, as previous work<sup>21</sup> had shown that a carboxy-group in this position exerts an electrostatic shielding effect which prevents attack of the borohydride ion from the  $\alpha$ -side of the molecule.

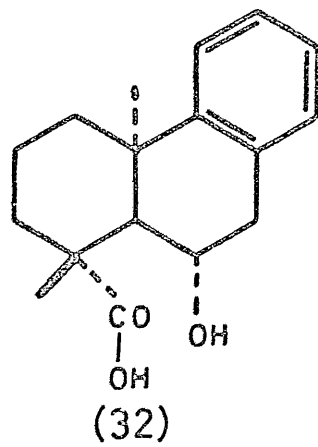
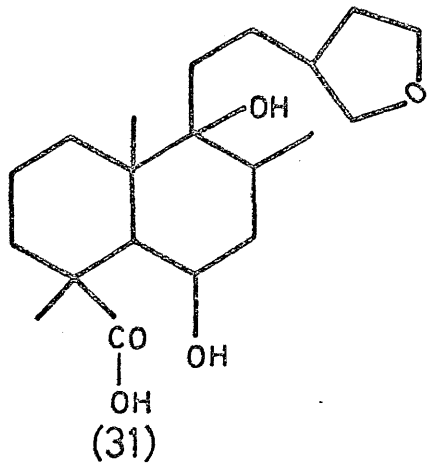
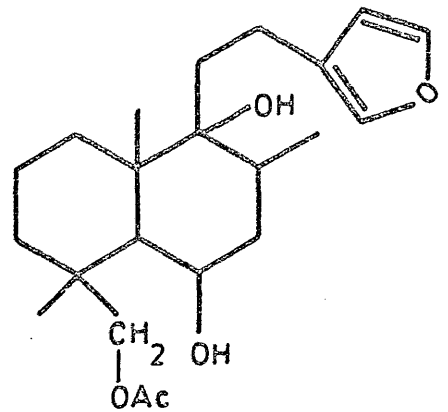
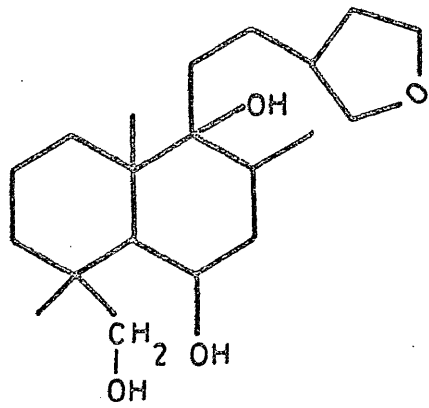
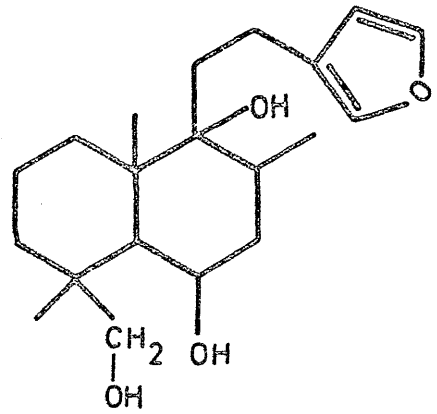
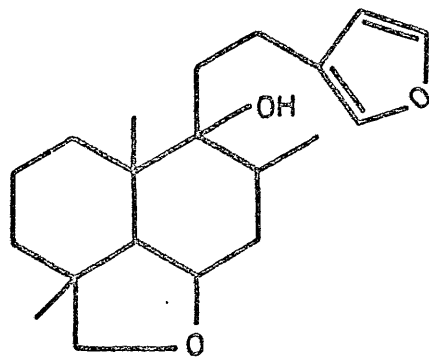
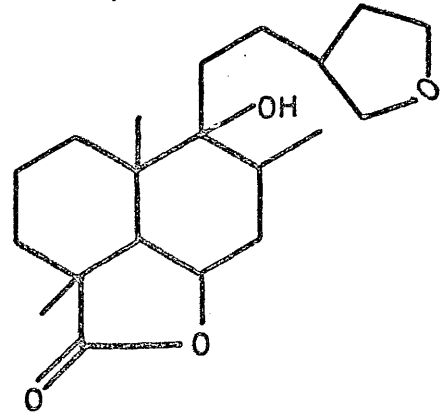
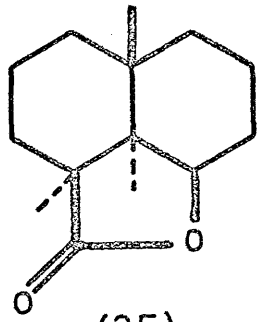
However, in a subsequent communication, Cocker et al.<sup>12</sup> stated that in their earlier paper<sup>8</sup> their interpretation of the rotational evidence for the configuration at C-6 was in error and that, since the lactone ring makes a positive contribution to the molecular rotation, it should be  $\beta$ .<sup>17</sup> Hence, by the same reasoning as before, they considered that the lactone ring should have the configuration shown in (25). This configuration was further supported by application of the rule of Klyne and Stokes<sup>22</sup> to the facts that (a) the C-6 hydroxy-group makes a negative contribution to the molecular rotation and (b) acetylation of this hydroxy-group leads to a negative shift in rotation.

Burn and Rigby,<sup>10</sup> although they did not consider the proposed  $\beta, \beta$ -lactone bridge as being necessarily incorrect, regarded this argument as unacceptable since: (i) There was no reason to believe that Klyne's lactone rule<sup>17</sup> would be valid for the structure favoured by Cocker et al. since data concerning its application to such structures apparently did not exist. (ii) Cocker et al.<sup>8</sup> had pointed out that vicinal effects could make the rule of Klyne and Stokes<sup>22</sup> invalid for the potential hydroxy-group at C-6 but had ignored such effects in their stereochemical deductions, even although they believed<sup>6</sup> them to be very strong in the related keto-acid (23). (iii) There was no

stereochemical reason for omitting from consideration a "skew" lactone bridge ( $4\alpha \rightarrow 6\beta$  or  $4\beta \rightarrow 6\alpha$ ) so that the configuration at C-4 should not follow from that at C-6 (or vice versa) as had been assumed.

With the development of nuclear magnetic resonance as a tool in the determination of organic structures, the configuration of the hydroxy-group was determined by Fulke and McCrindle<sup>23</sup> who concluded that the lactone ring in marrubiin was cis-fused and  $\beta$ -oriented. In the n. m. r. spectra of marrubiin, tetrahydromarrubiin (26) and the ether (27), the 6-H resonated at 4.75, 4.72 and 4.15 respectively as an ill-resolved triplet (multiplet width 10-14 Hz). Furthermore, in the n. m. r. spectra of marrubenol (28), marrubanol (29) and the oily monoacetate (30), this proton resonated as a broad singlet ( $W_{\frac{1}{2}} = 6-8$  Hz) at 4.22, 4.22 and 4.34 respectively. The narrowness of these resonances is diagnostic<sup>24</sup> of an equatorial proton at C-6, since the band width is compatible with one equatorial-equatorial and two axial-equatorial spin-spin couplings and both C-5 and C-7 carry an axial proton. The substituent at C-6 in marrubiin and its derivatives discussed above is, therefore, axial ( $\beta$ ).

Convincing evidence for the stereochemistry at C-4 was less readily obtained. Normally with diterpenoids, the n. m. r. shift values<sup>25, 26, 27</sup> for the protons in the functional groups  $-\text{CH}_2\text{OAc}$ ,  $-\text{CH}_2\text{OH}$  and  $-\text{CHO}$  and/or  $\text{pK}^*_{\text{MCS}}$  measurements<sup>28</sup> for the corresponding carboxylic acids allow the stereochemistry of the oxygenated methyl groups at C-4 to be deduced. In the present case, when utilising compounds readily available from marrubiin, such data



did not accord with either a normal axial or equatorial group. The  $pK^*_{MCS}$  of marrubic acid (10) and tetrahydromarrubic acid (31) were 6.66 and 6.71 respectively, both well below those expected for either axial (8.4) or equatorial (7.9) carboxy-groups, probably because of the stabilisation of the carboxylate ion by hydrogen bonding to the secondary hydroxy-group. Indeed, the two acids and their methyl esters showed strong intramolecular hydrogen bonding in their infrared spectra. (It is noteworthy that the closely related acid (32), which has  $pK^*_{MCS}$  6.35 showed, in infrared hydrogen bonding studies, properties very similar to those of marrubic acid). The n.m.r. spectra of readily accessible derivatives of marrubiin were also of little use in distinguishing between the two possible orientations of the lactone ring at C-4. In the keto-aldehyde (33) the aldehydic proton appeared as a barely resolved doublet ( $J$  1 Hz) at 10.42, a value considerably lower than that expected<sup>25,26,27</sup> for either an axial (9.8 approx.) or an equatorial (9.3 approx.) aldehyde group at C-4. This downfield shift must be due to the carbonyl group at C-6. A similar shift was apparent in the spectrum of the oily keto-acetate (34) which showed a 2-proton quartet ( $J$  12 Hz) centred at 4.68 which conformed<sup>25,26,27</sup> with neither an axial (4.10-4.30) nor an equatorial (3.65-3.85) primary acetate group at C-4. In the monoacetate (30) this methylene group appeared as a quartet ( $J$  12 Hz) at 4.56, the downfield shift being induced by the C-6 axial hydroxy-group. In this case, the shift is better rationalised as deriving from an axial, rather than an equatorial, primary acetate group. Reasoning along these lines, however, led only to tentative conclusions and to resolve the problem it was necessary to remove the oxygen function at C-6.

Thus an attempt was made to reduce the keto-acid (35) by the Wolff-Kishner method, but this led to the formation of a cyclic hydrazone.<sup>29</sup> The keto-acid was therefore reduced<sup>9</sup> by conversion to the enol-lactone (36) with refluxing acetic anhydride containing fused sodium acetate. The n. m. r. spectrum of this enol-lactone contained a resonance at 5.03 (quartet, J 2.5 Hz) attributable to one olefinic proton, confirming the presence of a  $\Delta^6$  rather than a  $\Delta^5$  double bond. Hydrogenation of the enol-lactone over Adam's catalyst in acetic acid afforded, as the major product, the acid (37), produced by hydrogenolysis of the vinylic oxygen atom and saturation of the double bond. Reduction of the methyl ester of the acid (37) with lithium aluminium hydride gave the oily diol (38), the derived monoacetate also being an oil. This latter compound showed, in the n. m. r. spectrum, a quartet (J 11 Hz), centred at 4.07 (-CH<sub>2</sub>OAc) as expected<sup>25, 26, 27</sup> for an axial primary acetate group at C-4. Furthermore, oxidation of the alcohol (38) with Sarett reagent<sup>30</sup> provided the unstable oily aldehyde (39), the n. m. r. spectrum of which showed a resonance at 9.86 (1H, singlet), a value which was acceptable for the proton in an axial aldehyde group at C-4. Similar results were obtained for the C-9-deoxy-series of compounds.

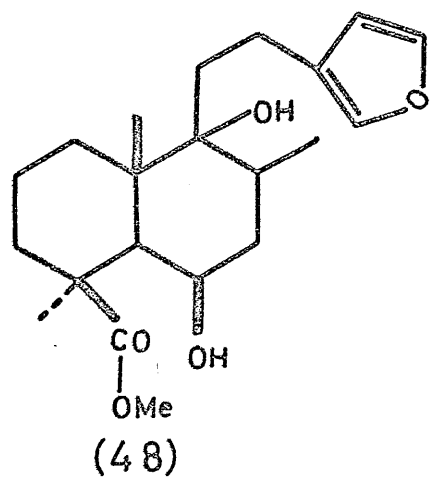
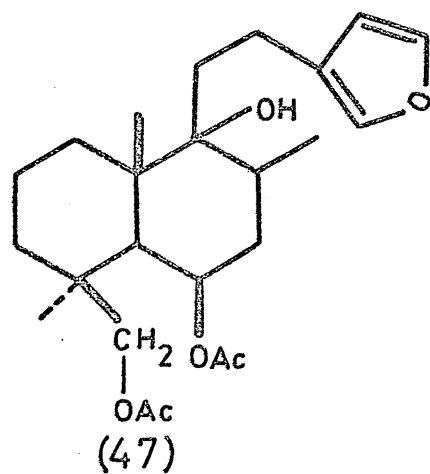
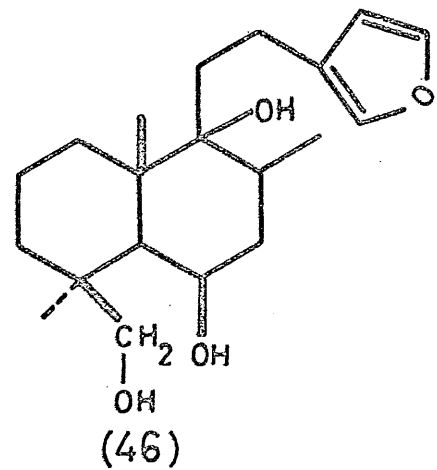
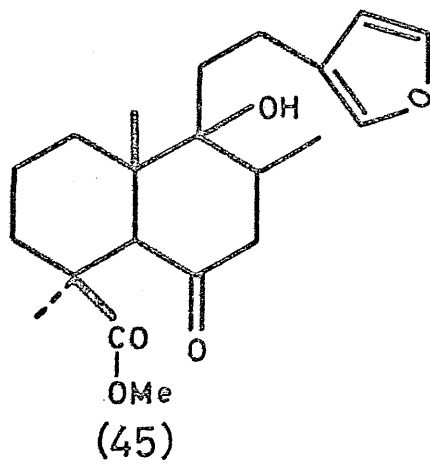
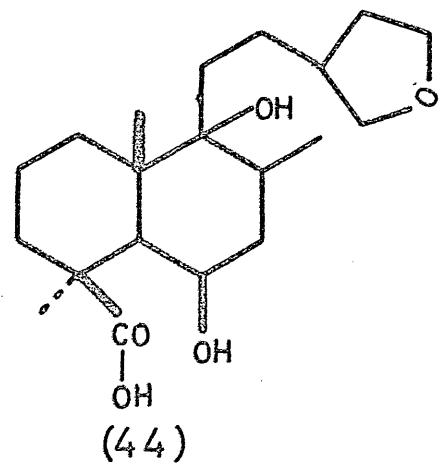
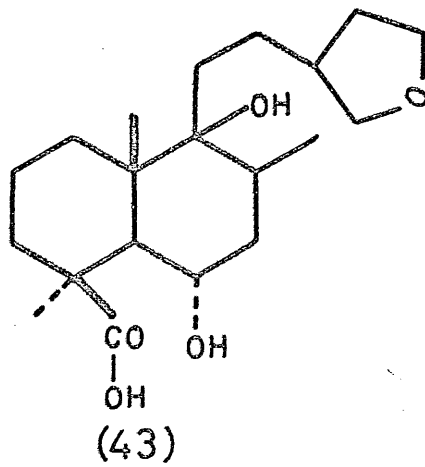
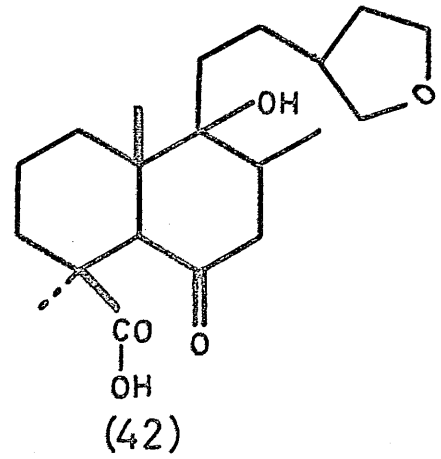
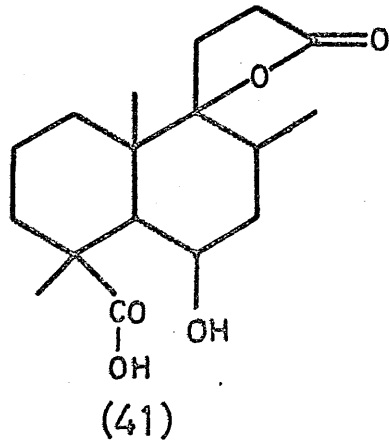
The validity of the  $\beta$ -cis-orientation, assigned mainly on spectroscopic grounds, was confirmed by purely chemical evidence. Thus, (a) the ease of formation of marrubiin from marrubic acid (10)<sup>6</sup> and that of the ether (27) on attempted p-bromobenzenesulphonation of marrubenol (28) could be rationalised, since in each case a 1, 3-diaxial non-bonded interaction was being removed; (b) the observation<sup>10</sup> that the olefin (40) was obtained in appreciable yield



(50%) from treatment of the hydroxy-acid (41) with toluene-*p*-sulphonyl chloride and pyridine at 20°, would indicate a smooth trans-diaxial (5 $\alpha$ , 6 $\beta$ ) elimination of toluene-*p*-sulphonic acid from the intermediate ester; (c) the secondary hydroxy-group in marrubenol (28) was fairly resistant to acetylation (it resisted acetylation with acetic anhydride and pyridine at room temperature for 14 hours), the product of acetylation under mild conditions being the monoacetate (30) which was, however, readily oxidised to the keto-acetate, i. e. reactivity typical of an axial secondary alcohol.

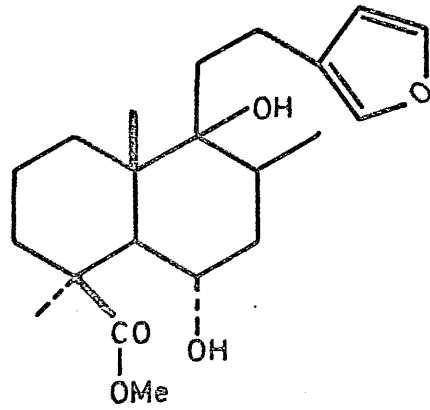
Wheeler, Wheeler, Fetizon and Castine,<sup>31</sup> although originally proposing that the lactone was fused  $\alpha, \alpha$ ,<sup>18</sup> independently reached the same conclusion as Fulke and McCrindle as a result of the following work. They originally planned to reduce the keto-acid (42) stereospecifically to the corresponding equatorial (43) and axial (44) alcohols. One of these should be tetrahydromarrubic acid and this would establish the configuration at C-6. Studies on the ease of lactonisation of tetrahydromarrubic acid and its C-6 epimer would then establish the arrangement at C-4. However, as stated earlier, reduction of the keto-acid (42) with either sodium borohydride or lithium in liquid ammonia afforded tetrahydromarrubic acid. Thus one of the reactions must have taken place in an unexpected fashion. To substantiate this, they decided to study the reduction of the methyl ester of the acid (42), but since this ester could not be crystallised, they turned from the tetrahydro- to the marrubiin series.

Attempts to reduce the keto-ester (45) with sodium borohydride were unsuccessful; at room temperature, starting material was recovered, while under reflux in isopropyl alcohol reduction was

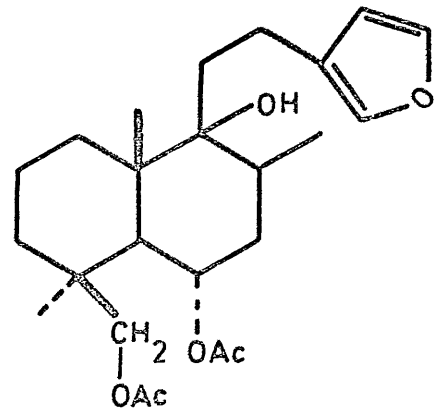


accompanied by cleavage of the ester. Accordingly, the keto-ester was reduced with lithium aluminium hydride to marrubenol (46), also obtained by direct reduction of marrubiin. Reduction of the keto-ester in methanol with lithium in liquid ammonia gave an oil whose infrared spectrum showed strong hydroxyl absorption and little carbonyl absorption. On acetylation, it gave a diacetate. A comparison of the infrared and n. m. r. spectra of this diacetate with those of the diacetate from marrubenol (47) left no doubt that the oil was the C-6 epimer of marrubenol. This showed that the anomalous reduction of the keto-acid (42) was that in which lithium in liquid ammonia was used, and thus established that the secondary hydroxy-group in marrubenol was axial ( $\beta$ ).

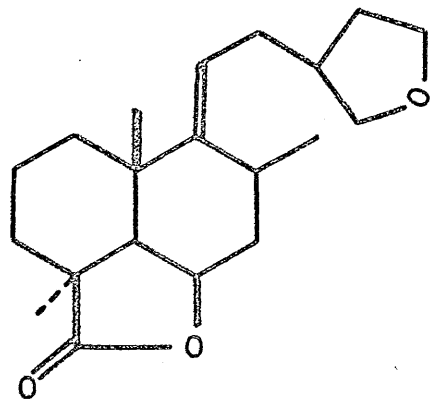
Bory and Fetizon<sup>32</sup> examined the infrared spectra of the methyl esters of a large number of di- and triterpenoids containing both a methyl and carboxy-group at C-4. They found that when the carbomethoxy-group was equatorial, the spectrum contained a single intense band at  $1245 \pm 4 \text{ cm}^{-1}$ . By contrast, compounds with axial carbomethoxy-groups showed little absorption at  $1245 \text{ cm}^{-1}$ , but had an intense peak at  $1145 \pm 5 \text{ cm}^{-1}$  which was accompanied by a less intense peak at  $1190 \pm 5 \text{ cm}^{-1}$  and a very weak one at  $1230 \pm 5 \text{ cm}^{-1}$ . Examination of the spectra of methyl marrubate (48), methyl 6-epimarrubate (49) and methyl dehydromarrubate (45) showed patterns which conformed to the axial ( $\beta$ ) arrangement of the carbomethoxy-group. The infrared spectrum of methyl marrubate also showed the carbonyl ester band at  $1697 \text{ cm}^{-1}$  with a small shoulder at  $1726 \text{ cm}^{-1}$  and an intense hydroxyl peak at  $3430 \text{ cm}^{-1}$  with a smaller band at  $3620 \text{ cm}^{-1}$ . The C-6 epimer of methyl marrubate (49) showed



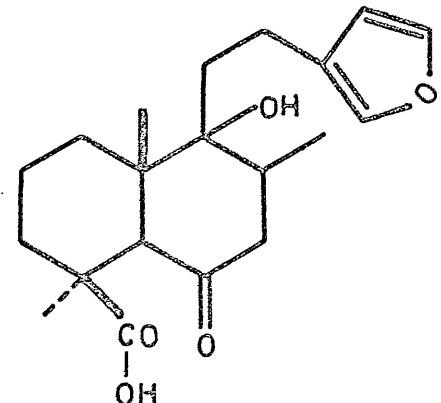
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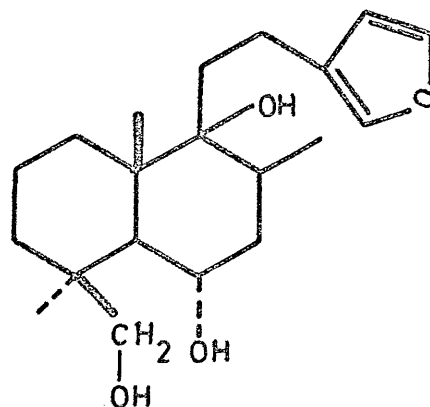
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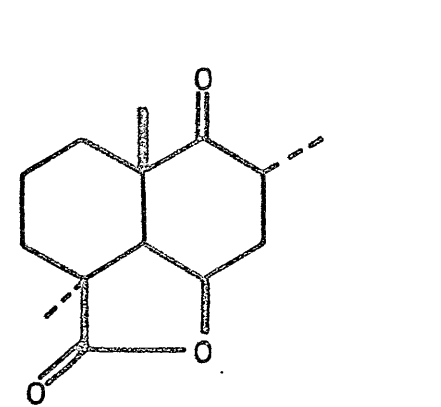
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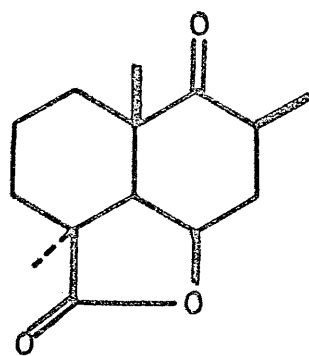
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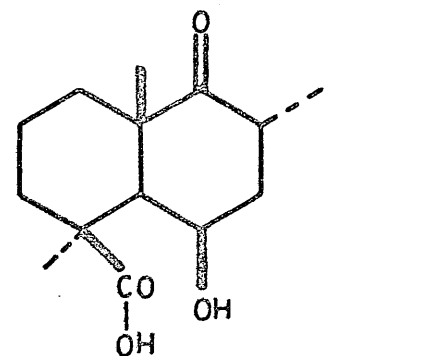
(53)



(54)



(55)



(56)

a carbonyl peak at  $1704\text{ cm}^{-1}$  which had a shoulder of almost equal intensity at  $1725\text{ cm}^{-1}$  and a broad hydroxyl peak at  $3630\text{ cm}^{-1}$  with a shoulder at  $3550\text{ cm}^{-1}$ . Clearly hydrogen bonding occurred in both compounds and was stronger in (48) than in (49). It followed that the C-6 hydroxy-group in (48) was cis to the axial carbomethoxy-group and was therefore  $\beta$ .

Conclusive evidence for the stereochemistry at C-4 and C-6 came from a study<sup>31</sup> of the n. m. r. spectra of the esters (48) and (49) and the acetates (47) and (50). The signals for the C-6 proton in the spectra of methyl marrubate and methyl 6-epimarrubate were at 4.40 ( $W_{\frac{1}{2}} = 6\text{ Hz}$ ) and 4.08 ( $W_{\frac{1}{2}} = 35\text{ Hz}$ ) respectively. Similarly, the C-6 proton appeared at 5.40 ( $W_{\frac{1}{2}} = 6\text{ Hz}$ ) and 5.10 ( $W_{\frac{1}{2}} = 25\text{ Hz}$ ) in the spectra of compounds (47) and (50) respectively. It is well known<sup>33</sup> that with both acetoxy- and hydroxy-pairs of epimers the signal for the C-6 proton should be narrower and occur at a lower field for a compound with the proton equatorial (oxygenated substituent axial) as in (47) and (48) than for the compound with the proton axial (oxygenated substituent equatorial) as in (49) and (50).

The shifts in the positions of the signals for the methyl groups with changes in structure also supported the proposed stereochemistry. (See Table I). As expected, the peaks for the tertiary methyl groups appeared in almost the same positions in the spectra of marrubiin, tetrahydromarrubiin, anhydrotetrahydromarrubiin (51) and the ester (48). In going from (48) to (49), the C-6 hydroxy-group goes from  $\beta$  to  $\alpha$  which leads to the observed shielding of the 20-methyl group and a deshielding of the 18-methyl group. Changing the hydroxy-group at C-6 to a ketone (52, 42 and 45) should lead to an

Table I<sup>31</sup>

Positions of the C-methyl peaks in the n. m. r. spectra of marrubiin and some derivatives (CDCl<sub>3</sub> solutions).<sup>a</sup>

Compound	18-Me	20-Me	17-Me <sup>b</sup>
marrubiin	78	64	58
tetrahydromarrubiin	77	63	55
anhydrotetrahydromarrubiin (51)	77	65	66 <sup>c</sup>
ester (48)	78	63	59
ester (49)	88	46	57
keto-acid (52)	76	52	66
keto-acid (42)	72	58	63 <sup>d</sup>
keto-ester (45)	74	67	62
triol (46)	63	79	58
triol (53)	75	62	58

<sup>a</sup>Shifts were obtained on a 60 MHz spectrometer and are given in Hz downfield from TMS.

<sup>b</sup>Except where indicated, this signal appears as a doublet with coupling constant J 6 Hz.

<sup>c</sup>J 8 Hz.

<sup>d</sup>Only one half of the doublet visible.

upfield shift of the 20-methyl resonance,<sup>34</sup> which was observed in the spectra of the acids (52) and (42), but in the spectrum of the ester (45) there was a slight downfield shift. The ester differed from the other compounds in that there was no hydrogen bonding between the substituents at C-4 and C-6. Thus in the acids (52) and (42), the carbonyl group of the acid was in the same plane as, but pointing away from, C-6, while in the ester, the ester carbonyl group was in the same plane as, but pointing towards, C-6. With compound (45) the dipole-dipole repulsion between the carbonyl groups may have caused the carbomethoxy-group to twist to a new conformation which affected the shielding of the angular methyl group, thus accounting for the anomalous shift. As expected, changing the hydroxy-group from  $6\beta$  to  $6\alpha$  (46  $\rightarrow$  53) deshielded the 18-methyl and shielded the 20-methyl groups. The shifts shown by the acetates paralleled those shown by the corresponding hydroxy-compounds.

#### 1. 4 Stereochemistry at C-8 and C-9

Cocker et al.,<sup>8</sup> supported by Castine, Wheeler and Wheeler,<sup>18</sup> put forward the suggestion that the 17-methyl group of the keto-lactone (54), obtained on ozonolysis of anhydrotetrahydromarrubiin (51), should be in the stable equatorial configuration shown, because it was not epimerised by boiling alkali. Since anhydrotetrahydromarrubiin was formed from marrubiin under conditions not likely to epimerise C-8, the 17-methyl group in marrubiin must be equatorial ( $\alpha$ ) and the 8-H must be axial. This argument was also found to be unacceptable by Burn and Rigby<sup>10</sup> although they did not deny the possibility of this

methyl group being, in fact, equatorial. They stated that if the methyl group in marrubiin was axial, the generated keto-lactone would be compound (55) and it was to be expected that this tri- $\alpha$ -substituted ketone would pass into its C-8 epimer with such ease that failure to isolate it should occasion no surprise. This was refuted by Stephens and Wheeler<sup>35</sup> who repeated the ozonolysis of anhydrotetrahydromarrubiin in MeOD and decomposed the ozonide with methyl sulphide. Under these conditions, epimerisation of C-8 would lead to the incorporation of deuterium in the keto-lactone. However, a study of the n. m. r. spectrum of the keto-lactone showed that incorporation of deuterium had not taken place, which indicated that the configuration of the 17-methyl group in compounds (51) and (54) was the same.

Contrary to the findings of Cocker et al.,<sup>8</sup> Mangoni and Adinolfi<sup>36</sup> observed that the keto-lactone (54) was transformed by alkali into its C-8 epimer. When hydrolysed with potassium hydroxide in methyl cellosolve, both epimers yielded the same hydroxy-acid which was assumed to have the 17-methyl group in the equatorial configuration as shown in (56). As this, under very mild conditions (ethyl chloroformate in triethylamine at 0°), quantitatively reformed the keto-lactone (54), the steric structure with the same configuration at C-8 had to be assigned to the latter and the structure (55) to the epimer. In order to rationalise the alkali-catalysed conversion of (54) to (55), formally involving an equatorial to axial epimerisation, the keto-lactone (55) was assumed to have ring B in a twist form. However, this did not necessarily imply that the 17- $\alpha$ -methyl hydroxy-acid (56) also had ring B in the same conformation, as the distortion due to the presence

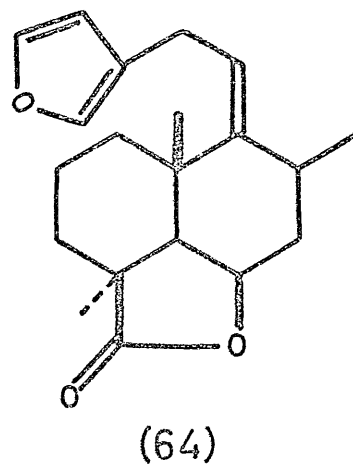
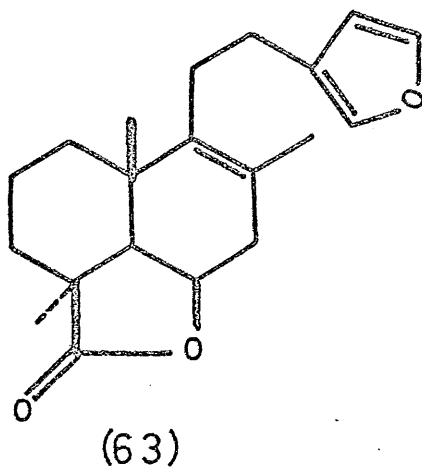
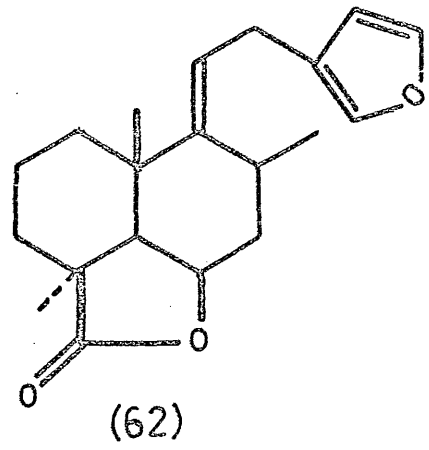
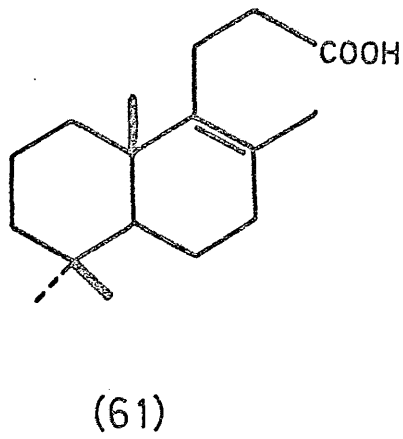
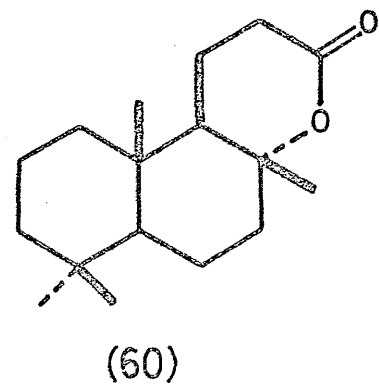
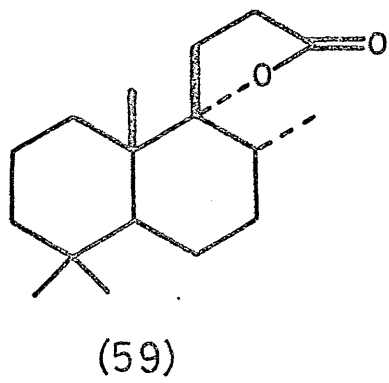
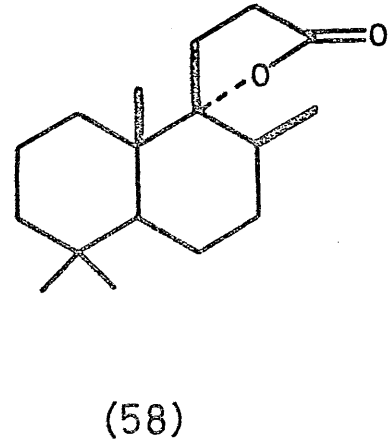
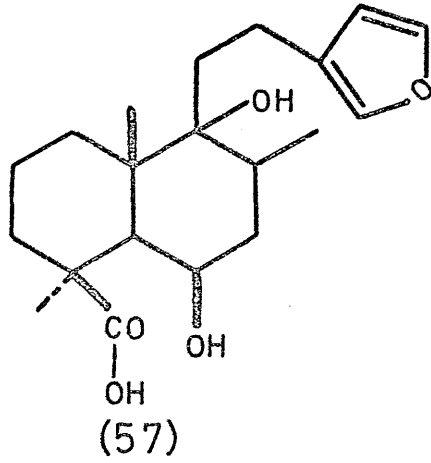
of the lactone bridge may have made compound (55) more stable than (54), even if, in the latter, ring B was in the normal chair form.

With the development of n.m.r., Appleton, Fulke, Henderson and McCrindle<sup>37</sup> assigned an  $\alpha$ - (equatorial) configuration to the 17-methyl group. Their evidence involved the assumption that the compounds discussed had ring B in the chair (or half chair) conformation. The major factor liable to lead to the preference for a ring B boat conformation would be the 1,3-diaxial interaction between a 17- $\beta$ -methyl and the 20- $\beta$ -methyl groups. However, from models it seemed likely that a downward rotation of C-8 would eventually produce even more severe steric interactions. Thus, assuming that ring B has a chair conformation, it was expected<sup>34</sup> that if the 17-methyl group was axial, the conversion of the monoacetate (30) to the keto-acetate (34) would produce a similar upfield shift of about 15-20 Hz for the resonances of the 17- and 20-methyl groups in their n.m.r. spectra. Although the observed upfield shift (22 Hz) in the resonance of the necessarily axial 20-methyl group did, indeed, accord reasonably well with expectation, the resonance due to the 17-methyl group suffered a downfield shift of 3.5 Hz. This group was therefore considered to be equatorial. The resonances of the 18- and 20-methyl groups in the spectrum of the keto-acetate (34) were identified by recording the spectra after progressive additions of benzene to the deuteriochloroform solution and utilisation of the "Plane Rule"<sup>38, 39</sup> which states that if a plane P is drawn through the carbon atom of a carbonyl group at right angles to the carbon-oxygen bond, then the protons close to P show very small shifts ( $\delta_{\text{benzene}} - \delta_{\text{CDCl}_3}$ );

protons on the same side of P as the oxygen atom are deshielded while protons on the other side of P are shielded.

Examination of the positions of the resonances of the 17-methyl group in the n. m. r. spectra of various other compounds<sup>31</sup> (see Table I) also led to the conclusion that this group is equatorial. The changes in the position of this resonance with structure did not parallel those shown by the 20-methyl resonance; for example, the exocyclic double bond in anhydrotetrahydromarrubiin had a deshielding effect (tetrahydromarrubiin  $\rightarrow$  anhydrotetrahydromarrubiin) on the 17-methyl group and practically no effect on the 20-methyl group; again, in going from compound (57) to compound (52), the peaks for the 17- and 20-methyl groups moved in opposite directions, indicating that the 17-methyl group was equatorial. However, it was also possible that this group could be equatorial and  $\beta$  with ring B in a non-chair form. In marrubiin itself and its derivatives possessing the C-4,C-6 lactone bridge, ring B is probably in a twist form. This was indicated by ORD evidence<sup>16</sup> of ring distortion in the ozonolysis product (54) from anhydrotetrahydromarrubiin. However, in compounds with the lactone ring open, the general pattern of shifts in peak positions suggested that the rings are in, or close to, the chair form.

This assignment of stereochemistry was opposite to that suggested by Mangoni and Belardini<sup>40</sup> who reported a partial synthesis of the isoambreinolide obtained from marrubiin.<sup>10</sup> They claimed that their synthesis established the stereochemistry of this compound as shown in (58), thus assigning the  $\beta$ -configuration to the alkyl groups at C-8 and C-9 in marrubiin. However, their assignment for C-8 was considered ambiguous<sup>31</sup> and should probably have been as shown in



(59). The formation of isoambreinolide from ambreinolide (60) was accompanied by the formation of the corresponding  $\Delta^8$  acid (61), considered to be an intermediate in the reaction. If this is so, then lactone closure to isoambreinolide would have taken place with the formation of compound (59), with the 17-methyl group in the equatorial configuration.

Cocker *et al.*<sup>8</sup> originally deduced from results of dehydration experiments on marrubiin<sup>3</sup> that the hydroxy-group at C-9 was equatorial ( $\beta$ ) since the only product (albeit in small yield) that they were able to identify was anhydromarrubiin (62). With an axial hydroxy-group at C-9, trans-elimination of water would have yielded some of the endocyclic product (63) as well. Accordingly, the C-9 side chain was considered to be axial, at variance with the usual orientation in the 7:8-seco-pimaranes. However, they did not consider this to be anomalous since it had been shown by Barton<sup>20</sup> that the accumulation of substituents at the positions  $\alpha$  and  $\alpha'$  to a group in a cyclohexane ring tends to reverse the order of stability and to make the axial configuration for the group the preferred one. With a system such as that in marrubiin, having three  $\alpha, \alpha'$  substituents, it was possible, therefore, that the equatorial configuration at C-9 was only slightly preferred and the situation was altered by the substitution of the hydroxy-group for the hydrogen atom.

Burn and Rigby<sup>9</sup> considered this proposal to be unsound since it had been assumed that, because anhydromarrubiin has an exocyclic double bond, marrubiin, on dehydration, gave predominantly this product. However, the yield of anhydromarrubiin was low and the major products in the dehydration were some other uncharacterised

(unsaturated) compounds. In the absence of evidence that exocyclic dehydration was favoured, any argument based on this assumption was considered speculative.

Fulke et al.<sup>41</sup> therefore repeated the dehydration of marrubiin with phosphorus trichloride in refluxing benzene and found that the n.m.r. spectrum of the total oily product was identical with that of crystalline anhydromarrubiin. The difficulty experienced in recovering even moderate yields of crystalline material from the oily product was probably due to one or both of the following reasons:

(a) Both geometric isomers (62) and (64) were produced and only one was crystalline. This explanation was unsatisfactory as the product appeared homogeneous on the basis of both t.l.c. and n.m.r. behaviour.

(b) Anhydromarrubiin would not crystallise from moist solvent. This appeared more acceptable since crystallisation of the product from dry methanol gave appreciably higher yields (63%) of crystalline material than those reported previously, and also the melting point of this material fell rapidly on exposure to atmospheric moisture (95-96° to 56-58° in 5 hours). Under conditions which were known to lead to the trans-elimination of water (phosphoryl chloride or phosphorus trichloride in refluxing pyridine) they obtained an oily mixture which ran as one symmetrical spot on t.l.c. and could not be separated into its individual components. However, a careful n.m.r. study led to the suggestion that the mixture consisted of two compounds, the  $\Delta^8$  olefin (63) (70%) and its  $\Delta^{9(11)}$  isomer (62) (30%). The possibility of a first-formed  $\Delta^{9(11)}$  isomer rearranging to its  $\Delta^8$  isomer under the dehydration conditions was considered. However, anhydromarrubiin (62) was recovered apparently unchanged (n.m.r. evidence) after

treatment with phosphoryl chloride in refluxing pyridine. Formation of a substantial proportion of the product with an endo olefinic bond indicated<sup>40</sup> the presence of an 8  $\beta$ -H and a 9 $\alpha$ -OH group, leading to the trans-elimination of water.

The arguments that Mangoni and Belardini<sup>40</sup> advanced in support of the stereochemistry at C-9 of their isoambreinolide were clearly sound and supported the conclusions of Fulke et al. However, this left the problem of explaining why the early dehydration experiments on marrubiin and tetrahydromarrubiin yielded largely unchanged starting material and very little product from endocyclic dehydration. This apparent anomaly was accounted for<sup>31</sup> by the suggestion that, since the lactone ring in marrubiin and tetrahydromarrubiin was closed, ring B was in a twist form; thus the hydrogen atom at C-8 and the hydroxy-group at C-9 were no longer trans and diaxial and endocyclic dehydration was not particularly favoured.

Closure of the lactone ring in marrubiin has been found to cause considerable distortion in ring B. Evidence for this was obtained by observing the solvent induced shifts of the 17-methyl resonances in the n.m.r. spectra of certain compounds.<sup>35</sup> Since the hydroxy-acid (56), obtained on hydrolysis of the keto-lactone (54), was recycled to compound (54) under mild conditions (dicyclohexylcarbodiimide at room temperature or ethyl chloroformate at 0°), both these compounds must have the 17-methyl group in the same configuration. The positions of the signals of the methyl groups in the n.m.r. spectra of compound (54) and the methyl ester of the hydroxy-acid (obtained from (56) with diazomethane) were observed in different solvents (see Table II).

Table II<sup>35</sup>

N. M. R. Signals of C-methyl peaks.<sup>a</sup>

Compound	CDCl <sub>3</sub>			(C <sub>6</sub> H <sub>6</sub> )		
	18-Me	20-Me	17-Me <sup>b</sup>	18-Me	20-Me	17-Me <sup>b</sup>
keto-lactone (54)	81	73	73.5	56	52	52
methyl ester of compound (56)	79	72	60	73	59	67

<sup>a</sup>Shifts obtained on a 60 MHz spectrometer and are given in Hz downfield from TMS.

<sup>b</sup>Doublet

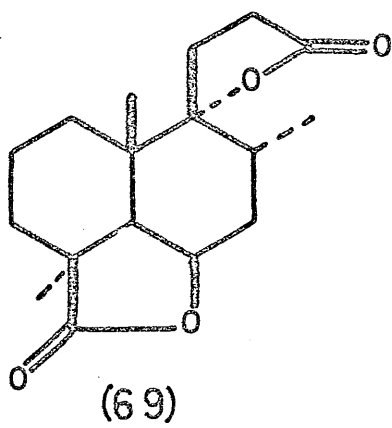
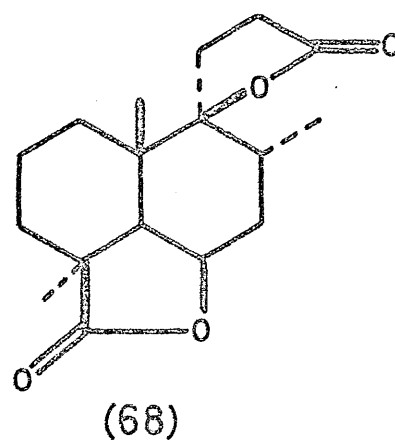
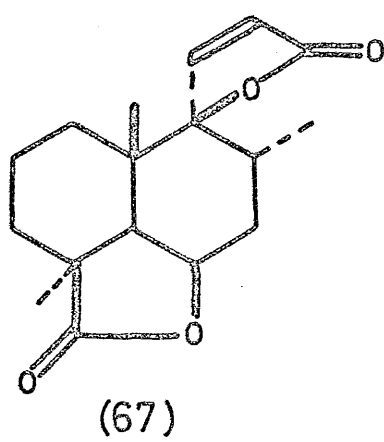
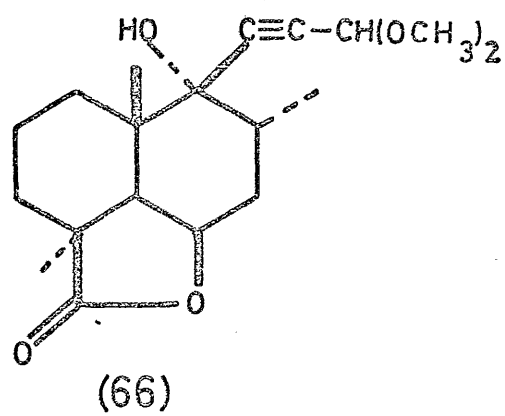
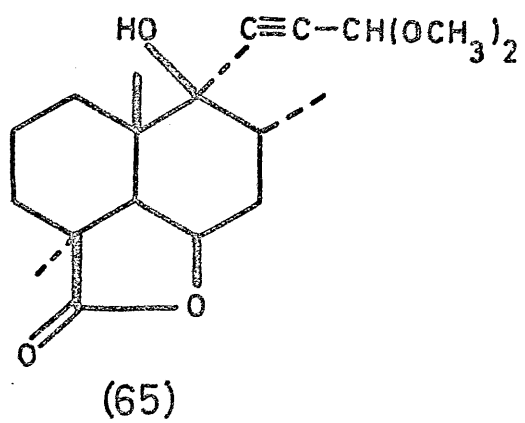
The solvent shifts (deuteriochloroform to benzene) shown by the 17-methyl resonance in these spectra were consistent with the group being axial in compound (54) and equatorial in the ester of compound (56).<sup>39, 42</sup> These results, combined with the interconversion of compounds (54) and (56), led to the conclusion that the 17-methyl group is  $\alpha$  in both compounds but that the lactone ring in compound (54) distorts ring B to a twist boat form.

The introduction of the  $\Delta^{9(11)}$  olefinic bond also produced, in ring B, a marked distortion which was reflected in the n. m. r. spectra of compounds containing this system.<sup>39, 41</sup> The spectra of marrubiin and derivatives containing a C-4, C-6 lactone ring showed a collapsed triplet at about 4.70 attributable to 6-H. In anhydromarrubiin this resonance had shifted to 5.03 and had a much wider spread (sextet,  $J_{5,6}$  5 Hz and  $J_{6,7}$  8 Hz). This suggested that A<sup>(1,3)</sup>-strain,<sup>43</sup> i. e. the steric interaction between the 17-methyl group and the side chain at C-9, had been relieved by a downward displacement of C-8 and an upward rotation of C-7, thus producing a quasi-boat ring B conformation.

The dihedral angles between the protons at C-5, C-6 and C-7 would be expected to result in spin-spin coupling constants of approximately the magnitude found.

The configurations at C-8 and C-9 were confirmed synthetically by Mangoni and Adinolfi<sup>36</sup> as follows. When the keto-lactone (54) was reacted with the lithio derivative of propargyl aldehyde dimethylacetal, it smoothly gave an 8:1 mixture of the epimeric acetals (65) and (66). Inspection of Dreiding models showed that the  $\beta$ -side of the keto-lactone (54) was considerably more hindered than the  $\alpha$ -side. Therefore structure (65) was assigned to the major product. Hydrogenation of this compound with palladium charcoal catalyst in alkaline methanol, followed by chromic acid oxidation in acetic acid, gave the unsaturated dilactone (67). Catalytic hydrogenation of this compound using platinum oxide in acetic acid gave the saturated dilactone (68), not identical with the dilactone obtained by chromic acid oxidation of marrubiin. Analogously, the oily less abundant, epimeric acetylenic acetal (66) was transformed to a saturated dilactone (69) identical in all respects with the dilactone from marrubiin. Thus the stereochemistry at C-8 and C-9 in marrubiin is certain.

The stereochemistry at all asymmetric centres in marrubiin has thus been established beyond all doubt and its structure is that shown in (3).



## 2. EXPERIMENTAL

All infrared spectra were run on a Beckman IR 8 Spectrometer and, unless otherwise stated, were taken in chloroform solution (6%). Thin layer chromatograms were run on plates of Merck silica gel G (according to Stahl) and were developed by placing in an atmosphere of iodine vapour. Alumina for chromatography was acid washed, neutralized and activated by heating at 170° for 18 hours. 100 MHz and 60 MHz nuclear magnetic resonance spectra were run on Varian HA-100 and Perkin-Elmer R12 Spectrometers respectively.

### 2. 1 Extraction of *Leonotis dubia*

The plant material was collected in different areas around Grahamstown, air-dried in the shade for approximately 6 weeks and extracted. The following experiment is typical.

The dried leaves and stems of *L. dubia* (Rhodes University Herbarium 19332), collected on 25.4.69 in the Belmont Valley approximately 5 miles from Grahamstown, were separated. The leaves (2.7 Kg) were steeped in acetone (45 l) at room temperature for two days, the acetone run off and the plant material washed with a further 37 l of acetone. The combined acetone extracts were concentrated by flash distillation to approximately 5 l and stirred with decolourising charcoal (BDH; 2 x 100 g) at room temperature for 3 hours. The solution was filtered through a Celite pad and evaporated to a dark brown gum on a rotary evaporator. The gum was dissolved in a minimum of refluxing ethanol (150 ml)

and allowed to stand at room temperature for 3 days, whereupon crystallisation took place. The crystals were filtered off, washed with a little cold ethanol and dried in a vacuum desiccator; yield 42.3 g. T.l.c. in ethyl acetate-hexane (1:1) showed that this material consisted almost entirely of one substance, dubiin, which was dissolved in chloroform (200 ml) and extracted with water (3 x 50 ml) to remove inorganic material. The combined water washings were extracted with chloroform (3 x 50 ml) and the combined chloroform solutions evaporated to a gum which was crystallised by dissolving in a minimum of refluxing ethyl acetate (100 ml) and allowing to stand at room temperature. The resulting crystals (8.8 g), m.p. 181-183°, were filtered off, washed with a little cold ethyl acetate and dried in a vacuum desiccator. The filtrate was concentrated to approximately 60 ml, hexane (20 ml) was added and the solution again allowed to stand at room temperature, whereupon a further crop of crystals (4.75 g), m.p. 181-185°, settled out. Similar work-up of the mother liquors yielded two further crops of crystals (4.1 g, 2.0 g), m.p. 178-180°; total yield 19.65 g, 0.72%.

Analysis: An analytical sample, recrystallised from ethyl acetate or aqueous ethanol, m.p. 187-188°, was dried at 100°/0.2 mm for 1 hour.  $[\alpha]_D^{21} = -17^\circ$  (c, 1.13 in chloroform).

Found : C = 67.85; H = 7.78

Molecular weight (mass spectrum) = 390

Calculated for  $C_{22}H_{30}O_6$ :

C = 67.67; H = 7.74%

Molecular weight = 390.

An extraction of finely crushed, dried stems of L. dubia carried out exactly as described above gave a gum (1.1% of dry stem weight) from which no crystalline material could be obtained.

Details of extractions carried out on L. dubia are given in Table III.

Table III

Date collected	Area	Weight of dried plants	Weight dubiin	Yield
3.10.68	Belmont Valley	6.8Kg (leaves + stems)	37.8g	0.56%
3.10.68	Belmont Valley	4.5 Kg (leaves)	25.4g	0.57%
15.4.69	Behind Livingstone House, Rhodes University	5.5Kg (leaves + stems)	-	-
25.4.69	Belmont Valley	2.7Kg (leaves)	19.7g	0.72%
27.4.70	Zeelie Heights	8.2Kg (leaves)	56.3g	0.69%
6.5.71	Zeelie Heights	5.5Kg (leaves)	54.6g	1.0%

## 2.2 Attempted dehydrogenation of dubiin

44

Dubiin (1.00 g) was mixed with 10% palladised charcoal (500 mg) in a long-necked 25 ml flask. The apparatus was flushed with nitrogen and then heated at 300-310° for 2 hours, during which time 122 ml of gas were evolved. The reaction product was extracted with boiling hexane (4 x 10 ml), the combined hexane extracts dried ( $\text{Na}_2\text{SO}_4$ ) and chromatographed on a column of Riedel-de Haen alumina (16g, 13 cm x 1 cm), eluting with dry hexane. Details of the chromatogram are as follows:

Fraction no. (50 ml)	Weight (mg)
1	185
2	12
3	3
4	2
5	1
6	4

Fraction 1 (185 mg) was dissolved in ethanol (1 ml) and 1, 3, 5-trinitrobenzene (50 mg) added. The mixture was boiled to solution whereupon the colour changed from pale yellow to dark orange. On standing at room temperature an oil separated out. This dissolved on addition of ethanol (2 ml) but only oily material separated out on standing at 0°. Attempts at obtaining crystalline material were fruitless.

### 2. 3 Attempted acetylation of dubiin

A solution of dubiin (34 mg) in acetic anhydride (3 ml) containing dry pyridine (0.1 ml) was refluxed for 1 hour. The solvent was removed under reduced pressure, the residue dissolved in methanol (2 ml) and water (1 ml) added. The resulting fine needles (13 mg), m.p. 186-187°, were filtered off and shown (mixed m.p. and infrared spectrum) to consist of unchanged dubiin.

#### 2. 4 Hydrogenation of dubiin

A mixture of dubiin (460 mg) and Paal catalyst<sup>45</sup> (877 mg) was shaken in ethanol (200 ml) under hydrogen for 30 minutes, during which time 46.8 ml of hydrogen (equivalent to 1.77 moles per mole of dubiin) were absorbed. The solution, after filtration through a Celite pad, was shown by t.l.c. in ethyl acetate-hexane (1:1) to contain a single compound. Removal of the solvent yielded an oil which crystallised from ethyl acetate-hexane as thick off-white needles (208 mg), m.p. 140-146°. Two further recrystallisations from the same solvent raised the melting point to 156.5-158°. The needles gave negative Ehrlich<sup>46</sup> and Liebermann-Buchard<sup>47</sup> test for furans; the infrared spectrum showed the absence of a furan peak at 872 cm<sup>-1</sup> and in the n.m.r. spectrum (CDCl<sub>3</sub>) the peaks due to the furan protons were missing.

Analysis: A sample was dried at 100°/0.2 mm for 1 hour.

$$[\alpha]_D^{21} = -11^\circ \text{ (c, 1.08 in chloroform).}$$

Found: C = 66.9; H = 8.7

Calculated for C<sub>22</sub>H<sub>34</sub>O<sub>6</sub>:

$$C = 66.98; H = 8.69\%.$$

#### 2. 5 Pyrolysis of dubiin

Dubiin (50 mg) was heated under nitrogen at 350° for two hours after which time the material had become a dark brown gum, most of which dissolved in ethanol. T.l.c. in ethyl acetate of the resulting solution showed a single spot corresponding to that of starting material.

## 2. 6 Oxidation of dubiin with chromium trioxide in acetic acid

A solution of  $\text{CrO}_3$  (1.2 g; 12 mmol) in water (6 ml) and acetic acid (20 ml) was added over a period of 5 minutes to an ice-cold solution of dubiin (1.5 g; 3.8 mmol) in acetic acid (7.5 ml). The solution was allowed to stand at room temperature for 4 days, water added and the acetic acid removed under reduced pressure. More water was added and the solution extracted continuously with ether for 4 hours. The product (560 mg) resulting on removal of the ether was dissolved in aqueous sodium carbonate and extracted continuously with ether to remove the neutral fraction which crystallised from benzene-hexane (204 mg), m. p. 212-213°. Analysis: A sample recrystallised from benzene-hexane, m. p. 213-214°, was dried at 100°/0.1 mm for 2 hours.  $[\alpha]_D^{21} = +5^\circ$  (c, 0.91 in chloroform).

Found: C = 65.24; H = 7.51

Calculated for  $\text{C}_{19}\text{H}_{26}\text{O}_6$ :

C = 65.12; H = 7.48%.

The infrared spectrum of this compound contained peaks at 1770 and 1731  $\text{cm}^{-1}$  indicating the presence of a  $\gamma$ - and a  $\delta$ -lactone. It contained no bands due to a furan ring.

The sodium carbonate layer was acidified with hydrochloric acid and extracted continuously with ether to give the acid fraction which crystallised from aqueous ethanol (66 mg), m. p. 215-219°.

Analysis: A sample, recrystallised from aqueous ethanol, m. p. 222-223°, was dried at 100°/0.1 mm for 2 hours.

$[\alpha]_D^{24} = 0^\circ$  (c, 0.66 in chloroform).

Found: C = 64.65; H = 7.24

Calculated for  $C_{19}H_{26}O_6$  :

C = 65.12; H = 7.48%.

## 2. 7 Attempted hydrolysis of dubiin in acid medium

Hydrochloric acid (0.25 ml) was added to a solution of dubiin (90 mg) in methanol (3 ml) and the solution refluxed on a water bath for 16 hours. The product, m.p. 174-177°, obtained on addition of water, was recrystallised from aqueous ethanol to afford needles, m.p. 184-186°, shown (mixed m.p. and infrared spectrum) to be unchanged dubiin. Hence no reaction had occurred.

## 2. 8 Attempted sodium borohydride reduction of dubiin<sup>48</sup>

Sodium borohydride (116 mg; 3.8 mmol) was added to a solution of dubiin (116 mg; 0.3 mmol) in methanol (5 ml), the mixture shaken at room temperature for 12 hours, poured into ice and extracted with benzene (5 x 20 ml). The combined benzene extracts were washed with water (3 x 20 ml) and dried ( $Na_2SO_4$ ). Removal of the solvent afforded a gum (110 mg) which crystallised on standing in air. Recrystallisation from ethyl acetate-hexane yielded a compound which was shown (m.p., mixed m.p. and infrared spectrum) to be identical with starting material.

The reaction was also carried out in the presence of potassium hydroxide as follows: Dubiin (112 mg) was dissolved in methanol (5 ml) and sodium borohydride (112 mg), followed by a solution of potassium hydroxide (100 mg) in a minimum of water, was added and the mixture shaken at room temperature for 24 hours. Work-up as before yielded a gum which would not crystallise. T.l.c. in

ethyl acetate-hexane (1:1) showed two spots of approximately equal intensity corresponding to those of dubiin and saponified dubiin. Clearly the lactone ring in dubiin is not attacked by sodium borohydride.

## 2. 9 Saponification of dubiin

A solution of dubiin (2.0 g; 5.1 mmol) in 0.7M ethanolic potassium hydroxide (43 ml; 30 mmol) was refluxed for 16 hours. Water (100 ml) was added and the alcohol removed under reduced pressure. The solution was acidified by adding concentrated hydrochloric acid (4 ml) dropwise with vigorous shaking to a pH of 1-2, whereupon a white precipitate formed. The mixture was allowed to stand in the fridge for 1 hour and the precipitate (1.707 g), m.p. 179-181<sup>o</sup>, filtered off, washed with a little cold water and dried in a vacuum desiccator.

Analysis: A sample of saponified dubiin (84), recrystallised from aqueous ethanol, m.p. 185-186<sup>o</sup>, was dried at 100<sup>o</sup>/0.1 mm for 1 hour.  $[\alpha]_D^{21} = +13^{\circ}$  (c, 1.20 in chloroform).

Found: C = 68.87; H = 8.16

Calculated for C<sub>20</sub>H<sub>28</sub>O<sub>5</sub>:

C = 68.94; H = 8.10%.

Later quantitative experiments on the saponification of dubiin showed that after 1 hour, 1.92 moles of potassium hydroxide had been consumed per mole of dubiin. However, the resulting hydroxy-acid could not be isolated due to the ease of relactonisation on acidification. Accordingly, in another experiment, the alkaline solution was acidified with acetic acid,<sup>49</sup> but again saponified dubiin was obtained.

2. 10 Attempted aminolysis of dubiin

A solution of dubiin (30 mg) in ethanol (2 ml) and 0.88 ammonia (1 ml) was heated in a sealed tube at 100° for 4 hours. T.l.c. in ethyl acetate-hexane (1:1) showed that the starting material was unchanged.

The experiment was therefore repeated as described above but was heated overnight. T.l.c. in ethyl acetate-hexane (1:1) indicated that the product consisted of a mixture of dubiin and saponified dubiin.

The reaction was repeated without ethanol. A mixture of dubiin (1.0 g) and 0.88 ammonia (50 ml) was heated in a sealed tube at 100° for 48 hours, by which time all the dubiin had gone into solution. The solution was allowed to cool whereupon it became cloudy and the product crystallised out. The crystalline product was filtered off and shown (m.p., mixed m.p. and infrared spectrum) to be identical with saponified dubiin. In a control experiment with ethyl benzoate, aminolysis was shown to have taken place under these conditions.

2. 11 Attempted tosylation of saponified dubiin

Freshly recrystallised tosyl chloride<sup>50</sup> (100 mg; 0.53 mmol) was added to an ice-cold solution of saponified dubiin (90 mg; 0.23 mmol) in dry pyridine (3 ml). The clear solution was allowed to stand in the deep freeze for 3 days, by which time it had become pale pink. Care was taken to see that no moisture entered the flask. Ice (3 g) was added, the mixture allowed to stand for 1 hour and extracted with ether (3 x 20 ml). The combined ethereal extracts

were washed successively with 5% hydrochloric acid (2 x 10 ml), water (2 x 10 ml), aqueous sodium bicarbonate (2 x 10 ml) and water (2 x 10 ml) and dried ( $\text{Na}_2\text{SO}_4$ ). Removal of the solvent yielded an oil (100 mg) which crystallised from aqueous methanol, m.p. 183-187 $^\circ$ , undepressed on admixture with saponified dubiin. A negative Lassaigne test on this material confirmed that no reaction had occurred.

## 2. 12 Acetylation of saponified dubiin

### (a) Using acetyl chloride

Redistilled acetyl chloride (0.4 ml) was added to an ice-cold solution of saponified dubiin (40 mg) in dry pyridine (1 ml). The solution was allowed to stand in ice for 1 hour and then at room temperature overnight. On addition of water, a white precipitate settled out which was shown by t.l.c. in ethyl acetate-hexane (1:1) to be an undefined mixture of products.

### (b) Using acetic anhydride

A solution of saponified dubiin (21 mg) in freshly redistilled acetic anhydride (1.5 ml) and dry pyridine (0.1 ml) was refluxed for 1 hour. Water (0.5 ml) was added, the solution allowed to stand at room temperature for 2 $\frac{1}{2}$  hours and concentrated to approximately 0.5 ml. Water (3 ml) was added and the solution allowed to stand in the refrigerator to afford colourless needles (19 mg), m.p. 182-183 $^\circ$ , shown (mixed m.p. and infrared spectrum) to be identical with dubiin. This indicated that treatment of dubiin with potassium hydroxide resulted in simple cleavage of the acetate group.

2. 13 Attempted determination of the configuration of the secondary hydroxy-group in saponified dubiin by Horeau's method.<sup>51, 52</sup>

No reference has been found to this method in which the esterification with  $\alpha$ -phenylbutyric anhydride was carried out under reflux.<sup>53, 54</sup> However the reaction would not proceed at room temperature and was therefore repeated under reflux.

A solution of saponified dubiin (70 mg; 0.2 mmol) and  $\alpha$ -phenylbutyric anhydride (186 mg; 0.6 mmol) in dry pyridine (5 ml) was refluxed for 24 hours. Water (1 ml) was added and the mixture allowed to stand at room temperature for 6 hours to allow complete hydrolysis of the excess anhydride to take place. Water (10 ml) was added and the aqueous solution extracted with ether (3 x 10 ml). The combined ethereal extracts were washed successively with water (1 x 20 ml), 5% aqueous sodium bicarbonate (3 x 20 ml), water (1 x 20 ml) and 5M hydrochloric acid (1 x 20 ml) and dried ( $\text{Na}_2\text{SO}_4$ ). Removal of the solvent yielded a gum (90 mg) which was not investigated further.

The combined sodium bicarbonate extracts were washed with chloroform (1 x 20 ml) to remove any neutral material, acidified (HCl) and extracted with chloroform. The resulting acidic material had  $[\alpha]_D^{25} = -0.5^\circ$  (c, 2.0 in benzene). This result was inconclusive and it appeared as though the elevated temperature of the reaction had caused racemisation of the unreacted anhydride. This was verified by repeating the reaction with euryopsonol under reflux. After work-up the excess acid had  $[\alpha]_D^{25} = +0.2^\circ$  (c, 1.7 in benzene), whereas reaction at room temperature yielded an acid with  $[\alpha]_D^{21} = +8^\circ$  (c, 2.5 in benzene).

2. 14 Attempted aminolysis of saponified dubiin

A solution of saponified dubiin (30 mg) in ethanol (2 ml) and 0.88 ammonia (1 ml) was heated in a sealed tube at 100° for 24 hours. T.l.c. in ethyl acetate-hexane (1:1) showed that the starting material was unchanged.

A further sample of saponified dubiin (30 mg) in 0.88 ammonia (2 ml) heated in a sealed tube at 100° for 96 hours remained unchanged.

2. 15 Treatment of saponified dubiin with sodamide

Liquid ammonia (50 ml) was prepared by passing ammonia gas, dried over barium oxide, into a flask fitted with a soda-lime tube and immersed in liquid air. Sodium was added with stirring until a permanent blue colour was observed. A few crystals of ferric nitrate were added whereupon the solution became colourless. Sodium (200 mg) was added in small pieces with stirring over a period of 30 minutes, followed by saponified dubiin (200 mg), and the solution stirred for 4 hours. Ethanol (15 ml) was added, the ammonia allowed to evaporate and, after addition of water, the ethanol removed under reduced pressure. The aqueous solution was extracted continuously with ether, acidified (HCl) and re-extracted with ether. Removal of the ether from the second extract yielded a gum (144 mg) which was shown by t.l.c. in ethyl acetate to consist almost entirely of unchanged starting material.

The experiment was repeated as described above but the saponified dubiin was dissolved in tetrahydrofuran (25 ml) before addition to the sodamide.<sup>55</sup> Work-up as before afforded unchanged starting material.

2. 16 L-alinate hydrobromide derivative of saponified dubiin<sup>56</sup>

L-alanine (45 mg) and phthalic anhydride (75 mg) were ground together in a pestle and mortar and then heated at 140-150° for 30 minutes. The product was converted to the acid chloride by refluxing with thionyl chloride (2 ml) for 2 hours. The excess thionyl chloride was removed under reduced pressure and dimethyl formamide (3 ml), pyridine (0.1 ml) and saponified dubiin (200 mg) were added. The mixture was allowed to stand at room temperature for 6 hours, ether (10 ml) added and the solution washed with water (4 x 20 ml). The dark brown gum, obtained on removal of the ether, was refluxed for 30 minutes with hydrazine hydrate (0.1 ml) in ethanol (5 ml). A large excess of water was added and the solution allowed to stand at room temperature whereupon crystallisation of phthalhydrazide took place. The mixture was filtered and the filtrate acidified with hydrobromic acid. Extraction of the solution with ethyl acetate (3 x 20 ml) and slow evaporation of the solvent afforded a crystalline product (260 mg) which was recrystallised from ethanol, m. p. > 300°.

Analysis: A sample was dried at 75°/0.1 mm for 3 hours.

Found: C = 58.45, 60.45

H = 3.74, 4.48

N = 17.01

Calculated for  $C_{23}H_{34}BrNO_6$ :

C = 55.2; H = 6.8; N = 2.8%.

Since the analyses were inconsistent and did not correspond to the calculated values for the L-alinate hydrobromide derivative of saponified dubiin, the product was not considered suitable for X-ray analysis.

2. 17 Attempted preparation of the methyl ester of saponified dubiin

A solution of dubiin (1.0 g; 2.6 mmol) in 0.52 M ethanolic potassium hydroxide (35 ml; 18 mmol) was refluxed on a water bath for 1 hour. The solution was allowed to cool to room temperature and the excess potassium hydroxide titrated with 0.205M nitric acid using phenolphthalein as indicator; 1.9 moles of potassium hydroxide were absorbed per mole of dubiin. A solution of silver nitrate (500 mg; 2.9 mmol) in water (3 ml) was added which caused the formation of a precipitate. The alcohol was removed under reduced pressure and the mixture allowed to stand at room temperature overnight. The light brown precipitate (800 mg) was filtered off, washed successively with water, ethanol and ether and dried in a vacuum desiccator. This silver salt was refluxed with redistilled methyl iodide (5 ml) on a glycerine bath for 7 hours after which time a yellow precipitate of silver iodide had formed. Ether (20 ml) was added and the mixture allowed to stand at room temperature for 2 hours with occasional shaking and then filtered. Removal of the solvent yielded a gum (570 mg) which was shown by t.l.c. in ethyl acetate-hexane (1:1) to be a mixture of at least 5 compounds, the major spot corresponding to that of saponified dubiin.

2. 18 Treatment of saponified dubiin with lead tetra-acetate<sup>57, 58</sup>

A solution of saponified dubiin (500 mg; 1.4 mmol), lead tetra-acetate<sup>59</sup> (2.8 g; 6.3 mmol), iodine (750 mg) and benzene (70 ml) was refluxed for 60 hours. A 20% excess of potassium iodide in water was added and the liberated iodine destroyed with aqueous sodium thiosulphate. The layers were separated, the aqueous layer was extracted with benzene (4 x 30 ml), the combined

benzene extracts were washed with water (3 x 50 ml) and dried ( $\text{Na}_2\text{SO}_4$ ). Removal of the solvent yielded the product which settled out as an oil from ethyl acetate-hexane. This was dissolved in ethanol (2 ml) and water (2 ml), concentrated hydrochloric acid (0.1 ml) was added and the solution heated on a water bath for 1 hour. Water (5 ml) was added and the ethanol removed under reduced pressure. The aqueous layer was extracted with ether (2 x 10 ml), the ether extract dried ( $\text{Na}_2\text{SO}_4$ ) and evaporated to afford a gum whose n.m.r. spectrum showed the absence of both acidic and aldehydic protons. The experiment was therefore discarded.

## 2. 19 Oxidation of saponified dubiin

### (a) Using Jones reagent<sup>60</sup>

A solution of saponified dubiin (100 mg) in acetone (10 ml) (distilled over potassium permanganate) was cooled in ice and 8N  $\text{CrO}_3/\text{H}_2\text{SO}_4$  (0.15 ml) was added dropwise from a micro-burette until an orange colour was obtained which persisted for 5 minutes. A few drops of ethanol were added to destroy the excess  $\text{CrO}_3$ , followed by water (10 ml) and the acetone removed under reduced pressure, whereupon the solution became cloudy and was allowed to stand in the refrigerator overnight. The resulting colourless needles of the ketone (85) (55 mg; 55%), m.p. 143-145°, were filtered off, washed with water and dried in a vacuum desiccator.

Analysis: A sample recrystallised from ethyl acetate-hexane m.p. 157-159°, was dried at 100°/0.2 mm for 4 hours.  $[\alpha]_D^{21} = -51^\circ$  (c, 1.50 in chloroform).

Found: C = 68.96; H = 7.55

Molecular weight (mass spectrum) = 346

Calculated for  $C_{20}H_{26}O_5$ :

C = 69.34; H = 7.57%

Molecular weight = 346.

(b) Using Sarett reagent<sup>30</sup>

$CrO_3$  (600 mg; 6 mmol) was dissolved slowly, with stirring, in ice-cold pyridine (6 ml) to give a bright yellow complex. To this was added a solution of saponified dubiin (730 mg; 2.1 mmol) in a minimum of dry pyridine (3 ml). The mixture was stirred in ice for 45 minutes, left overnight at room temperature and extracted with chloroform (5 x 50 ml). The combined chloroform extracts were washed successively with M hydrochloric acid (4 x 25 ml) and water (2 x 25 ml) and dried ( $Na_2SO_4$ ). Removal of the solvent yielded a dark brown gum which was passed through a column of 50-200 mesh silica, eluting with benzene. Removal of the solvent yielded a gum which crystallised as needles from benzene (503 mg; 69%), m.p. 157-159°. This product was identical (mixed m.p. and infrared spectrum) with compound (85) obtained by Jones oxidation of saponified dubiin.

(c) Using Collins reagent<sup>61</sup>

$CrO_3$  (3.50 g; 35 mmol) was added in one portion to a mechanically stirred solution of pyridine (5.5 g) in redistilled methylene chloride<sup>62</sup> (100 ml) at room temperature. The mixture was stirred for 15 minutes and a solution of saponified dubiin (2.0 g; 5.8 mmol) in methylene chloride (20 ml) was added. Stirring was continued for a further 15 minutes, the solution decanted and the residue washed thoroughly with ether (150 ml). The combined organic solutions were washed successively with 5% aqueous sodium

hydroxide (3 x 50 ml), 5% hydrochloric acid (3 x 50 ml), 5% aqueous sodium bicarbonate (1 x 50 ml), saturated aqueous sodium chloride (1 x 50 ml) and water (2 x 50 ml) and dried ( $\text{Na}_2\text{SO}_4$ ). Removal of the solvent yielded a pale yellow gum which crystallised from ethyl acetate-hexane as needles (1.76 g; 88%), m.p. 157-159°. This product was also identical (mixed m.p. and infrared spectrum) with that from the Jones oxidation of saponified dubiin.

(d) Using chromic acid in ether<sup>63</sup>

A solution of chromic acid was made up by dissolving sodium dichromate dihydrate (20 g) in water (60 ml) in a 100 ml volumetric flask. Concentrated sulphuric acid (14.7 ml) was added dropwise from a burette with cooling and the solution diluted to the mark. This solution was made up so that 500 ml would oxidise 1 mole of an alcohol.

Saponified dubiin (696 mg; 2 mmol) was dissolved in ether (50 ml) and the solution stirred mechanically at room temperature. The above chromic acid solution (1.1 ml) was added dropwise over a period of 15 minutes, keeping the flask in a water bath at room temperature. Stirring was continued for a further 2 hours, water (50 ml) added and the ether layer separated. The aqueous layer was extracted with ether (2 x 30 ml), the combined ethereal extracts were washed successively with saturated aqueous sodium bicarbonate (3 x 30 ml) and water (2 x 30 ml) and dried ( $\text{Na}_2\text{SO}_4$ ). Removal of the ether yielded a gum which crystallised from ethyl acetate-hexane as needles (600 mg; 86%), m.p. 157-159°. This product was identical (mixed m.p. and infrared spectrum) with those from the previous three experiments.

2. 20 Deuteration of the ketone (85)

A solution of the ketone (58 mg), potassium hydroxide (85 mg) and deuterium oxide (2 ml) was refluxed in a dry atmosphere for 8 hours. The solution was allowed to stand at room temperature overnight and then acidified by adding hydrochloric acid (0.5 ml) in water (1 ml), causing the product to precipitate. The mixture was centrifuged and the supernatant liquid sucked off. The precipitate was dissolved in ethanol (1.5 ml) to back-exchange the -OD to -OH, and water was added to reprecipitate the product which crystallised as needles, m.p. 161-164°. Thin layer chromatography in ethyl acetate-hexane (1:1) showed the product to be a single compound with the same mobility as starting material. Mass spectrometry showed that a maximum of 3 deuterium atoms had been incorporated into the molecule, the molecular ion peak being shifted from 346 to 349.

2. 21 Attempted preparation of the 2,4-dinitrophenylhydrazone of the ketone (85)<sup>64</sup>

A solution of 2,4-dinitrophenylhydrazine (20 mg) in hot diglyme (0.5 ml) was added to a solution of the ketone (85) (20 mg) in methanol (0.5 ml). One drop of concentrated hydrochloric acid was added and the solution allowed to stand at room temperature for 5 days. No crystallisation took place.

The reaction was therefore repeated as follows: Freshly recrystallised 2,4-dinitrophenylhydrazine (20 mg) was refluxed to solution in ethanol (3 ml) containing 1 drop of sulphuric acid.

Ketone (85) (30 mg) was added, the mixture refluxed for 1 hour, allowed to stand at room temperature and finally in the refrigerator. No crystallisation occurred.

2. 22 Attempted preparation of the oxime of the ketone (85)<sup>65</sup>

A solution of hydroxylamine hydrochloride (60 mg) in water (0.2 ml) was added to a solution of the ketone (85) (50 mg) in dimethyl sulphoxide (4 ml). The mixture was heated on a water bath for 1 hour and water added, whereupon crystallisation took place. The colourless needles (40 mg), m.p. 157-158°, were filtered off and shown (mixed m.p. and infrared spectrum) to be identical with starting material.

2. 23 Attempted preparation of the thioketal of the ketone (85)

To a solution of the ketone (18 mg) in redistilled acetic acid (0.4 ml) were added ethanedithiol (0.15 ml) and boron trifluoride-acetic acid (0.10 ml). The solution was allowed to stand at room temperature for 5 days. No crystallisation took place. T.l.c. in ethyl acetate showed that the solution contained only unchanged starting material.

2. 24 Attempted phosphorus trichloride dehydration of the ketone (85)

A solution of redistilled phosphorus trichloride (0.3 ml; 3.4 mmol) in benzene (1 ml) was added to a boiling solution of the ketone (310 mg; 0.9 mmol) in benzene (20 ml), the mixture refluxed for 30 minutes and poured into water. The two layers were separated and the aqueous layer was extracted with benzene (2 x 50 ml). The combined

benzene extracts were washed successively with 10% sodium hydroxide (3 x 50 ml) and water (3 x 50 ml), dried ( $\text{Na}_2\text{SO}_4$ ) and evaporated to a gum (200 mg) which was shown by t.l.c. in ethyl acetate to consist only of unchanged starting material.

2. 25 Attempted Wolff-Kishner reduction of the ketone (85)<sup>66</sup>

Sodium (2 g) was dissolved in anhydrous methanol<sup>67</sup> (30 ml) and the solution concentrated to approximately 20 ml. To this was added the ketone (85) (400 mg) and anhydrous hydrazine<sup>68</sup> (2 ml) and the solution heated in a sealed tube at 200° for 16 hours.

(Warning: Nitrogen is given off during the reaction causing a high pressure to be built up inside the tube). Water (10 ml) was added, the solution refluxed for 30 minutes, acidified (HCl) and extracted with ether (3 x 20 ml). The combined ethereal extracts were washed with water (3 x 20 ml) and dried ( $\text{Na}_2\text{SO}_4$ ). Removal of the solvent yielded a brown gum (215 mg) which was shown by t.l.c. in ethyl acetate to be a mixture of at least six compounds.

The reaction was therefore repeated by another method.<sup>69</sup>

A mixture of the ketone (85) (500 mg), redistilled diethylene glycol (10 ml) and potassium hydroxide (200 mg) was heated on a metal bath and anhydrous hydrazine<sup>68</sup> distilled in until the mixture refluxed freely at 180°. Refluxing was continued for 2 hours. The condenser was removed and hydrazine distilled off until the temperature of the mixture had risen to 220°. Refluxing was continued for a further 6 hours, the mixture allowed to cool and extracted with benzene (4 x 30 ml). The combined benzene extracts were washed successively with dilute hydrochloric acid (3 x 30 ml) and water (2 x 30 ml),



dried ( $\text{Na}_2\text{SO}_4$ ) and evaporated to a brown gum (330 mg) which was shown by t.l.c. in ethyl acetate to be a mixture of at least four compounds, only one of which had a higher  $R_f$  value than the starting material. This component (50 mg) was separated by means of chromatography on neutral alumina (5 g), eluting with benzene. It could, however, not be induced to crystallise.

The experiment was repeated exactly as described above except that it was refluxed under nitrogen. However, this produced the same complex mixture obtained before.

The technique of the experiment was tested by attempting to reduce the Jones oxidation product of marrubic acid. A crystalline product was obtained which was shown by elemental analysis to be a cyclic hydrazone.<sup>29</sup>

The reaction was therefore repeated by a different procedure.<sup>68</sup> Throughout this experiment great care was taken to keep all traces of moisture out of the apparatus. Sodium (200 mg) and redistilled diethylene glycol (10 ml) were placed in a flask and heated on a metal bath. Anhydrous hydrazine was distilled into the solution until it refluxed freely at  $180^\circ$ . The ketone (85) (840 mg) was added and the solution refluxed overnight at  $180^\circ$ . Hydrazine was then allowed to distil off until the temperature of the solution had risen to  $210^\circ$ . Refluxing was continued at this temperature for a further 24 hours. The solution was allowed to cool, water added and the mixture extracted with benzene (4 x 20 ml). The combined benzene extracts were washed with water (2 x 20 ml) and dried ( $\text{Na}_2\text{SO}_4$ ). Removal of the solvent afforded a straw coloured gum (512 mg) which was

shown by t.l.c. in ethyl acetate to be a mixture of three compounds in approximately equal proportions.

2. 26 Attempted Clemmensen reduction of the ketone (85)

Granulated zinc (3 g) and mercuric chloride (300 mg) were stirred with concentrated hydrochloric acid (0.15 ml) and water (4 ml) for 5 minutes. Approximately one-tenth of the zinc was removed, placed in a flask with water (0.5 ml), hydrochloric acid (1.2 ml), toluene (1 ml) and the ketone (85) (250 mg) and the mixture refluxed for 48 hours. A control experiment, set up exactly as described above but containing no zinc, was run concurrently. T.l.c. of the toluene layer, by now dark brown-black, showed that the starting material in both the experiment and control was considerably degraded by the acid. This method of reduction was therefore clearly unsuitable because of the instability of the furan ring under acid conditions.

2. 27 Attempted transesterification of the ketone (85)

(a) Using boron trifluoride diethyl etherate<sup>70</sup>

A solution of the ketone (85) (200 mg), boron trifluoride diethyl etherate (1 ml) and anhydrous methanol<sup>67</sup> (10 ml) was refluxed for 15 hours, cooled, water (20 ml) added and the solution extracted with ether (3 x 15 ml). The combined ethereal extracts were washed with saturated aqueous sodium chloride (2 x 15 ml) and dried ( $\text{Na}_2\text{SO}_4$ ). Removal of the solvent yielded a gum which crystallised from aqueous ethanol and was shown (m. p. and mixed m. p.) to be identical with starting material.

(b) Using diazomethane<sup>71</sup>

The ketone (85) (200 mg) was saponified by refluxing overnight with methanolic potassium hydroxide (200 mg). The solution was cooled in a mixture of ice and salt and an excess of diazomethane<sup>72</sup> was added. The solution was acidified by adding dilute hydrochloric acid dropwise over a period of 15 minutes with vigorous stirring and then allowed to stand at room temperature while the excess diazomethane evaporated. Water was added, the solution extracted with ether (2 x 20 ml) and the combined ethereal extracts were washed with water (2 x 20 ml) and dried ( $\text{Na}_2\text{SO}_4$ ). The ether solution was concentrated to a small volume to afford crystalline material, shown (m. p., mixed m. p. and infrared spectrum) to be identical with starting material.

(c) Using RONA

Sodium (400 mg) was dissolved in anhydrous ethanol<sup>67</sup> (70 ml) and the ketone (85) (500 mg) was added. The solution was heated in a sealed tube at  $140^\circ$  for 72 hours, diluted with water (20 ml), acidified (HCl) and extracted with ether (3 x 20 ml). The combined ethereal extracts were washed with saturated aqueous sodium chloride (2 x 30 ml), dried ( $\text{Na}_2\text{SO}_4$ ) and evaporated to a gum (480 mg) which was shown by t. l. c. in ethyl acetate-hexane (1:1) to consist largely of a single compound, different from the starting material. A more convenient method of transesterification was found whereby the sodium was dissolved in n-hexanol. After addition of the ketone, the solution was refluxed for 48 hours by which time the reaction had proceeded to completion. Work-up as described above yielded a similar product as before. This gum could not be induced

to crystallise and was therefore dried, dissolved in dry pyridine (10 ml), the solution cooled in ice and treated with freshly recrystallised tosyl chloride<sup>50</sup> (400 mg). The solution was allowed to stand in the refrigerator for 3 days, ice (20 g) was added, the mixture left at room temperature for 2 hours and extracted with ether (3 x 20 ml). The combined ethereal extracts were washed successively with 3M hydrochloric acid (4 x 20 ml), water (1 x 20 ml), aqueous sodium bicarbonate (3 x 20 ml) and water (2 x 20 ml) and dried ( $\text{Na}_2\text{SO}_4$ ). Removal of the solvent yielded a straw-coloured gum which was shown by t.l.c. in ethyl acetate-hexane (1:1) to be unchanged starting material. Lassaigne sodium fusion test on the product showed that sulphur was absent.

## 2. 28 Reduction of dubiin with lithium aluminium hydride

Dubiin (500 mg; 1.3 mmol) was dissolved in tetrahydrofuran (50 ml) which had been freshly redistilled over lithium aluminium hydride. Lithium aluminium hydride (250 mg; 6.6 mmol) was added and the mixture refluxed on a glycerine bath. After 4 hours, a further quantity of lithium aluminium hydride (250 mg) was added and the mixture refluxed for a further 15 hours. The mixture was cooled in ice and the excess lithium aluminium hydride destroyed with ethyl acetate (5 ml). Ether (50 ml) and 10% sulphuric acid (50 ml) were added and the layers separated. The aqueous layer was extracted continuously with ether for 24 hours and the combined ethereal extracts were washed successively with aqueous sodium carbonate (3 x 50 ml) and water (2 x 50 ml), dried ( $\text{Na}_2\text{SO}_4$ ) and evaporated. The resulting tetraol (88) crystallised from ethanol as prisms (450 mg), m.p. 217-219°.

Analysis: A sample recrystallised from ethanol, m.p. 219-221°, was dried at 100°/0.1 mm for 1 hour.  $[\alpha]_D^{18} = -17^\circ$  (c, 0.92 in ethanol).

Found: C = 68.10; H = 9.10

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

C = 68.15; H = 9.15%.

2. 29 Attempted partial reduction of dubiin and saponified dubiin with lithium aluminium hydride<sup>73, 74</sup>

A standard solution of lithium aluminium hydride was prepared as follows: Lithium aluminium hydride (200 mg) was added to tetrahydrofuran (20 ml) and the mixture stirred for 5 hours. The suspension was filtered through a scintered glass disc (porosity 2) and the clear solution standardised by introducing measured amounts, by means of a hypodermic syringe, through a septum cap into a two-necked flask containing water. The amount of hydrogen produced was measured and the molarity of the lithium aluminium hydride solution calculated.

Experiments were carried out under different conditions; the results are summarised in Table IV. The general method used was as follows: Dubiin (or saponified dubiin) was dissolved in tetrahydrofuran and the lithium aluminium hydride solution was added with stirring, very slowly by means of a hypodermic syringe through a septum cap. Stirring was continued for a further period, ice was added, the solution acidified (HCl), extracted with ether, the combined ethereal extracts washed with aqueous sodium carbonate and water and dried ( $Na_2SO_4$ ). The products of the reaction were identified either by crystallisation or by t.l.c. in ethyl acetate-hexane (1:1).

Table IV

Attempted partial reduction of dubiin and saponified dubiin with lithium aluminium hydride

Starting Material	Moles LAH/ Moles S. M.	Addn of LAH		Further reaction		Products	Method of Identification
		Time for addn.	Temp.	Time	Temp.		
dubiin	0.25	40 min.	ice/salt	1 hr.	room	dubiin	m. p., i. r. spectrum
dubiin	0.50	1 hr.	room	1 hr.	room	dubiin (66%) tetraol (33%)	t. l. c.
dubiin	0.375	1 hr.	0°	1 hr.	room	dubiin + tetraol	t. l. c.
sap. dubiin	0.25	½ hr.	ice/salt	1 hr.	room	saponified dubiin	t. l. c.
sap. dubiin	0.50	½ hr.	0°	1½ hr.	room	saponified dubiin	t. l. c.
sap. dubiin	1.0	½ hr.	ice/salt	1 hr.	room	sap. dubiin (50%) + tetraol (50%)	t. l. c.
sap. dubiin	1.0	1 hr.	room	1 hr.	room	sap. dubiin + tetraol + 1 other	t. l. c.
sap. dubiin	1.0	40 min.	room	¾ hr.	reflux	sap. dubiin + tetraol + 1 other	t. l. c.
sap. dubiin	1.0	40 min.	room	1½ hr.	reflux	sap. dubiin + tetraol + 1 other	t. l. c.

## 2. 30 Periodate oxidation of the tetraol (88)

The tetraol (44 mg) was dissolved in ethanol (10 ml) in a 25 ml volumetric flask, 0.1008M aqueous sodium periodate (5.0 ml) was added and the solution made up to the mark with water. A blank experiment containing no tetraol was run concurrently. At set intervals aliquots (5 ml) were withdrawn, water (20 ml) added to each and the alcohol removed under reduced pressure. To each solution was added sodium bicarbonate (2 g), 0.0102N sodium arsenite (40 ml) and potassium iodide (2 g) and the mixture allowed to stand at room temperature for 10 minutes. It was then titrated against 0.0099N iodine solution, using starch as an indicator. This showed that no periodate was consumed after 3 hours.

## 2. 31 Attempted dehydrogenation of the tetraol (88)

The tetraol (962 mg) was mixed with 10% palladised charcoal<sup>44</sup> (500 mg) in a long-necked 25 ml flask. The apparatus was flushed with nitrogen and then heated at 300-310° for 2½ hours, during which time 185 ml of gas were evolved. The reaction product was extracted with boiling hexane (6 x 10 ml), the combined hexane extracts were dried (Na<sub>2</sub>SO<sub>4</sub>), concentrated and passed through a column of Riedel-de Haen alumina (16 g, 1 cm x 13 cm), eluting with hexane (225 ml). Removal of the solvent afforded a gum (320 mg) which was dissolved in ethanol (6 ml). On addition of 1, 3, 5, - trinitrobenzene (50 mg) the solution changed colour from pale yellow to dark orange. The solution was concentrated to approximately 3 ml when a dark red oil separated out, but no crystallisation took place even after the solution had been allowed to stand at 0° for several days.

2. 32 Acetylation of the tetraol (88)(a) Using acetyl chloride

Acetyl chloride (0.4 ml) was added to an ice-cold solution of the tetraol (100 mg) in dry pyridine (2 ml). The solution was allowed to stand overnight, slowly approaching room temperature as the ice melted. Water was added, the mixture allowed to stand at room temperature for 2 hours and then extracted with ether (4 x 15 ml). The combined ethereal extracts were washed successively with aqueous sodium carbonate (3 x 15 ml) and water (3 x 15 ml) and dried ( $\text{Na}_2\text{SO}_4$ ). Removal of the solvent gave a gum which was shown by t.l.c. in ethyl acetate to be a mixture of two compounds, both different from starting material and thought to arise from incomplete acetylation. Therefore, the reaction product was treated with a further portion of acetyl chloride as before. This yielded a product which was shown by t.l.c. in ethyl acetate to consist of a single compound. However, it could not be induced to crystallise.

(b) Using acetic anhydride

A solution of the tetraol (88) (57 mg) in freshly redistilled acetic anhydride (1.5 ml) containing dry pyridine (0.1 ml) was refluxed on a glycerine bath for 3 hours. Water (1 ml) was added, the solution allowed to stand at room temperature for 3 hours, concentrated to approximately 0.5 ml and water (3 ml) added, the resulting slightly coloured crystals which settled out were filtered off (90 mg), dried in a vacuum desiccator, dissolved in benzene and passed through a small column of neutral alumina, eluting with benzene. Removal of the solvent yielded a gum which crystallised as colourless needles (89) from ethyl acetate-hexane, m.p. 127-128°.

Analysis: A sample recrystallised from ethyl acetate-hexane, m.p. 127-128°, was dried at 50°/0.1 mm for 4 hours.  $[\alpha]_D^{25} = -40^\circ$  (c, 1.04 in chloroform).

Found : C = 64.8; H = 7.8

Calculated: For  $C_{26}H_{38}O_8$ , (3 acetyl groups):

C = 65.27; H = 7.95

For  $C_{24}H_{36}O_7$ , (2 acetyl groups):

C = 66.03; H = 8.31%.

Overnight saponification of this triacetate at room temperature afforded the parent alcohol, m.p. 218-220°, undepressed on admixture with authentic material.

## 2. 33 Attempted partial hydrolysis of the triacetate (89)

Attempts were made to hydrolyse the primary acetate groups without removal of the secondary acetate group. The general method used for the reaction was as follows: The triacetate was dissolved in ethanol, an ethanolic solution of the alkali added and the reaction followed by t.l.c. in ethyl acetate-hexane (1:1). The experiments and results are summarised in Table V. It would appear that partial hydrolysis had occurred but that the reaction could not be stopped at this intermediate stage.

## 2. 34 Silver carbonate-Celite oxidation of the tetraol (88)<sup>75, 76</sup>

The Celite (Johns-Manville, 535) was purified by washing with methanol containing 10% hydrochloric acid, then with water until neutral and drying at 120°. Purified Celite (30 g) was added to a mechanically stirred solution of silver nitrate (34 g) in water (200 ml) followed by a solution of  $Na_2CO_3 \cdot 10H_2O$  (30 g) in water (300 ml).

Table V

Attempted partial hydrolysis of the triacetate (89)

Alkali used	Moles alkali/ Moles triacetate	Temp.	Time	T. l. c. showed:
KOH	40	room	10 min.	3 spots corresp. to starting material, tetraol and an intermediate.
			30 min.	2 spots corresp. to starting material and tetraol.
			60 min.	1 spot corresp. to tetraol.
$\text{Na}_2\text{CO}_3$	2.2	room	24 hrs.	3 spots corresp. to starting material (5x), tetraol (2x) and an intermediate (1x).
$\text{Na}_2\text{CO}_3$	20	room	12 hrs.	3 spots corresp. to starting material (3x), tetraol (2x) and intermediate (1x)
$\text{NaHCO}_3$	2.2	room	12 hrs.	1 spot corresp. to starting material.
$\text{NaHCO}_3$	2.2	reflux	12 hrs.	3 spots corresp. to starting material (5x), tetraol (2x) and intermediate (1x)
$\text{NaHCO}_3$	20	reflux	12 hrs.	3 spots corresp. to starting material (3x), tetraol (2x) and intermediate (1x)

The resulting yellow-green precipitate was filtered off and dried in a vacuum desiccator in the dark for 3 days. The reagent contains 1 mmol of silver carbonate per 0.57 g.

A mixture of the tetraol (88) (850 mg; 2.4 mmol) and silver carbonate-Celite (19 g; 33 mmol) in benzene (200 ml) was refluxed for 1 hour. (After 30 minutes the silver carbonate-Celite had become dark grey-black). T.l.c. of the solution in ethyl acetate-hexane (1:1) showed a single spot which did not correspond to that of the starting material. The mixture was filtered, the residue washed with benzene and the filtrate washed with water (4 x 50 ml) and dried ( $\text{Na}_2\text{SO}_4$ ). Removal of the benzene yielded a gum which crystallised from aqueous ethanol as thick colourless needles (600 mg; 70%), m.p. 153-154°. Recrystallisation from aqueous ethanol afforded thick needles, m.p. 182-183°, shown (mixed m.p. and infrared spectrum) to be identical with saponified dubiin.

#### 2.35 Iodination of the tetraol (88) with triphenyl phosphite methiodide<sup>77</sup>

Triphenyl phosphite was prepared<sup>78</sup> and converted to the methiodide,<sup>79</sup> which was found to be extremely unstable even when stored in a dark bottle in a vacuum desiccator which had been flushed out with nitrogen. It was therefore stored under ether in a dry box containing nitrogen. When required, it was dried between two sheets of filter paper and weighed directly into the flask.

This method of iodination, followed by lithium aluminium hydride reduction, gave satisfactory results when tested with cholestanol.

A solution of the tetraol (88) (500 mg; 1.4 mmol) and triphenyl phosphite methiodide (1.27 g; 2.8 mmol) in dimethyl formamide (10 ml) was allowed to stand at room temperature for 15 minutes. Methanol (1 ml) was added, the solution allowed to stand for a few minutes and then diluted with chloroform. The solution was washed successively with dilute aqueous sodium thiosulphate (2 x 25 ml) and water (2 x 25 ml), dried ( $\text{Na}_2\text{SO}_4$ ) and evaporated to a pale straw-coloured oil which was dissolved in a minimum of benzene and chromatographed on a column of silica gel, eluting with hexane. The first fraction (100 ml) was evaporated to a gum (700 mg) which was shown by t.l.c. in ethyl acetate to consist mainly of one compound different from starting material and giving a positive Beilstein test for halogen.

This gum was dissolved in tetrahydrofuran (30 ml) and lithium aluminium hydride (800 mg) was added. The mixture was refluxed overnight, cooled, the excess lithium aluminium hydride destroyed with ethyl acetate and the solution acidified (HCl). The ether extract (3 x 30 ml) of this solution was washed successively with aqueous sodium bicarbonate (2 x 30 ml) and water (2 x 30 ml) and dried ( $\text{Na}_2\text{SO}_4$ ). Removal of the solvent afforded a gum which contained no halogen (Beilstein, Lassaigne) or phosphorus and was shown by t.l.c. in ethyl acetate to be a mixture of two compounds which were separated on a column of silica gel, eluting with hexane. However neither of these compounds could be induced to crystallise.

## 2. 36 Oxidation of the tetraol (88) with Collins reagent<sup>61</sup>

$\text{CrO}_3$  (5.1 g; 51 mmol) was added to methylene chloride<sup>62</sup> (50 ml) at room temperature and the solution stirred mechanically

for 15 minutes. A solution of the tetraol (88) (1.0 g; 2.8 mmol) in methylene chloride (300 ml) was added in one portion and stirring continued for 1 hour. The solution was decanted and the tarry residue washed with ether (400 ml). The combined organic solutions were washed successively with 5% aqueous sodium hydroxide (3 x 100 ml), 5% hydrochloric acid (3 x 100 ml), 5% aqueous sodium bicarbonate (1 x 100 ml), saturated aqueous sodium chloride (1 x 100 ml) and water (2 x 100 ml) and dried ( $\text{Na}_2\text{SO}_4$ ). Removal of the solvent afforded a gum (800 mg) which would not crystallise but which was shown by t.l.c. in ethyl acetate to consist of a single compound. The n.m.r. spectrum of this gum contained a broad 1-proton singlet at 9.72.

2. 37 Wolff-Kishner reduction of the Collins oxidation product of the tetraol (88)<sup>68</sup>

Sodium (200 mg) was added to a solution of the gum (800 mg) (obtained from the previous experiment) in redistilled diethylene glycol (10 ml). The mixture was heated on a metal bath and anhydrous hydrazine distilled in until the solution refluxed freely at 180°. Refluxing was continued for 24 hours, the condenser removed and hydrazine distilled off until the temperature had risen to 210°. Refluxing was continued for a further 24 hours, the mixture cooled, water added and the solution extracted with benzene (4 x 20 ml). The combined benzene extracts were washed successively with 2M hydrochloric acid (2 x 20 ml) and water (2 x 20 ml), dried ( $\text{Na}_2\text{SO}_4$ ) and evaporated to a gum which was shown by t.l.c. in ethyl acetate to consist largely of unchanged starting material with a small amount of a slower moving spot. These compounds were separated on a column of neutral alumina eluting with benzene, but neither could be induced

to crystallise.

2. 38 Attempted tosylation of the tetraol (88) by initial preparation of the toluenesulphinat ester<sup>80, 81</sup>

The method was first tested by converting *p*-toluenesulphinyl chloride, prepared<sup>82</sup> from sodium *p*-toluenesulphinate,<sup>83</sup> to the *p*-toluenesulphinat ester of benzyl alcohol, followed by oxidation with *m*-chloroperbenzoic acid to benzyl tosylate, m.p. 51°.

*p*-Toluenesulphinyl chloride (3 ml; 17 mmol) was added dropwise with stirring to an ice-cold solution of the tetraol (88) (500 mg; 1.4 mmol) in dry ether (25 ml) and pyridine (15 ml), giving a pale yellow solution and a white precipitate. The mixture was stirred for 2 hours and placed in the fridge overnight. Ice was added whereupon the precipitate dissolved. The solution was allowed to stand for 2 hours, the ether layer separated and washed successively with 2M hydrochloric acid (2 x 25 ml), water (1 x 25 ml), aqueous sodium bicarbonate (2 x 25 ml) and water (2 x 25 ml) and dried (Na<sub>2</sub>SO<sub>4</sub>). Removal of the ether yielded a pale yellow oil which was shown by t.l.c. in ethyl acetate to consist of a single compound, different from starting material. The infrared spectrum showed a small sharp peak in the hydroxyl region, considerably smaller than that in spectrum of the starting material, which was probably due to the tertiary hydroxy-group.

A solution of *m*-chloroperbenzoic acid (290 mg; 1.7 mmol) in methylene chloride (10 ml) was added dropwise with stirring over a period of 20 minutes to a solution of the oil (obtained above) in the same solvent (25 ml). The mixture was left in the fridge overnight, washed successively with aqueous sodium carbonate (2 x 30 ml) and

water (2 x 20 ml) and dried ( $\text{Na}_2\text{SO}_4$ ). T.l.c. in ethyl acetate of the resultant product showed that the oil was approximately 90% unchanged. However, there was a less intense ( $\pm 10\%$ ) slower moving spot. The infrared spectrum of the product was almost identical with that of the starting oil except that it contained a small peak in the carbonyl region. This was thought to arise from oxidation of the furan ring to a lactone in the presence of the peracid. The experiment was therefore abandoned.

## 2. 39 Tosylation of the tetraol (88)

Freshly recrystallised tosyl chloride<sup>50</sup> (600 mg; 3.2 mmol) was added to an ice-cold solution of the tetraol (100 mg; 0.3 mmol) in dry pyridine (3 ml) and the solution allowed to stand in the deep freeze for 3 days, care being taken to see that no moisture entered the flask. Ice (3 g) was added, the mixture allowed to stand for 2 hours and extracted with ether (3 x 20 ml). The combined ethereal extracts were washed successively with 5% hydrochloric acid (2 x 20 ml), water (2 x 20 ml), aqueous sodium bicarbonate (2 x 20 ml) and water (2 x 20 ml) and dried ( $\text{Na}_2\text{SO}_4$ ). Removal of the solvent afforded an oil which crystallised from methanol (310 mg), m.p. 71-73<sup>o</sup>, raised on recrystallisation from the same solvent to 79-83<sup>o</sup>. A Lassaigne test for the presence of sulphur was positive. This product was unstable and decolourised on standing at room temperature overnight.

Analysis:

Found: C = 64.80, 64.74, 64.60

H = 7.9, 7.8, 7.8

Calculated: For  $C_{41}H_{50}S_3O_{11}$  (tritosylate):  
 C = 60.50; H = 6.15  
 For  $C_{34}H_{44}S_2O_9$  (ditosylate):  
 C = 61.81; H = 6.66  
 For  $C_{27}H_{38}SO_7$  (monotosylate):  
 C = 64.02; H = 7.56  
 For  $C_{27}H_{36}SO_6$  (monotosylate - water):  
 C = 66.38; H = 7.43  
 For  $C_{34}H_{42}S_2O_8$  (ditosylate - water):  
 C = 63.55; H = 6.54%.

The non-agreement between experimental and calculated figures could possibly be due to the instability of the compound.

## 2. 40 Reduction of this tosylate with lithium aluminium hydride

A solution of the tosylate of the tetraol (200 mg) (obtained from the previous experiment) in tetrahydrofuran (15 ml) was added dropwise to a mixture of lithium aluminium hydride (200 mg) in tetrahydrofuran (40 ml). The mixture was stirred at room temperature for 1 hour, refluxed on a glycerine bath for 6 hours, cooled in ice and the excess lithium aluminium hydride destroyed with ethyl acetate (5 ml). Ether (50 ml) and 10% sulphuric acid (50 ml) were added and the layers separated. The aqueous layer was extracted with ether (5 x 50 ml) and the combined ethereal extracts were washed successively with aqueous sodium bicarbonate (2 x 50 ml) and water (2 x 50 ml) and dried ( $Na_2SO_4$ ). Removal of the solvent gave a gum which was passed through a small column of neutral alumina, eluting with benzene. Removal of the benzene yielded a gum which crystallised

from hexane (44 mg), m.p. 114-115°. A Lassaigne test for the presence of sulphur was negative.

Analysis: A sample recrystallised from hexane, m.p. 114-115°, was dried at 50°/0.1 mm for 4 hours.  $[\alpha]_D^{25} = +20^\circ$  (c, 1.0 in chloroform).

Found: C = 75.18; H = 9.46

Molecular weight (mass spectrum) = 318

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

C = 75.43; H = 9.50%

Molecular weight = 318.

2. 41 N.m.r. experiments using the paramagnetic shift reagent  
tris (dipivaloylmethanato) europium III,  $Eu(dpm)_3$

The europium complex was prepared as follows:<sup>84</sup> Europium oxide  $Eu_2O_3$  (500 mg; 1.42 mmol) was dissolved in concentrated nitric acid (1.0 ml) and the solution placed over potassium hydroxide pellets in a vacuum desiccator for 7 days, giving a white crystalline residue of europium nitrate  $Eu(NO_3)_3$  (1.19 g), which was dissolved in 50% aqueous ethanol (10 ml). This solution was added with stirring to a solution of dipivaloylmethane (2, 2, 6, 6-tetramethyl-3, 5-heptanedione) (1.57 g; 8.52 mmol) in ethanol (5 ml). A 2.58M sodium hydroxide solution in 50% aqueous ethanol (3.3 ml; 8.52 mmol) was added, the apparatus evacuated with a water pump, sealed off and stirring continued for a further 2½ hours. The mixture was concentrated under reduced pressure to approximately half the volume and water (50 ml) added. The product settled out as an oil which formed pale yellow needles (1.73 g; 87%), purified by sublimation in vacuo at 180° and recrystallisation from hexane, m.p. 187-189°.

The 60 MHz n.m.r. spectra were run using deuteriochloroform as solvent. Spectra were taken after successive additions of small amounts of  $\text{Eu(dpm)}_3$  to a solution of the compound. From this series of spectra the changes in chemical shift of each peak with changes in concentration of  $\text{Eu(dpm)}_3$  could be observed. Graphs illustrating these changes were drawn.

## 2. 42 Extraction of *Leonotis leonitis*

*L. leonitis* (Rhodes University Herbarium 21877) was collected next to the Grahamstown-Kingwilliamstown road, between 15 and 25 miles from Grahamstown. The plants were air-dried in the shade for approximately 6 weeks, the leaves and stems separated and the leaves extracted. The following experiment is typical.

Dried leaves of *L. leonitis* (6.35 Kg), collected on 16.12.70, were steeped in acetone (25 l) at room temperature for 2 days. The acetone solution was run off, the leaves were washed with a further 10 l of acetone and the combined extracts concentrated to approximately 8 l by flash distillation. The solution was decolourised by stirring with charcoal (BDH; 2 x 150 g) for 3 hours, the mixture filtered through a Celite pad and the filtrate evaporated to a dark brown gum (235 g), which was dissolved in chloroform (3 l) and the solution washed with water (4 x 500 ml) to remove inorganic material; on shaking with water, emulsions formed which took several hours to break. The chloroform solution was evaporated to a gum which was crystallised by dissolving in a minimum of refluxing ethyl acetate (100 ml) and allowing to stand at room temperature for 7 days. The dark brown crystals (29.7 g; 0.5% of dry leaf weight) were filtered off, washed with a little cold ethyl acetate and dried. T.l.c. in ethyl

acetate showed a single spot. The crystals were decolourised by dissolving in ethanol (1.5 l), boiling with charcoal and filtering. The filtrate was allowed to stand in the fridge for 2 days and the light brown crystals were filtered off, washed with a little cold ethanol and dried, m.p. 239-240°.

Analysis: An analytical sample, recrystallised from ethanol as colourless needles, m.p. 242-243°, was dried at 100°/0.1 mm for 1 hour.  $[\alpha]_D^{21} = 0^\circ$  (c, 1.0 in chloroform).

Found: C = 65.43; H = 7.44

Molecular weight (mass spectrum) = 406

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

C = 65.01; H = 7.44%

Molecular weight = 406.

Details of extractions carried out on L. leonitis are given in Table VI.

Table VI

Date collected	Weight of dried plants	Weight leonitin	Yield
25.4.70	3.2 Kg (leaves + stems)	7.4g	0.23%
16.12.70	6.35 Kg (leaves)	29.7g	0.47%
25.5.71	2.7 Kg (leaves)	11.8g	0.44%

#### 2.43 Saponification of leonitin

A solution of leonitin (400 mg; 1 mmol) in 0.3M ethanolic potassium hydroxide (35 ml; 10.5 mmol) was refluxed for 24 hours. Water (60 ml) was added and the alcohol removed under reduced pressure. The solution was acidified (HCl) to pH 1 and extracted continuously for 3 hours.

Removal of the solvent afforded a gum which crystallised from benzene-hexane as needles (385 mg). The melting point of this product, the  $\gamma, \delta$ -dilactone (109), obtained from different experiments, varied between  $224^{\circ}$  and  $238^{\circ}$ .

Analysis: A sample m. p.  $238^{\circ}$ , was dried at  $100^{\circ}/0.1$  mm for 1 hour.  $[\alpha]_D^{24} = +39^{\circ}$  (c, 1.0 in chloroform).

Found: C = 65.8; H = 8.0

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

C = 65.92; H = 7.70%.

#### 2. 44 Acetylation of saponified leonitin

A solution of saponified leonitin (50 mg) in redistilled acetic anhydride (3 ml) containing pyridine (0.1 ml) was refluxed for 16 hours. Water (3 ml) was added, the solution allowed to stand at room temperature for 2 hours, concentrated to approximately half the volume and allowed to stand in the refrigerator. The resulting crystals (33 mg) were filtered off and recrystallised from chloroform-hexane, m. p.  $300-301^{\circ}$ .

Analysis: A sample recrystallised from ethanol as needles, m. p.  $300-301^{\circ}$ , was dried at  $100^{\circ}/0.1$  mm for 1 hour.  $[\alpha]_D^{24} = +26^{\circ}$  (c, 0.8 in chloroform).

Found: C = 64.51; H = 7.53

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

C = 65.01; H = 7.44%.

#### 2. 45 Attempted partial hydrolysis of leonitin<sup>85</sup>

A solution of potassium carbonate (17 mg; 0.13 mmol) in water

(2 ml) was added dropwise, over a period of 30 minutes, to a refluxing solution of leonitin (50 mg; 0.12 mmol) in dioxane (16 ml). The solution was refluxed for a further 1 hour, extracted continuously with ether for 2 hours and evaporated to a gum which crystallised from ethanol and was shown (m.p., mixed m.p. and infrared spectrum) to be unchanged starting material.

Further attempts were made to hydrolyse the acetate group in leonitin without the formation of the  $\gamma, \delta$ -dilactone. The general method of hydrolysis was as follows: Leonitin (50 mg) was dissolved in ethanol (10 ml) and an ethanolic solution of the alkali was added. After a certain reaction time, water was added and the alcohol removed under reduced pressure. The solution was acidified (HCl) and extracted continuously with ether. Removal of the ether gave the product which was identified by the melting point or infrared spectrum. The results of the experiments are summarised in Table VII.

Table VII

Alkali or acid used	Moles alkali/ Moles leonitin	Time (hours)	Temperature	Product
$K_2CO_3$	4	22	room	leonitin
$K_2CO_3$	4	2	reflux	saponified leonitin
$NaHCO_3$	3.9	24	room	leonitin
$NaHCO_3$	3.9	2	reflux	saponified leonitin
$H_2SO_4$	0.2 ml/30 mg	1	reflux	leonitin
$H_2SO_4$	0.2 ml/30 mg	5	reflux	saponified leonitin

2. 46 Sodium borohydride reduction of saponified leonitin

Sodium borohydride (2 g) was added to an ice-cold solution of saponified leonitin (960 mg) in methanol (60 ml). The mixture was stirred at room temperature for 4 days during which time 2 further portions of sodium borohydride (2 g each) were added. Water (50 ml) was added, the methanol removed under reduced pressure, the mixture acidified (HCl) and extracted continuously with ether for 12 hours. The product crystallised from the ether solution, was filtered off and recrystallised from methanol as off-white prisms (775 mg; 81%), m.p. 261-263°. The infrared spectrum (KBr disc) of this compound, the hemi-acetal (112) contained only one peak in the carbonyl region due to a  $\delta$ -lactone.

Analysis: A sample, recrystallised from methanol, m.p. 262-263°, was dried at 100°/0.1 mm for 2 hours.  $[\alpha]_D^{25} = +25^\circ$  (c, 0.56 in ethanol).

Found: C = 65.82; H = 8.62

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

C = 65.55; H = 8.25%.

2. 47 Tosylation of the hemi-acetal (112)<sup>41</sup>

A solution of the hemi-acetal (75 mg; 0.2 mmol) and freshly recrystallised tosyl chloride<sup>50</sup> (100 mg; 0.53 mmol) in dry pyridine (10 ml) was refluxed overnight. The solution was cooled, ice (10 g) added and the mixture allowed to stand at room temperature for 2 hours. The solution was extracted with ether (4 x 20 ml) and the combined ethereal extracts washed successively with 5M hydrochloric acid (4 x 20 ml), water (1 x 20 ml), 5% aqueous sodium bicarbonate (3 x 20 ml) and water (3 x 20 ml) and dried ( $Na_2SO_4$ ). Removal of the solvent

afforded a gum which was shown by t.l.c. in ethyl acetate to be a mixture of two compounds in the ratio of approximately 5:1, the minor spot corresponding to that of saponified dubiin in a number of solvents. The major component was separated and crystallised from ether as plates (40 mg), m.p. 259-261<sup>o</sup>, shown (mixed m.p. and infrared spectrum) to be unchanged starting material.

2. 48 Attempted acetoxylation of leonitin<sup>86</sup>

A solution of leonitin (20 mg; 0.05 mmol) and lead tetra-acetate<sup>59</sup> (100 mg; 0.23 mmol) in benzene (10 ml) was refluxed for 10 days. The solution was washed with water (3 x 10 ml), dried (Na<sub>2</sub>SO<sub>4</sub>) and evaporated to a gum which crystallised from aqueous ethanol. The product was shown (m.p., mixed m.p. and infrared spectrum) to be unchanged starting material.

2. 49 Lithium aluminium hydride reduction of leonitin

Lithium aluminium hydride (1.0 g; 26 mmol) was added to a solution of leonitin (1.5 g; 3.7 mmol) in freshly redistilled tetrahydrofuran (100 ml) and the mixture refluxed. After 4 hours, a further quantity of lithium aluminium hydride (500 mg; 13 mmol) was added and refluxing continued for a total of 18 hours. The mixture was cooled in ice, the excess lithium aluminium hydride destroyed with ethyl acetate, and ether (50 ml) and dilute hydrochloric acid (50 ml) were added. The two layers were separated and the aqueous layer was extracted continuously with ether for 24 hours. The combined organic layers were dried (Na<sub>2</sub>SO<sub>4</sub>) and evaporated to yield the crystalline pentaol (113) which was recrystallised from methanol as needles (1.19 g; 89%), m.p. 166-168<sup>o</sup>. Naidu<sup>99</sup> has recorded the m.p. as 158<sup>o</sup>.

Analysis: A sample, recrystallised from methanol, m.p. 167-168°, was dried at 75°/0.2 mm for 1 hour.  $[\alpha]_D^{24} = -2^\circ$  (c, 1.0 in ethanol).

Found: C = 64.04; H = 9.75

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

C = 64.49; H = 9.74%.

2. 50 Tosylation of the pentaol (113)

A solution of freshly recrystallised tosyl chloride<sup>50</sup> (4.0 g; 21 mmol) in dry pyridine (10 ml) was added to an ice-cold solution of the pentaol (113) (500 mg; 1.36 mmol) in dry pyridine (20 ml). The solution was allowed to stand in the deep freeze for 48 hours, ice (30 g) was added and, after standing at room temperature for 2 hours, the mixture was extracted with ether (5 x 30 ml). The combined ethereal extracts were washed successively with dilute hydrochloric acid (4 x 30 ml), water (1 x 30 ml), 5% aqueous sodium carbonate (4 x 30 ml) and water (3 x 30 ml), dried ( $Na_2SO_4$ ) and evaporated to a gum (836 mg) which was not purified further.

2. 51 Lithium aluminium hydride reduction of the tosylate of the pentaol (113)

Lithium aluminium hydride (1.0 g) was added to a solution of the crude tosylate gum (836 mg, obtained from the previous experiment) in freshly redistilled tetrahydrofuran (100 ml) and the mixture refluxed for 6 hours, cooled in ice and worked up by the dropwise addition of water (1 ml), 15% sodium hydroxide (1 ml) and water (3 ml).<sup>87</sup> The thick white precipitate which formed was filtered off, washed with ether and the solvent removed from the filtrate to yield a clear gum which was shown by t.l.c. in ethyl acetate to consist of a single compound.

The gum was dissolved in benzene and passed through a small column of neutral alumina, eluting with benzene. The product separated as an oil from a number of different solvents.<sup>99</sup>

2. 52 Acetylation of the pentaol (113)

A solution of the pentaol (100 mg) in freshly redistilled acetic anhydride (4 ml) and dry pyridine (0.15 ml) was refluxed for 5 hours, cooled, water (4 ml) added, the mixture allowed to stand at room temperature for 2 hours and concentrated to 1 ml. Water was added and the solution allowed to stand in the refrigerator. The product separated out as an oil which would not crystallise.

2. 53 Extraction of Leonotis microphylla

L. microphylla (1.12 Kg), collected in July, 1971 by the Botanical Research Institute, Pretoria, consisting of twigs (70% approx.) and small leaves (30% approx.), was air-dried and steeped in acetone (20 l) at room temperature for 7 days. The solution was run off, the plants washed with a further 4 l of acetone and the combined organic solutions were concentrated to 3 l by flash distillation and decolourised by stirring with charcoal (BDH; 50 g) at room temperature for 6 hours. Filtration through a Celite pad and evaporation afforded a brown gum which was dissolved in chloroform (200 ml), the solution washed with water (5 x 50 ml) to remove inorganic material and dried ( $\text{Na}_2\text{SO}_4$ ). Evaporation of the solvent afforded a gum (13.4 g; 1.2%) which could not be induced to crystallise.

2. 54 Extraction of Euryops tenuissimus

E. tenuissimus (Rhodes University Herbarium 21878)

was collected on Sugar Loaf Hill on the outskirts of Grahamstown during November, 1970, after good spring rains. The freshly picked leaves and stems were separated and the stems (14.5 Kg), mostly about 2-5 mm in diameter, were steeped in acetone (100 l) at room temperature for 4 days. The acetone solution was run off and the plant material washed with a further 30 l of acetone. The combined extracts were evaporated to a dark brown gum (150 g) which was dissolved in ethanol (1.2 l) and refluxed overnight with a solution of potassium hydroxide (60 g) in water (20 ml). Water (500 ml) was added and the alcohol removed under reduced pressure. The aqueous solution was extracted with ether (4 x 50 ml) and the combined ethereal extracts washed with water (3 x 50 ml) and dried ( $\text{Na}_2\text{SO}_4$ ). The ether solution was concentrated to approximately 75 ml and the resulting fine white needles of euryopsol (6.68 g, 0.05% of undried plant weight) were filtered off, dried and recrystallised from ethyl acetate, m.p. 171-174°.

Analysis: A sample recrystallised from ethanol, m.p. 174-175°, was dried at 100°/0.1 mm for 2 hours.  $[\alpha]_D^{21} = +14^\circ$  (c, 1.0 in ethanol).

Found: C = 67.59; H = 8.33

Molecular weight (mass spectrum) = 266

Calculated for  $\text{C}_{15}\text{H}_{22}\text{O}_4$ :

C = 67.65; H = 8.33%

Molecular weight = 266.

Similar extractions were performed on E. spathaceus (Rhodes

University Herbarium 21879) collected next to the Grahamstown - Cradock road 5 miles from Grahamstown. Plant material collected in October, 1968, a year of drought, yielded no crystalline material, although plants collected in November, 1963 and extracted by Woolard<sup>88</sup> (previously called E. tenuissimus - see later) yielded 0.7% euryopsol.

## 2. 55 Extraction of E. floribundus

E. floribundus consisting mostly of stems 0.5-3 cm in diameter was collected in November, 1969, on the farm "Sondagsrivierhoek" near Graaff-Reinet. The undried stems (27 Kg) were cut into small pieces and steeped in acetone (100 l) for 4 days. The acetone was run off, the plants covered with a further 100 l of acetone and soaked for a further 4 days. The combined acetone extracts were evaporated to a gum (490 g) which was dissolved in ethanol (3 l) and saponified by refluxing for 4 hours with ethanolic potassium hydroxide (150 g). Water was added and the ethanol removed under reduced pressure. The aqueous solution was extracted with ether (5 x 300 ml) and the combined ethereal extracts were washed with water (2 x 500 ml) and dried ( $\text{Na}_2\text{SO}_4$ ). On concentration of the ethereal solution, crystallisation of euryopsol (4.0 g), m.p. 170-174<sup>o</sup>, took place. The ultraviolet spectrum of this material showed that it contained less than 1% euryopsonol. Plants extracted during 1968 and 1971 also yielded no euryopsonol. Details of extractions carried out on Euryops species are given in Table VIII.

Table VIII

Date Collected	Species	Weight un-dried stems	Weight euryopsol	Yield
4.10.68	<u>E. spathaceus</u>	13 Kg	-	-
15.10.68	<u>E. spathaceus</u>	7.3 Kg	-	-
12.11.68	<u>E. floribundus</u>	27 Kg	4.0 g	0.01%
10.2.71	<u>E. floribundus</u>	31 Kg	8.1 g	0.03%
19.10.70	<u>E. tenuissimus</u>	14.5 Kg	6.7 g	0.05%

### 2. 56 Oxidation of euryopsol with Sarett reagent<sup>30</sup>

Chromium trioxide (620 mg; 6.2 mmol) was added slowly with stirring to ice-cold pyridine (6 ml) to give a bright yellow suspension. A solution of euryopsol (275 mg; 1.0 mmol) in pyridine (3 ml) was added, the mixture stirred in ice for 30 minutes and then allowed to stand at room temperature overnight. The mixture was diluted with water and extracted with ether (4 x 25 ml). The combined ethereal extracts were washed successively with M hydrochloric acid (4 x 30 ml) and water (2 x 30 ml), dried ( $\text{Na}_2\text{SO}_4$ ) and evaporated to a gum (52 mg) which was dissolved in benzene, passed through a small column of 50-200 mesh silica gel and the product sublimed at  $120^\circ/0.1$  mm. Recrystallisation from benzene-hexane afforded colourless needles (119) (18 mg), m.p.  $150-152^\circ$ , which gave a negative Ehrlich test<sup>46</sup> (cf. euryopsonol<sup>88</sup>).

Analysis: A sample was dried at  $75^\circ/0.2$  mm for 1 hour.

Found: C = 69.0; H = 7.1

Molecular weight (mass spectrum) = 262

Calculated for  $C_{15}H_{18}O_4$ :

C = 68.69; H = 6.92%

Molecular weight = 262.

#### 2.57 Manganese dioxide oxidation of euryopsol

A mixture of euryopsol (253 mg; 1 mmol), manganese dioxide<sup>89</sup> (7.45 g; 85 mmol) and acetone (100 ml) was refluxed for 1½ hours, filtered, the residue washed with ethanol and the filtrate evaporated to a gum (95 mg). This product could not be purified by crystallisation or sublimation but its ultraviolet spectrum contained bands at 284 ( $\epsilon$  10,400) and 214 nm ( $\epsilon$  4,500) and the infrared spectrum showed strong carbonyl absorption.

#### 2.58 Periodate oxidation of euryopsol

The general method used for the reaction was as follows: An accurately known weight of euryopsol (approx. 260 mg) was dissolved in ethanol (50 ml) in a 100 ml volumetric flask. Standard sodium periodate solution was added, the solution made up to the mark with water and allowed to stand in the dark at room temperature. At set intervals, aliquots (10 ml) were withdrawn and sodium bicarbonate (5 g), sodium arsenite (standard amount) and a solution of potassium iodide (3 g) in water were added to each. The ethanol was removed under reduced pressure and the excess arsenite titrated against standard iodine solution, using starch as indicator. (In the presence of ethanol, unsatisfactory end points were obtained<sup>90</sup>). A blank experiment containing no euryopsol was run concurrently. In this way the amount of periodate consumed per mole of euryopsol could be calculated. The results are summarised in Table IX.

Table IX

Moles periodate added per mole euryopsol	Time (hours)	Moles periodate consumed per mole euryopsol
2	1	1.17
	2	1.35
	5	1.54
	12	1.59
	48	1.67
2.75	$\frac{1}{2}$	1.06
	1	1.29
	2	1.38
	3	1.45
	$5\frac{1}{2}$	1.62
4	24	1.65
	48	1.83
	72	2.20

Solutions of euryopsol (1 mmol) and sodium periodate (2-8 mmol) in methanol (30 ml) and water (12 ml) were allowed to stand at room temperature overnight. Water was added, the methanol removed under reduced pressure and the aqueous solution extracted with ethyl acetate (2 x 30 ml). The combined extracts were dried ( $\text{Na}_2\text{SO}_4$ ) and evaporated to a gum which was shown by t. l. c. in ethyl acetate to consist chiefly of one component. This gum could not be induced to crystallise or form any of the usual carbonyl derivatives. Its infrared spectrum, however, contained a strong carbonyl peak at  $1720 \text{ cm}^{-1}$ .

### 3. DISCUSSION

Leonotis species, of the family Labiatae, are found growing in widespread areas in South Africa. The most common, Leonotis leonurus, is known as "wild dagga" or "wildedagga" although this is a misnomer since the name "dagga" refers to Cannabis species. Species which have been reported are L. leonurus (L.) Ait., L. dubia E. Mey., L. leonitis R. Br., L. dysophylla Benth., L. microphylla Skan, L. nepetaefolia R. Br., L. mollis Benth. and L. latifolia Guerke, although not all of these are found in South Africa. Some species are used medicinally and it seems they are eaten selectively by animals, since no plants were found in areas where livestock had been grazing although frequently found in areas between the road and fences.

Diterpenoids with a labdane skeleton (2), including marrubiin, have been found in a number of these species.<sup>2, 91-95</sup>

#### 3. 1 Dubiin

L. dubia, from which the diterpenoid acetate, dubiin, has been isolated, is found quite commonly in areas around Grahamstown although it is not as common as L. leonurus. The main areas of supply have been the Belmont Valley and Zeelie Heights on the Kingwilliamstown - Queenstown road. The plants grow to about three to four feet in height and have deltoid-shaped leaves. The flowers are orange-brown, differing in colour from the bright orange flowers of L. leonurus. Plants were collected during summer and autumn and yields of dubiin differed with the season; the highest yield was obtained from plants collected in May, 1971, a year of good spring and summer rains. A sample, collected in April, 1969, during a very dry summer yielded

no crystalline material.

The aerial portions of L. dubia were picked and air-dried in the shade for 3-6 weeks. The leaves and stems were separated and the leaves extracted by steeping in acetone for 3 days. Filtration, concentration of the green solution by flash distillation, decolourisation with charcoal and evaporation under reduced pressure yielded a dark brown sticky gum which was dissolved in a minimum of refluxing ethanol. The resulting semi-solid was filtered and the slightly coloured crystals shown by thin layer chromatography to consist almost entirely of a single compound. This was dissolved in chloroform and washed with water to remove inorganic material. The product was recrystallised from ethyl acetate-hexane to give pure, pale yellow dubiin,  $C_{22}H_{30}O_6$ , m.p. 187-188°. Recrystallisation from aqueous ethanol gave colourless needles of the same melting point.

Similar extractions performed on finely crushed, dried stems of L. dubia yielded no diterpenoids at all, indicating that they only occur in the leaves.

The molecular formula of dubiin was confirmed by both its mass spectrum and elemental analysis. The infrared spectrum (Figure 1) showed a peak at  $3630\text{ cm}^{-1}$  due to a hydroxy-group which was presumably tertiary since it resisted acetylation. A peak at  $872\text{ cm}^{-1}$  was indicative of a furan ring, confirmed by a band in the ultraviolet spectrum at  $213\text{ nm}$  ( $\epsilon\ 5,000$ )<sup>96</sup> and an intense pink colour obtained with Ehrlich reagent.<sup>46</sup> The spectroscopic properties and colour tests of furan rings in terpenoids have recently been thoroughly reviewed.<sup>97</sup> The infrared spectrum also contained a strong band at  $1724\text{ cm}^{-1}$  with a shoulder at  $1736\text{ cm}^{-1}$  which indicated the

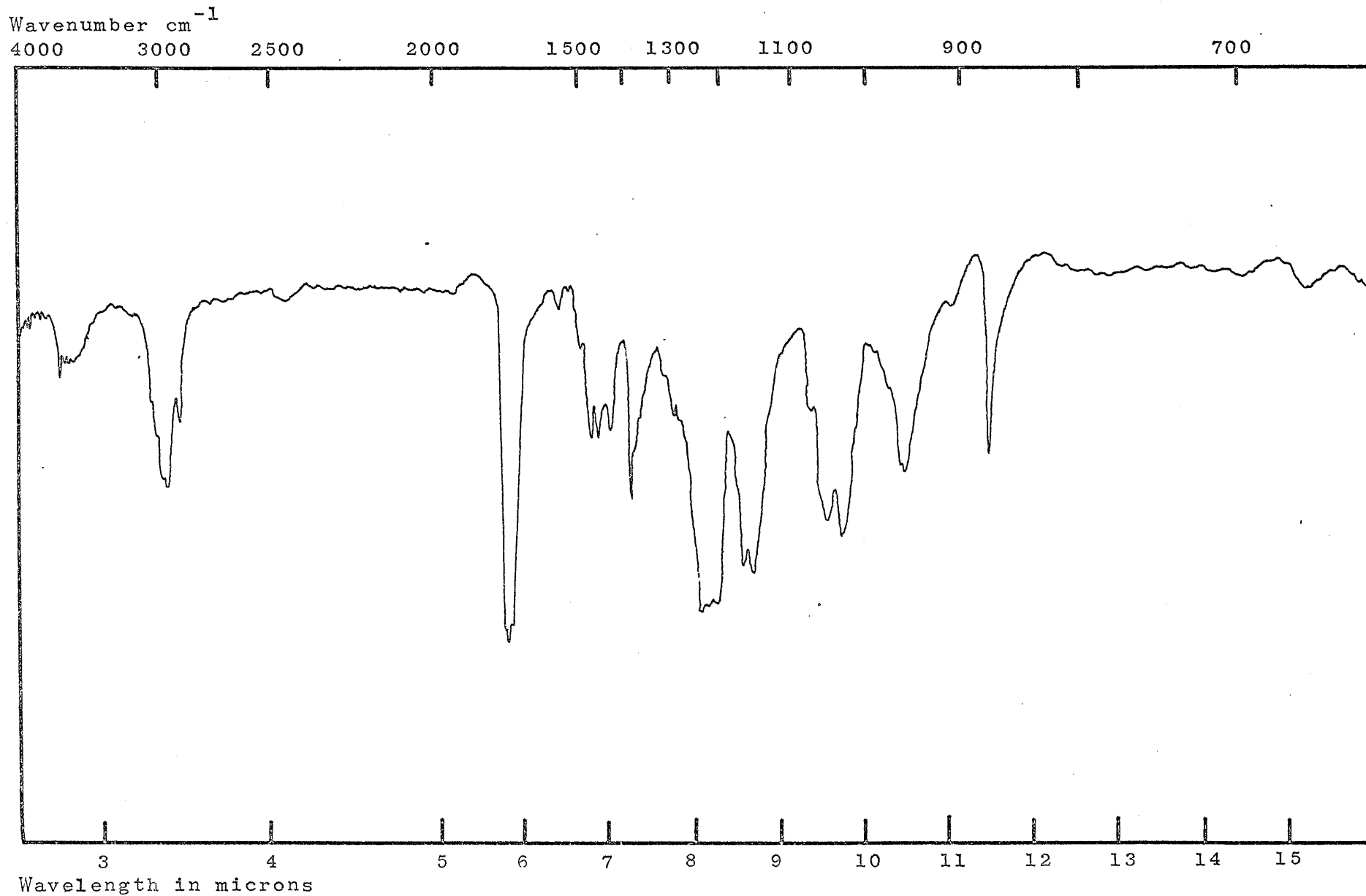


Figure 1: Infrared Spectrum of Dubiin

presence of an ester and a  $\delta$ -lactone; there was no peak at  $1770\text{ cm}^{-1}$  which would have indicated a  $\gamma$ -lactone as in marrubiin.

The mass spectrum of dubiin (Figure 2), besides giving the molecular ion peak at  $m/e$  390, had the base peak at  $m/e$  81 and a strong (65%) peak at  $m/e$  95 which are characteristic of a  $\beta$ -substituted furan side chain.<sup>98</sup> These peaks are also contained in the spectra of marrubiin and compound Y<sup>93</sup> and are due to cleavage between C-11 and C-12 and between C-9 and C-11, giving rise to the fragments (70) and (71). This confirms the presence of a furan side chain in dubiin similar to that in marrubiin and compound Y. In addition, there was a significant peak at  $m/e$  330 (33%) ( $M - \text{CH}_3\text{COOH}$ ) with a corresponding metastable peak at  $m/e$  279, and a peak at  $m/e$  235 (27%) ( $M - 95 - 60$ ), this fragmentation ( $295 \rightarrow 235$ ) being confirmed by a metastable peak at  $m/e$  188.

The 100 MHz n.m.r. spectrum (DMSO- $d_6$ ) of dubiin (Figure 3) confirmed the presence of an acetate group (1.90, 3H, singlet) and a  $\beta$ -substituted furan ring (7.52, 1H, triplet, J 1 Hz; 7.42, 1H, doublet, J 0.5 Hz; 6.38, 1H, triplet, J 1 Hz). In addition, there were peaks at ~~0.99~~<sup>0.87</sup> (3H, doublet, J 6 Hz), ~~1.14~~<sup>0.77</sup> (3H, singlet), 4.20 (1H, singlet), 5.02 (1H, quartet, J 3 Hz) and an AB quartet centred at 4.46 (2H, J 12 Hz) which indicated gem coupling. This resonance is characteristic of a methylene group adjacent to the oxygen atom of an ester (or lactone) group. The resonances of all the other protons were contained in the "methylene envelope". On addition of deuterium oxide, the peak at 4.20 disappeared and must therefore be due to the tertiary hydroxy-group. The 1-proton quartet at 5.02 is characteristic of a proton adjacent to an ester group, the narrowness of the signal

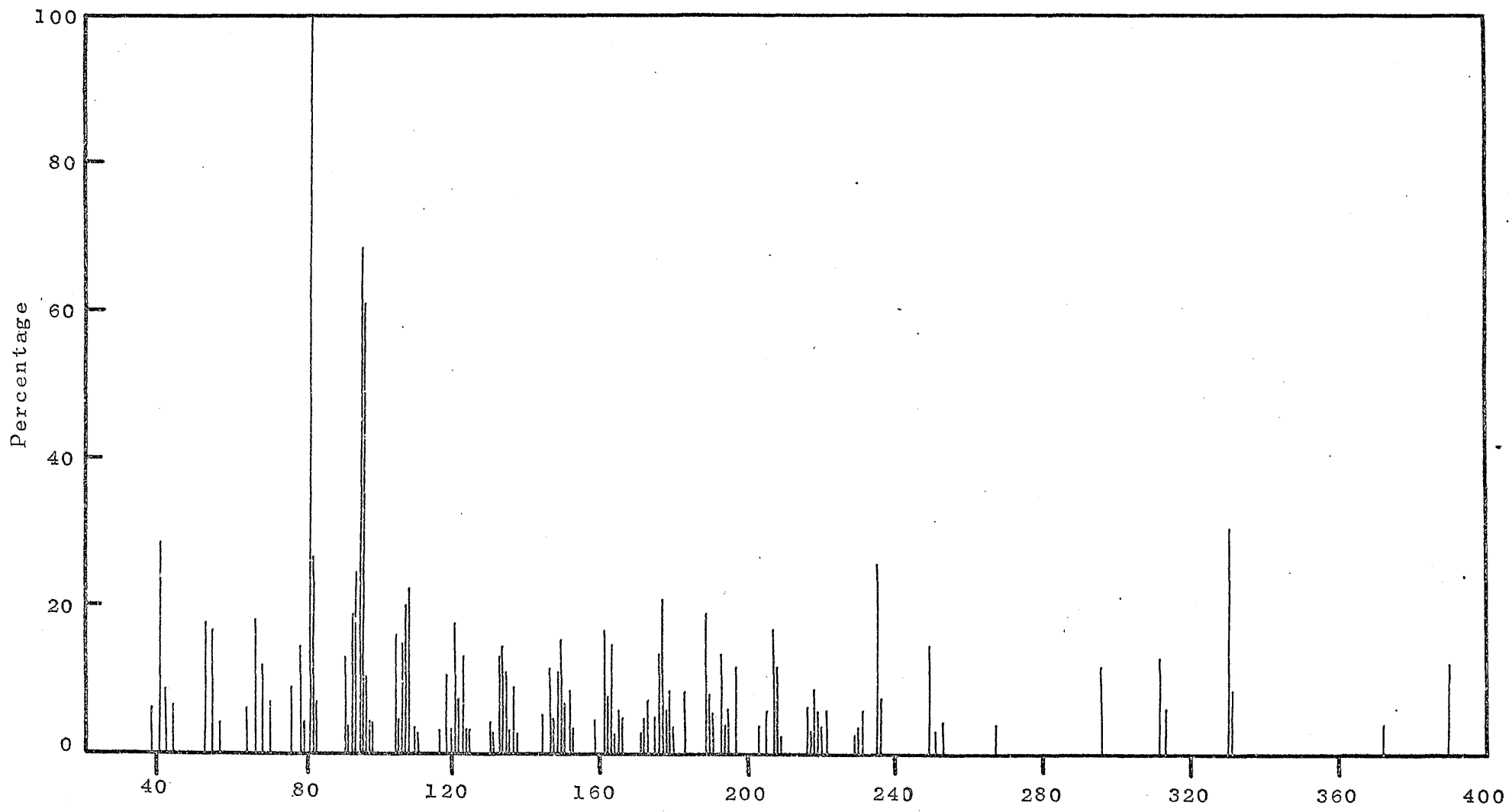


Figure 2: Mass Spectrum of Dubiin

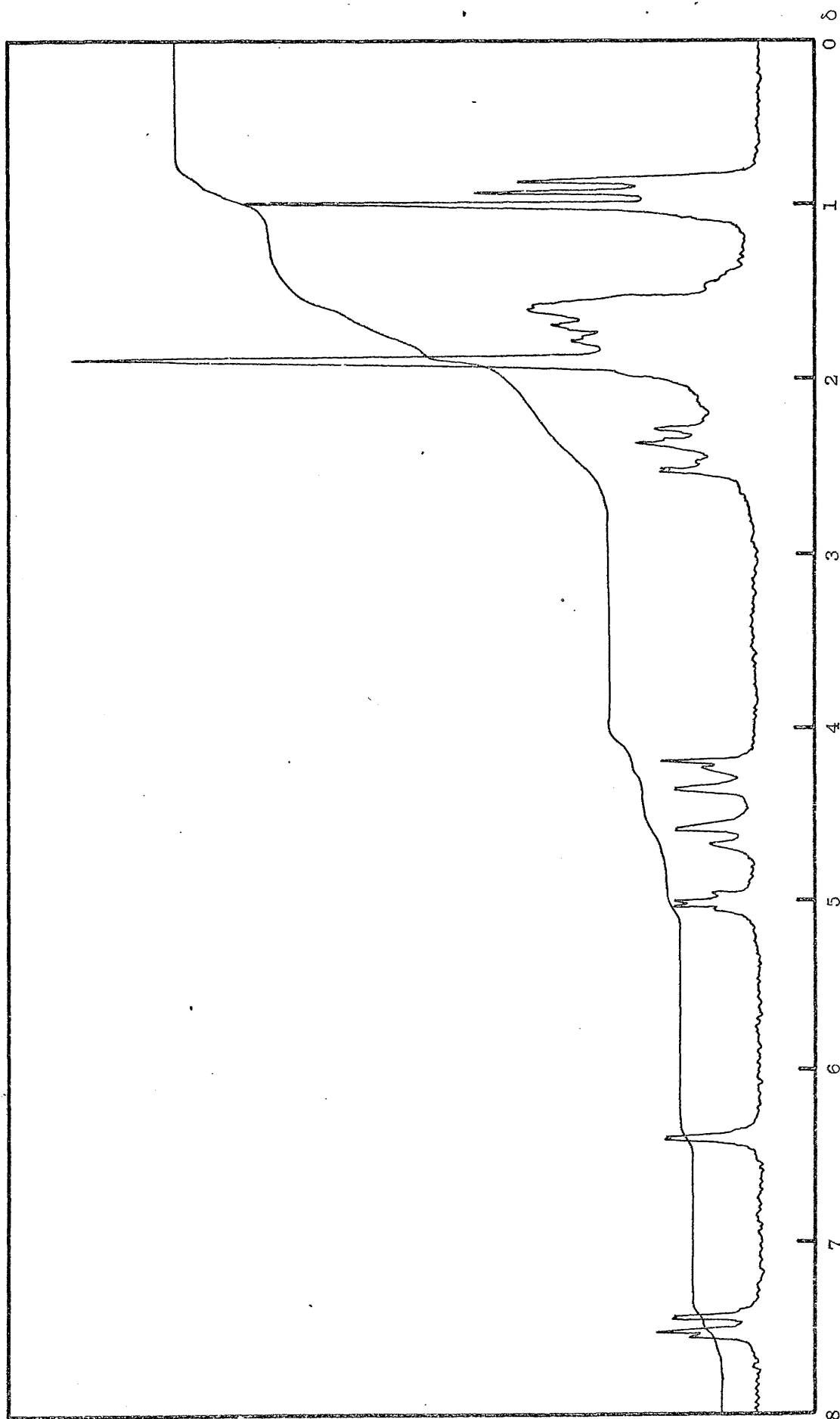


Figure 3: 100 MHz N.M.R. Spectrum of Dubilin (DMSO-d<sub>6</sub>)

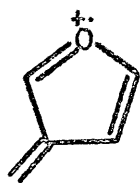
( $W_{\frac{1}{2}} = 8 \text{ Hz}$ ) being indicative of a proton in an equatorial configuration.<sup>24</sup>

Thus, although it has not been possible to prove the carbon skeleton of dubiin, either by dehydrogenation with palladium on charcoal to a substituted naphthalene, or by comparison of a degradation product of dubiin with a compound of known structure, the spectroscopic evidence suggests a structure closely related to that of marrubiin.

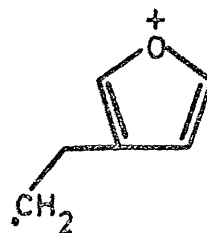
The dehydrogenation product of neither dubiin nor its lithium aluminium hydride reduction product formed crystalline adducts with 1, 3, 5-trinitrobenzene as has been observed with marrubiin and compound Y (72).<sup>96</sup> 8-Hydroxymarrubiin (73), on dehydrogenation, also gave no crystalline 1, 3, 5-trinitrobenzene derivative.<sup>99</sup> The reason for these failures is not known.

Treatment of dubiin with Paal catalyst (10% palladium hydroxide-barium sulphate) under hydrogen resulted in the uptake of two moles of hydrogen and gave tetrahydrodubiin,  $C_{22}H_{34}O_6$ , m.p. 156.5-158°. This compound presumably consists of a mixture of epimers at C-13 as observed with marrubiin.<sup>31, 100</sup> It gave negative Ehrlich<sup>46</sup> and Liebermann-Buchard<sup>47</sup> tests for furans and its infrared spectrum did not contain a peak at  $870 \text{ cm}^{-1}$ . This indicated that the only unsaturation in dubiin is present in the furan ring.

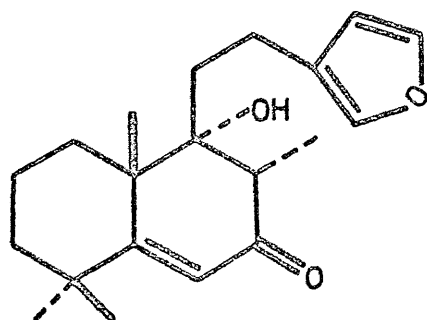
Treatment of dubiin with alkali yielded a crystalline compound  $C_{20}H_{28}O_5$ , m.p. 185-186°, which resisted tosylation and acetylation with acetyl chloride at room temperature, but was acetylated by boiling acetic anhydride in pyridine to give dubiin. This showed that saponification had resulted in the simple cleavage of an acetate group and that the secondary hydroxy-group of saponified dubiin is hindered. Quantitative saponification showed that two moles of alkali were absorbed per mole



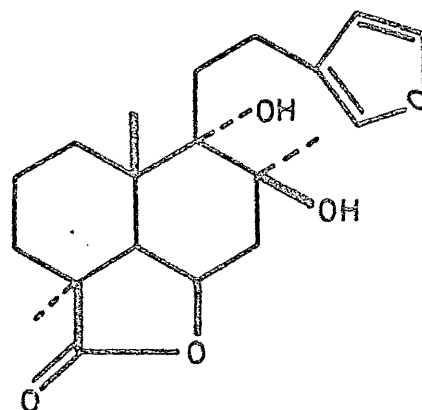
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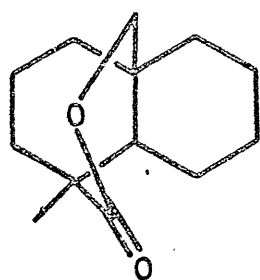
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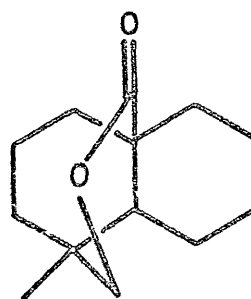
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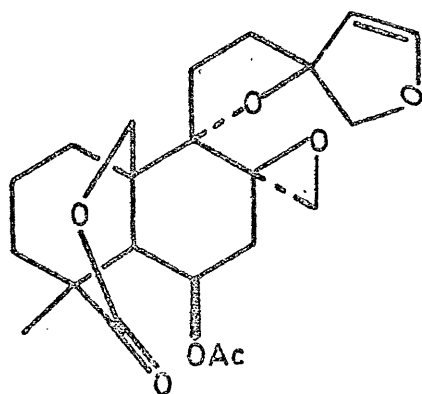
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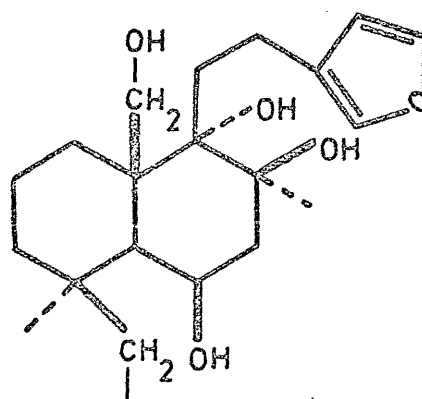
(74)



(75)



(76)



(77)

of dubiin, indicating that cleavage of the lactone ring had also taken place. The free acid resulting from this fission could, however, not be isolated due to the ease of relactonisation, thus confirming that the lactone ring is 6-membered.

The n.m.r. spectrum of saponified dubiin was similar to that of dubiin except that the singlet due to the acetate methyl group had disappeared and the quartet at 5.02 had moved upfield to 3.92, indicating that this resonance is due to a proton attached to a carbon atom carrying a secondary hydroxy-group. On saponification of an acetate ester to a secondary alcohol, it is expected<sup>101</sup> that the resonance due to the adjacent hydrogen atom should shift upfield by about 1.1 ppm. The narrowness of this signal ( $W_{\frac{1}{2}} = 8 \text{ Hz}$ ) is characteristic of a proton in the equatorial configuration;<sup>24</sup> the hydroxy-group is therefore axial.

The infrared spectrum of saponified dubiin contained peaks at 3620 (hydroxy-group), 872 (furan) and 1712 ( $\delta$ -lactone)  $\text{cm}^{-1}$ . Assuming that dubiin has the same carbon skeleton as marrubiin, the lactone group (-CO-O-) can only be situated between C-19 and C-20, giving the partial structure (74) or (75). Structure (74) is preferred since saponification of dubiin causes quite a large upfield shift (0.16 ppm.) in the resonance of one of the methylene protons in the AB quartet. Models show that a methylene group at C-20 would be affected to a greater extent by the saponification of (74) than a C-19 methylene group by the saponification of (75). X-ray studies have shown that the -C-CO-O-C- group of a  $\delta$ -lactone is planar<sup>102, 103</sup> and thus the lactone must exist either in the half-boat or half-chair form.<sup>104</sup> Attempts to determine the conformation of the lactone ring

by ORD studies<sup>105</sup> have been unsuccessful since the ORD spectrum of saponified dubiin does not exhibit any distinct maxima or minima.

The presence of an oxygen function at C-20 in labdane diterpenoids is unusual and it has thus far proved impossible to remove this function and convert dubiin to a compound of known structure. White and Manchand<sup>94</sup> have recently reported the isolation, from L. nepetaefolia, of a compound (76) with this lactone system. They stated that they were able to remove the oxygen function from C-20 in the following way. Compound (76) was warmed with chloroform to afford a furano-compound. This was reduced with lithium aluminium hydride to give compound (77) which, on tosylation, gave two primary monotosylates. Hydrogenolysis with lithium aluminium hydride in tetrahydrofuran, afforded compound (78) "probably by way of an intermediate cyclic ether." An analogous series of experiments with dubiin gave very different results (see later).

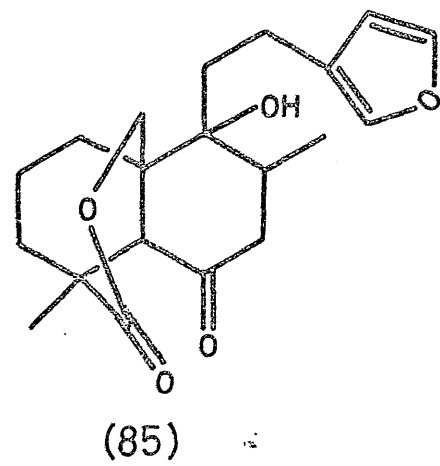
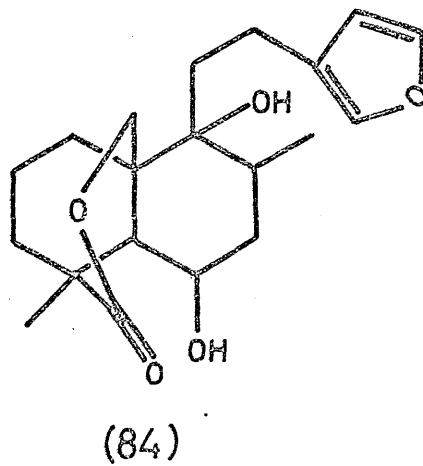
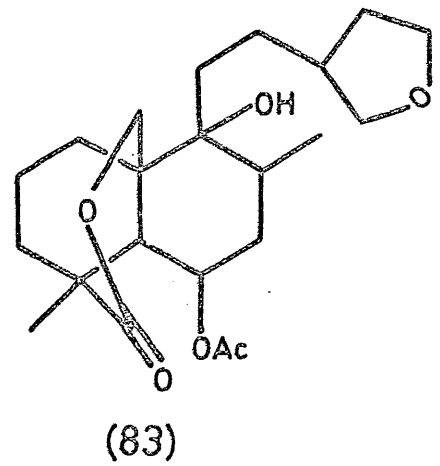
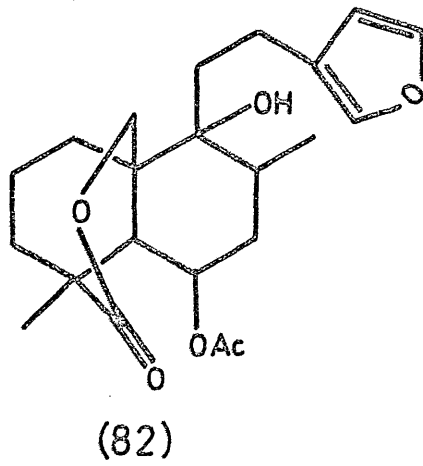
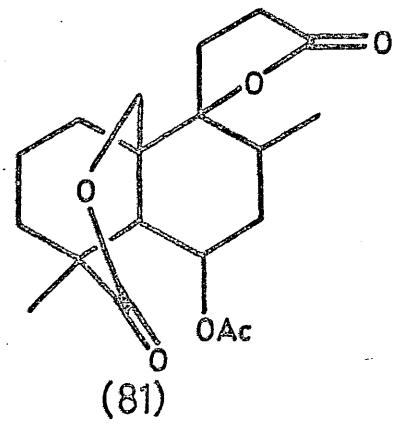
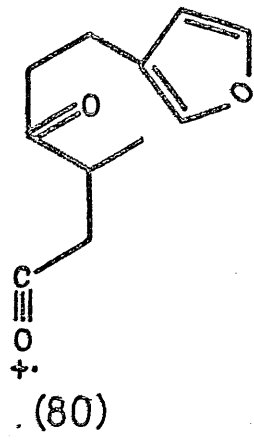
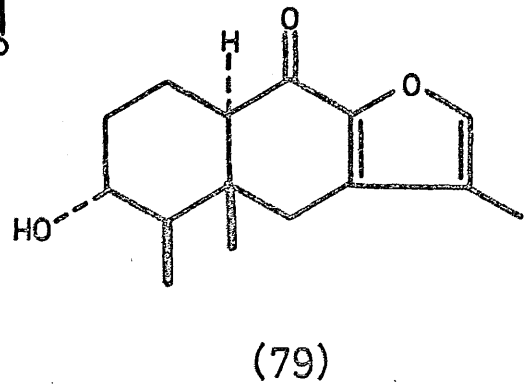
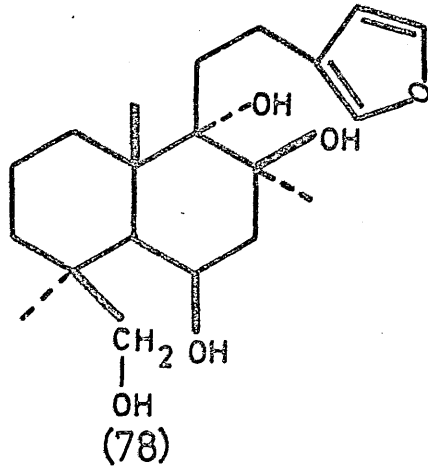
An attempt was made to confirm the configuration of the secondary hydroxy-group in saponified dubiin by the method of Horeau.<sup>51-54</sup> According to the literature, esterification of the alcohol with  $\alpha$ -phenylbutyric anhydride is always carried out at room temperature. However, saponified dubiin, on treatment with  $\alpha$ -phenylbutyric anhydride at room temperature for 10 days, was recovered unchanged. The reaction was therefore repeated under reflux. Isolation of the excess acid gave a product whose specific rotation was effectively zero. It was thought that the elevated temperature had possibly caused racemisation of the excess anhydride. This was confirmed by the fact that the excess acid isolated from the reaction of euryopsonol (79) with  $\alpha$ -phenylbutyric anhydride under reflux also had a rotation

of zero. When euryopsonol was treated with  $\alpha$ -phenylbutyric anhydride at room temperature the excess acid recovered had  $[\alpha]_D^{21} = + 8^\circ$ .

Oxidation of saponified dubiin with Jones,<sup>60</sup> Sarett<sup>30</sup> and Collins<sup>61</sup> reagents and with chromic acid in ether<sup>63</sup> yielded the same crystalline compound  $C_{20}H_{26}O_5$ , m.p. 157-159<sup>o</sup>. It was presumed that oxidation had converted the secondary hydroxy-group to a ketone which, however, gave a negative Zimmerman test<sup>106</sup> and would not form any of the usual carbonyl derivatives. This is usual<sup>107, 108</sup> for compounds containing a 6-keto-group and indicated that the keto-group in this compound, and hence the acetate group in dubiin, was at C-6. This suggestion was supported by the fact that the mass spectrum of this compound contained a strong (56%) peak at  $m/e$  193, probably arising from  $\alpha$ -cleavage of the ketone. Such cleavage<sup>109</sup> of a compound containing a 6-keto-group would give rise to fragment (80) which has  $m/e$  193. The mass spectrum also showed a molecular ion peak at  $m/e$  346 and the usual strong peaks at  $m/e$  81 and 95. The presence of a 6-keto-group was confirmed by deuterium exchange in the presence of alkali, which resulted in the incorporation of a maximum of three deuterium atoms per molecule. A carbonyl group at C-7 would also result in the uptake of three deuterium atoms, but this would cause the doublet due to the 17-methyl group in the n.m.r. spectrum to collapse to a singlet, which is not the case. A carbonyl group at either C-1 or C-3 would lead to the uptake of two deuterium atoms while a carbonyl group at C-2 would lead to the incorporation of a maximum of four deuterium atoms. In the mass spectrum of the

deuterated compound, the molecular ion peak had shifted from  $m/e$  346 to  $m/e$  349. The peak at  $m/e$  193 had shifted to  $m/e$  195, confirming that this peak was due to a fragment arising from  $\alpha$ -cleavage of the ketone. In the n. m. r. spectrum (DMSO- $d_6$ ) of the ketone the AB quartet had collapsed to a triplet at 4.28 (2H, J 13 Hz) with a very large central peak. It appeared as though the two doublets of the AB quartet in the spectra of dubiin and saponified dubiin had shifted closer together in the spectrum of the ketone so that they overlapped. This lent further support to the suggestion that the lactone methylene group is at C-20 as shown in partial structure (74), since models show that the two hydrogen atoms of the 20-methylene group are almost equidistant from the keto- and lactone carbonyl groups respectively, resulting in the resonances due to these protons having almost identical chemical shifts. The rest of the n. m. r. spectrum of the ketone was similar to that of saponified dubiin except that, as expected, the 1-proton quartet at 3.92 had disappeared.

Oxidation of dubiin with chromium trioxide in acetic acid gave a mixture of an acidic and a neutral compound which was easily separated. The infrared spectrum of the neutral compound,  $C_{19}H_{26}O_6$ , m. p. 212-214 $^{\circ}$ , showed two bands in the carbonyl region at 1770 and 1731  $cm^{-1}$  due to  $\gamma$ - and  $\delta$ -lactones respectively. Since the oxidation results in the loss of three carbon atoms from the molecule, oxidation of the furan ring must have taken place with the formation of a carboxy-group. This must have undergone cyclisation with a  $\gamma$ -hydroxy group, giving rise to a  $\gamma$ -lactone and



the compound must have structure (81). This confirms the presence of a furan ring with a  $\gamma$ -hydroxy-group, a system which is also present in marrubiin. The hydroxy-group in dubiin must, therefore, be at C-9 and dubiin must have the structure (82). The structures for tetrahydrodubiin (83), saponified dubiin (84) and the oxidation product of saponified dubiin (85) follow.

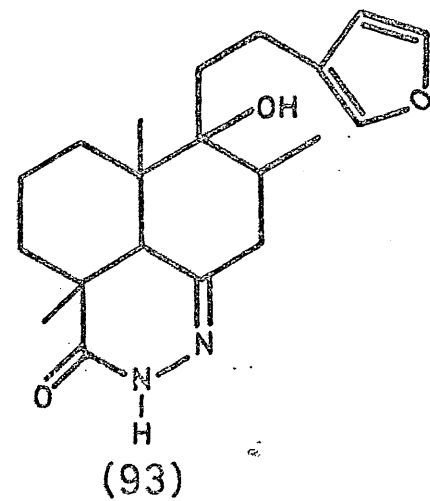
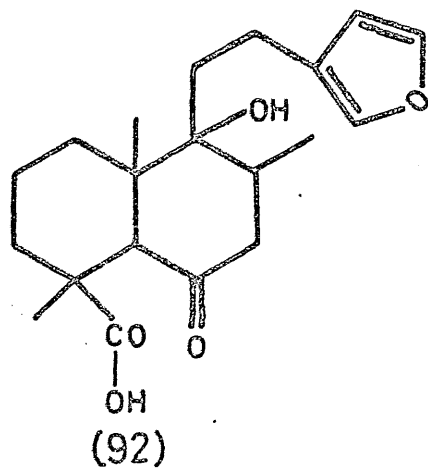
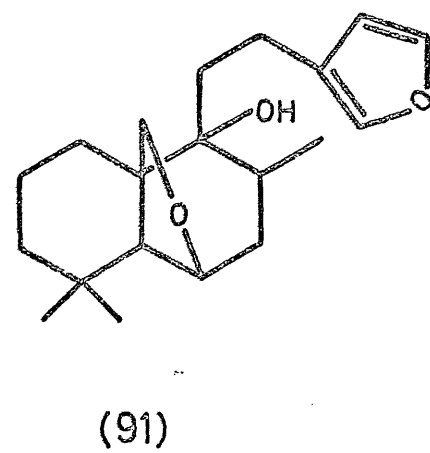
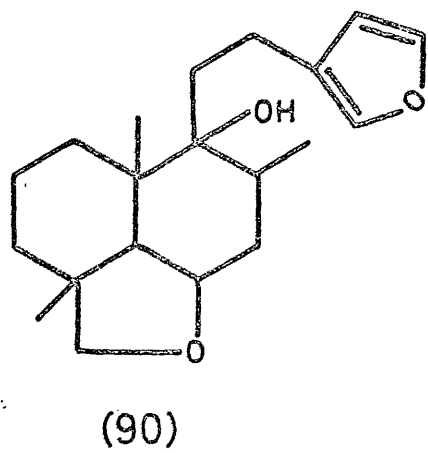
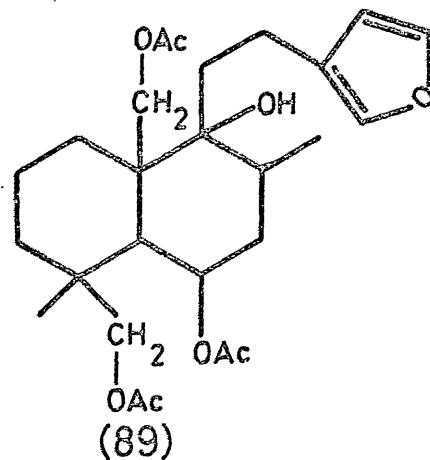
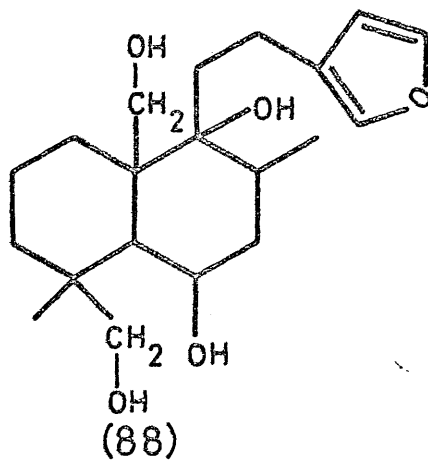
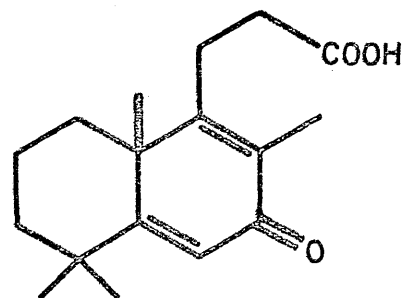
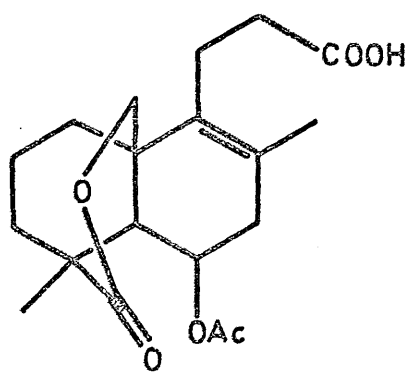
The acidic compound,  $C_{19}H_{26}O_6$ , m. p. 222-223 $^{\circ}$ , obtained from the chromium trioxide-acetic acid oxidation of dubiin is considered to be compound (86), similar to the acidic compound (87) obtained in an analogous manner from compound Y.<sup>96</sup>

Reduction of dubiin with lithium aluminium hydride yielded the tetraol (88)  $C_{20}H_{32}O_5$ , m. p. 219-221 $^{\circ}$ , whose infrared spectrum (KBr disc) contained a large hydroxyl peak and no bands in the carbonyl region. An attempt was made to dehydrogenate this compound with 10% palladium on charcoal to a substituted naphthalene. However, no crystalline 1,3,5-trinitrobenzene adduct of the product could be obtained. The tetraol resisted cleavage by sodium periodate; acetylation with acetyl chloride afforded a gum which would not crystallise, but treatment with acetic anhydride in pyridine under reflux yielded the crystalline triacetate (89). Despite several attempts partial hydrolysis of this triacetate could not be achieved. When the tetraol was treated with Collins reagent for 1 hour, a product was obtained which would not crystallise but whose n. m. r. spectrum contained a broad 1-proton resonance at 9.72 attributable to one aldehydic proton. This showed that oxidation of only one of the primary hydroxy-groups had taken place. This gum was subjected to Wolff-Kishner reduction conditions<sup>68</sup> which are known to reduce 11-keto-steroids, but was recovered unchanged (t. l. c. evidence),

indicating that the aldehydic group is highly unreactive.

Treatment of the tetraol with silver carbonate-Celite<sup>75, 76</sup> in benzene under reflux for 1 hour yielded a crystalline product which was shown (m. p., mixed m. p. and infrared spectrum) to be identical with saponified dubiin. Under these conditions, secondary hydroxy-groups are known to be oxidised more readily than primary, although primary diols which are separated by 4-6 carbon atoms are oxidised to lactones.<sup>110</sup> It was, therefore, expected that the tetraol (88) should yield the keto-lactone (85) when treated in this way. The fact that the secondary hydroxy group was not attacked confirms that it is highly hindered.

Since marrubiin, on treatment with lithium aluminium hydride, yielded marrubenol which, on tosylation afforded the ether (90),<sup>96</sup> an attempt was made to synthesise this ether from dubiin by an analogous pathway. The tetraol (88) was tosylated, yielding a nicely crystalline compound, m. p. 79-81<sup>o</sup>, which gave a positive sulphur test. However, after repeated carbon and hydrogen analyses, consistent figures were obtained which did not correspond to any of the following: tritosylate, ditosylate, ditosylate - water, monotosylate, monotosylate - water. The reason for this is unknown but is probably due to the fact that this compound is unstable and turns brown even on standing at room temperature overnight. Reduction of this compound with lithium aluminium hydride yielded a compound, m. p. 112.5-114<sup>o</sup>, which contained no sulphur. Carbon and hydrogen analysis and mass spectrum showed the molecular formula to be C<sub>20</sub>H<sub>30</sub>O<sub>3</sub>, the same as that of the ether from marrubiin. However, the n. m. r. spectra of these two compounds were different, but both



contained resonances due to two tertiary methyl groups, one secondary methyl group and three lowfield protons. The two compounds had slightly different infrared spectra and a depression of their melting points on mixing. The ether from dubiin is therefore considered to possess the structure (91), arising from cyclisation between C-6 and C-20 and removal of the tosyl group from C-19. White and Manchand,<sup>94</sup> on tosylation of compound (77), obtained a mixture of two primary monotosylates which, on hydrogenolysis with lithium aluminium hydride afforded compound (78). Since a tosyl ester is such a good leaving group, it is difficult to see how they could have obtained a mixture of primary monotosylates. One would expect that the "intermediate cyclic ether" obtained on hydrogenolysis of the monotosylates would, in fact, be the final product.

A series of 60 MHz n. m. r. spectra ( $\text{CDCl}_3$ ) of the ether (91) was run after progressive additions of small amounts of the paramagnetic shift reagent  $\text{Eu}(\text{dpm})_3$  to the solution (see Figure 4) and the change in chemical shift for each peak was noted. Graphs were plotted of chemical shift vs molar ratio of  $\text{Eu}(\text{dpm})_3$  to ether for each peak and are shown in Figure 5. These indicated that the europium complexes preferentially with the ether oxygen atom rather than that of the hydroxy-group<sup>111, 112</sup> since the slope of the graphs is greatest for the peaks due to the three lowfield protons and least of all (except for peaks of the furan protons which are hardly shifted at all) for the secondary methyl group. To verify this, a similar series of spectra of the ether from marrubiin was run; a similar tendency was found in the change of chemical shift of the peaks of

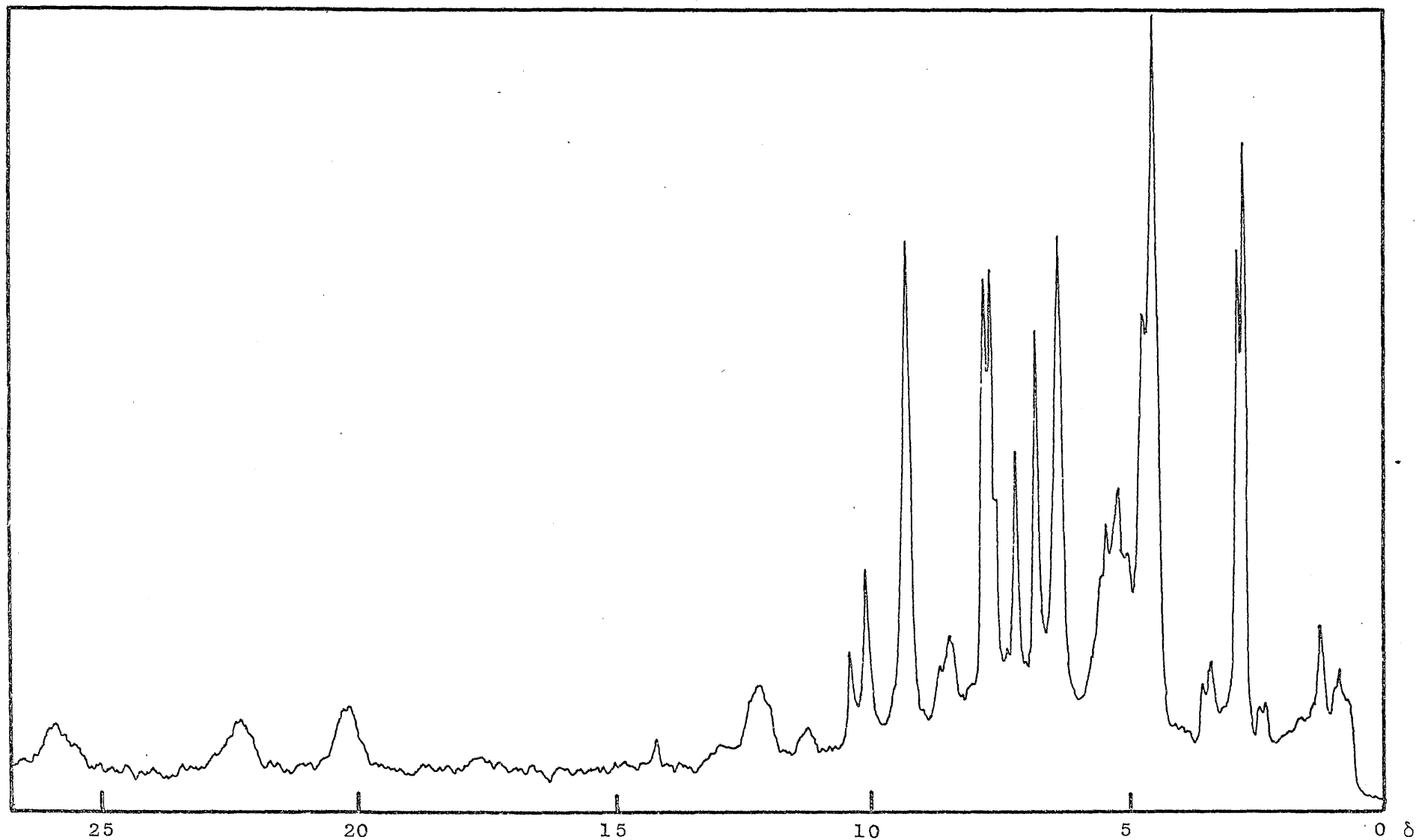


Figure 4: 60 MHz N.M.R. Spectrum of Ether (91) with 0.77 moles  $\text{Eu}(\text{dpm})_3$

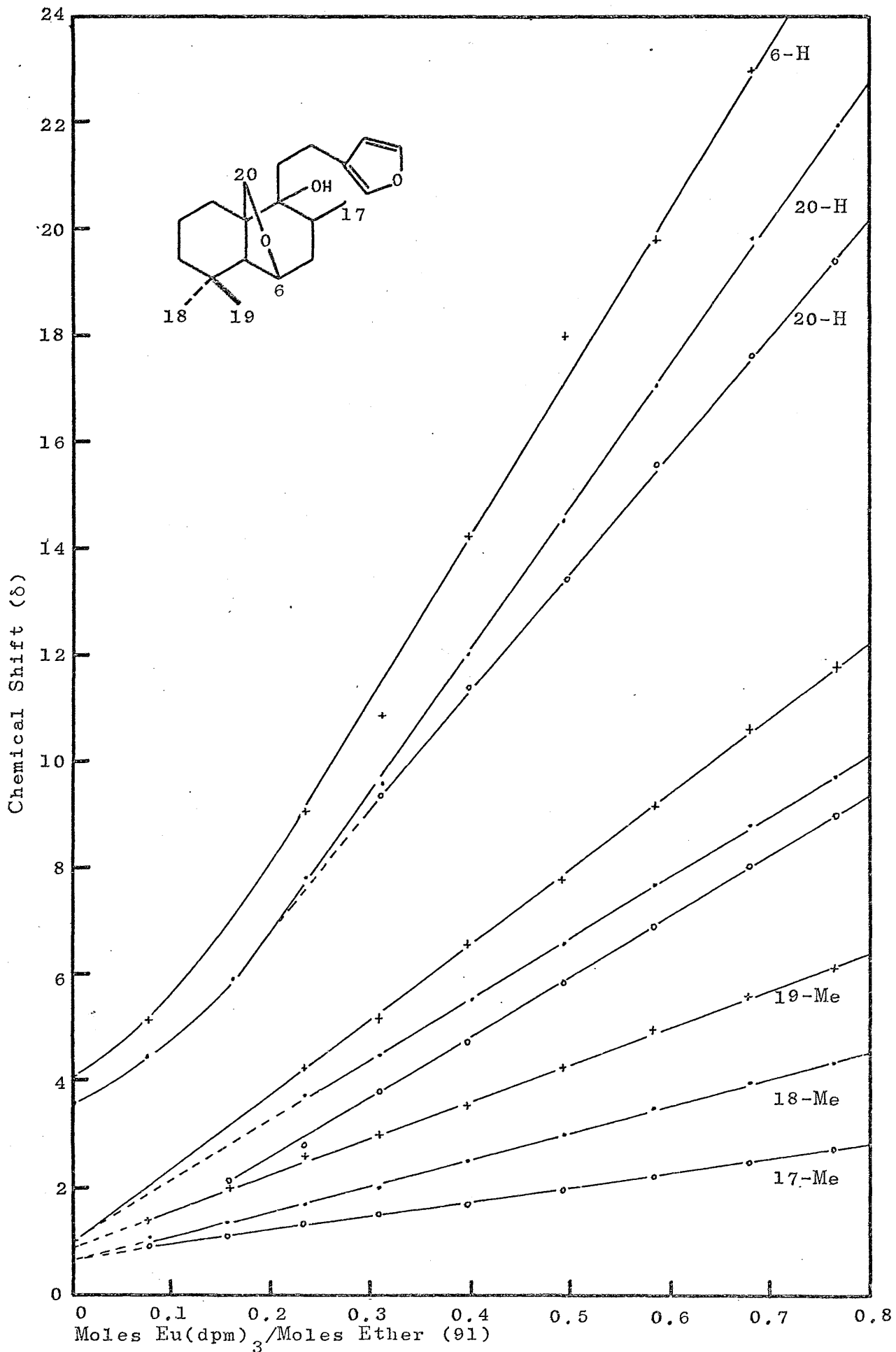


Figure 5

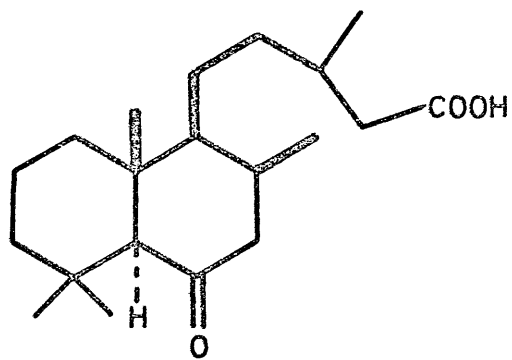
this compound on addition of the shift reagent, confirming that complexation takes place with the ether oxygen atom. Since the europium seemed to show a reluctance to complex with the oxygen atom of the tertiary hydroxy-group which, from models, is not particularly hindered, it was decided to run a series of spectra of saponified dubiin with  $\text{Eu}(\text{dpm})_3$  to see if the europium would complex with the secondary hydroxy-group. However, it was found that after a molar ratio of  $\text{Eu}(\text{dpm})_3$  to saponified dubiin of only 0.4 had been reached, no more  $\text{Eu}(\text{dpm})_3$  could be induced to dissolve in the deuteriochloroform solution. Since in this case only about half the amount of  $\text{Eu}(\text{dpm})_3$  which had been added in the previous two cases could be dissolved, it seemed to indicate that the solubility of the  $\text{Eu}(\text{dpm})_3$  is related to the structure of the compound already in solution.

Other attempts were made to compare degradative products of dubiin and marrubiin by removal of the oxygen function at C-6 from both compounds. If this could be achieved and the lactone ring cleaved with lithium aluminium hydride, then tosylation and reduction could be carried out without ring closure to form an ether. However, although this was tried by various methods, all proved fruitless. Saponified dubiin resisted tosylation at C-6 and the oxidation product of saponified dubiin resisted thioketal formation. On Wolff-Kishner (Huang Minlon) reduction<sup>66, 68, 69</sup> of this ketone, gums were obtained which were shown by t. l. c. in ethyl acetate to be complex mixtures. Marrubic acid, obtained on saponification of marrubiin, was oxidised with Jones reagent to compound (92). Wolff-Kishner (Huang Minlon) reduction of this yielded a crystalline compound which contained nitrogen and proved to be the cyclic hydrazone (93) previously

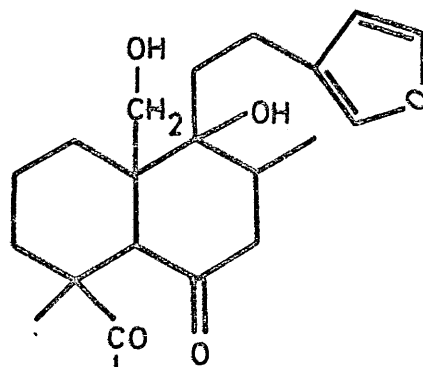
obtained by Hardy, Rigby and Moody.<sup>29</sup> (Halsall and Moyle<sup>107</sup> were, however, able to reduce the dihydro derivative of 6-oxocativic acid (94) to dihydrocaticvic acid by the Wolff-Kishner procedure). Clemmensen reduction of dehydromarrubic acid and the keto-lactone (85) from dubiin resulted in considerable degradation of the starting material, probably due to the instability of the furan ring under acid conditions.

An attempt was, therefore, made to transesterify the oxidation product of saponified dubiin to give the ester (95). This could possibly be tosylated and reduced to give marrubenol, obtained on lithium aluminium hydride reduction of marrubiin. However, treatment of the ketone (85) with boron trifluoride diethyl etherate<sup>70</sup> in methanol gave a gummy product which was shown by t. l. c. in ethyl acetate to be a mixture of at least four components. This was probably also due to the instability of the furan ring under acid conditions. Attempts to transesterify the ketone by saponification and treatment of the alkaline solution with diazomethane, followed by slow acidification<sup>71</sup> resulted in the recovery of starting material. This method has recently been used successfully to convert (96) to (97). Treatment of compound (85) with sodium methoxide or sodium ethoxide in a sealed tube at 140° for 72 hours yielded a compound which could not be induced to crystallise but which, presumably, had the structure (95); treatment of the ketone (85) with sodium in refluxing hexanol for 48 hours yielded a similar product, which, with tosyl chloride in pyridine gave unchanged starting material (95).

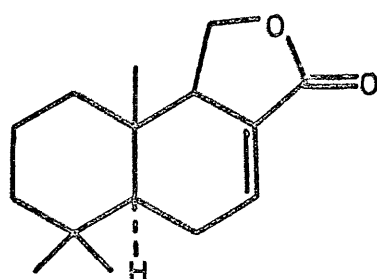
An attempt was also made to synthesise the di-iodide (98) from



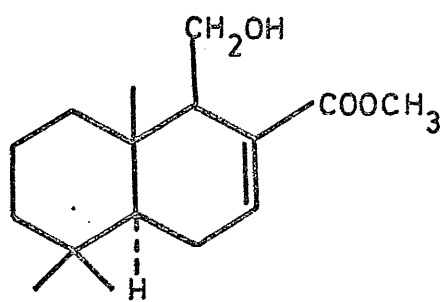
(94)



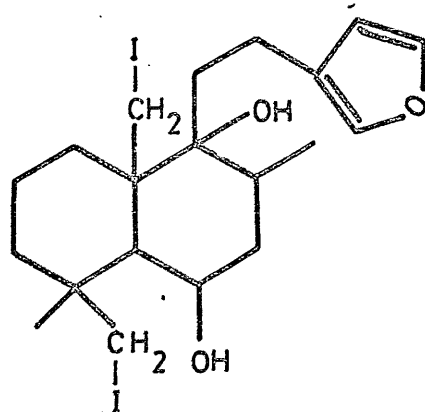
(95)



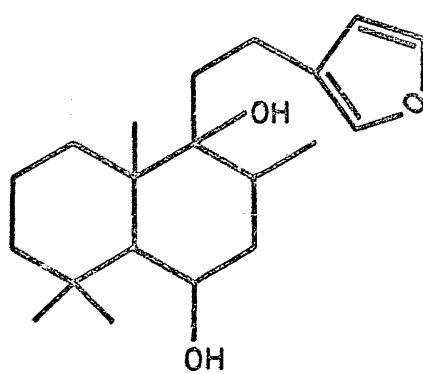
(96)



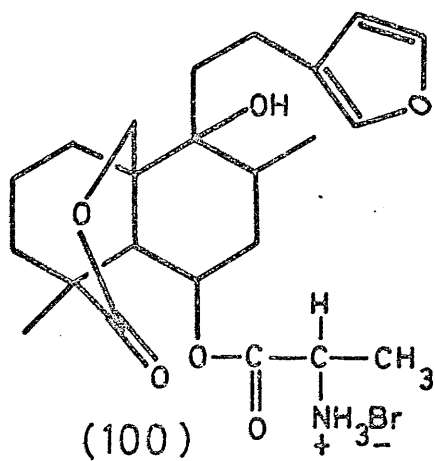
(97)



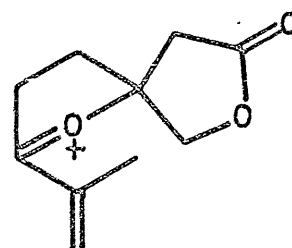
(98)



(99)



(100)



(101)

the tetraol (88) with triphenyl phosphite methiodide.<sup>77</sup> This should be reducible with lithium aluminium hydride to the compound (99). Similar treatment of marrubenol should yield the same compound. On iodination, a gum was obtained which gave a positive Beilstein test, but which could not be induced to crystallise and was therefore reduced with lithium aluminium hydride. The product contained no iodine but t.l.c. showed it to be a mixture. The components were separated on a column of silica gel but could not be induced to crystallise.

It was then decided to try and introduce a heavy atom into saponified dubiin in order to render it suitable for X-ray crystallographic studies. An attempt was made to synthesise the L-alinate hydrobromide derivative (100) of saponified dubiin.<sup>56</sup> A crystalline derivative was obtained and analysed for carbon and hydrogen. However, the values obtained were inconsistent and far removed from the calculated values. The reason for this is unknown but the product was considered unsuitable for X-ray analysis.

Therefore, although the proposed structure (82) for dubiin has not been proved, all the spectroscopic and chemical evidence suggest that this structure is the correct one.

### 3. 2 Leonitin

L. leonitis, from which the diterpenoid acetate leonitin has been isolated, is less common than L. dubia. Plants are scattered and are found growing in rocky areas. The main source of supply has been the area next to the Grahamstown-Kingwilliamstown road, between 15 and 25 miles from Grahamstown. The plants are small, growing to about two feet in height, with orange-brown thorny flowers similar to those of L. dubia. The small deltoid-shaped leaves are sparse and grow mainly along the ground with a few scattered ones up the stems. The yields of leonitin varied with the season in which the plants were picked; some crops yielded no crystalline material.

The leaves and stems were separated and the leaves were air-dried in the shade for 3-6 weeks. The extraction was carried out in a similar manner to that of L. dubia; the dried leaves were steeped in acetone for 2-3 days, the acetone run off and the green solution concentrated by flash distillation. Decolourisation with charcoal and evaporation of the solvent yielded a dark brown gum which was crystallised by dissolving in a minimum of refluxing ethyl acetate and allowing to stand at room temperature. The crystalline material, shown by t. l. c. in ethyl acetate to consist almost entirely of a single compound, was filtered off, dissolved in chloroform and extracted with water to remove inorganic material. After removal of the chloroform, the gum was crystallised from ethanol to give darkly coloured leonitin. This was decolourised with charcoal and recrystallised from ethanol to give colourless

needles, m. p. 242-243°.

Leonitin was shown by elemental analysis and mass spectrum to have the molecular formula  $C_{22}H_{30}O_7$ . The infrared spectrum (Figure 6) contained a strong band at  $1770\text{ cm}^{-1}$  ( $\gamma$ -lactone) with a shoulder at  $1742\text{ cm}^{-1}$  (ester carbonyl). There was no hydroxyl peak and no band in the region of  $870\text{ cm}^{-1}$ , indicating the absence of a furan ring, which was confirmed by the fact that leonitin gave a negative Ehrlich test<sup>46</sup> and was transparent to ultraviolet light. Leonitin absorbed no hydrogen on catalytic hydrogenation showing that there was no unsaturation in the molecule which must, therefore, be pentacyclic.

The mass spectrum of leonitin (Figure 7), besides containing a molecular ion peak at  $m/e$  406, had the base peak at  $m/e$  181 and a strong (35%) peak at  $m/e$  183 attributable to fragments (101) and (102) respectively. There was a peak at  $m/e$  347 corresponding to  $(M-CH_3COO\cdot)$ . As expected, peaks at  $m/e$  81 and 95, due to the presence of a furan ring, were absent.

The 100 MHz n. m. r. spectrum ( $CDCl_3$ ) of leonitin (Figure 8) was very similar to that of compound X (103).<sup>93</sup> The spectra were almost identical in the following respects: Both spectra contained a secondary methyl doublet at 0.88 (J 6 Hz, 17-Me) and a 1-proton multiplet at 4.68 (J 5 Hz, 6-H); the spectrum of compound X contained a 3-proton singlet at 1.28 (18-Me) and an AB quartet centred at 2.72 (J 17 Hz, 14- $H_2$ ) while that of leonitin contained a 3-proton singlet at 1.27 and an AB quartet centred at 2.76 (J 17 Hz).

The spectra were different in the following respects:

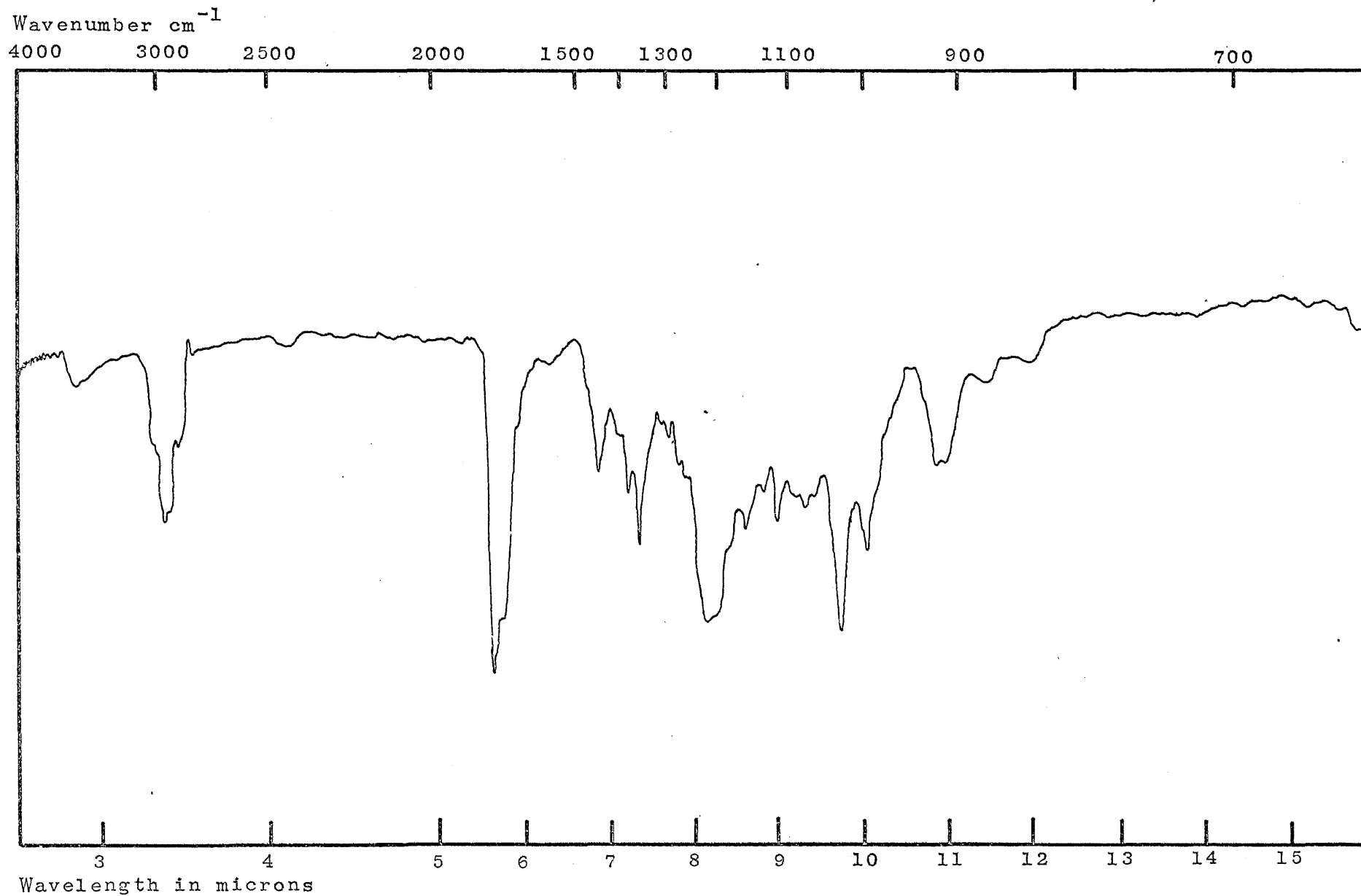


Figure 6: Infrared Spectrum of Leonitin

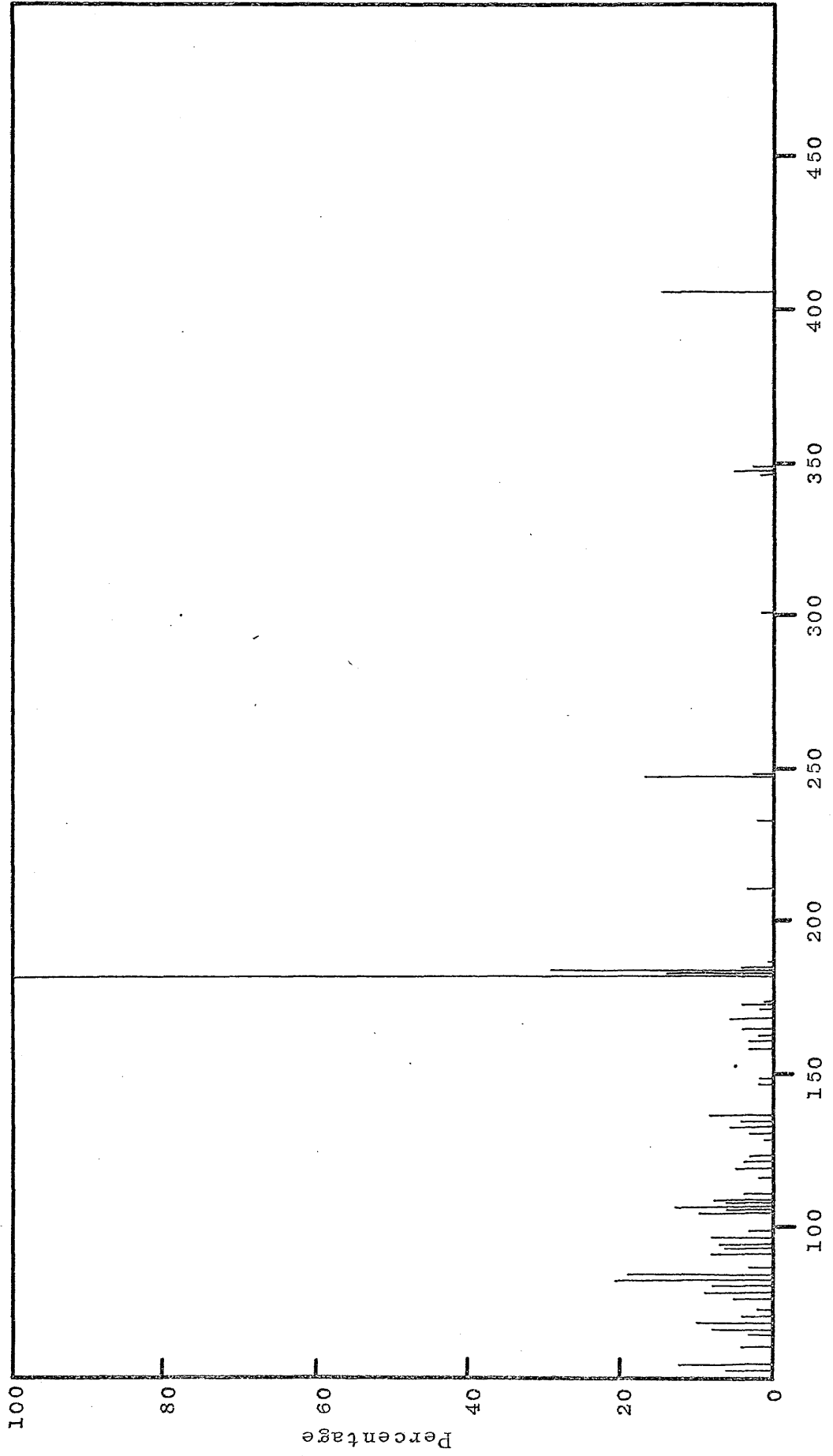


Figure 7: Mass Spectrum of Leonitin

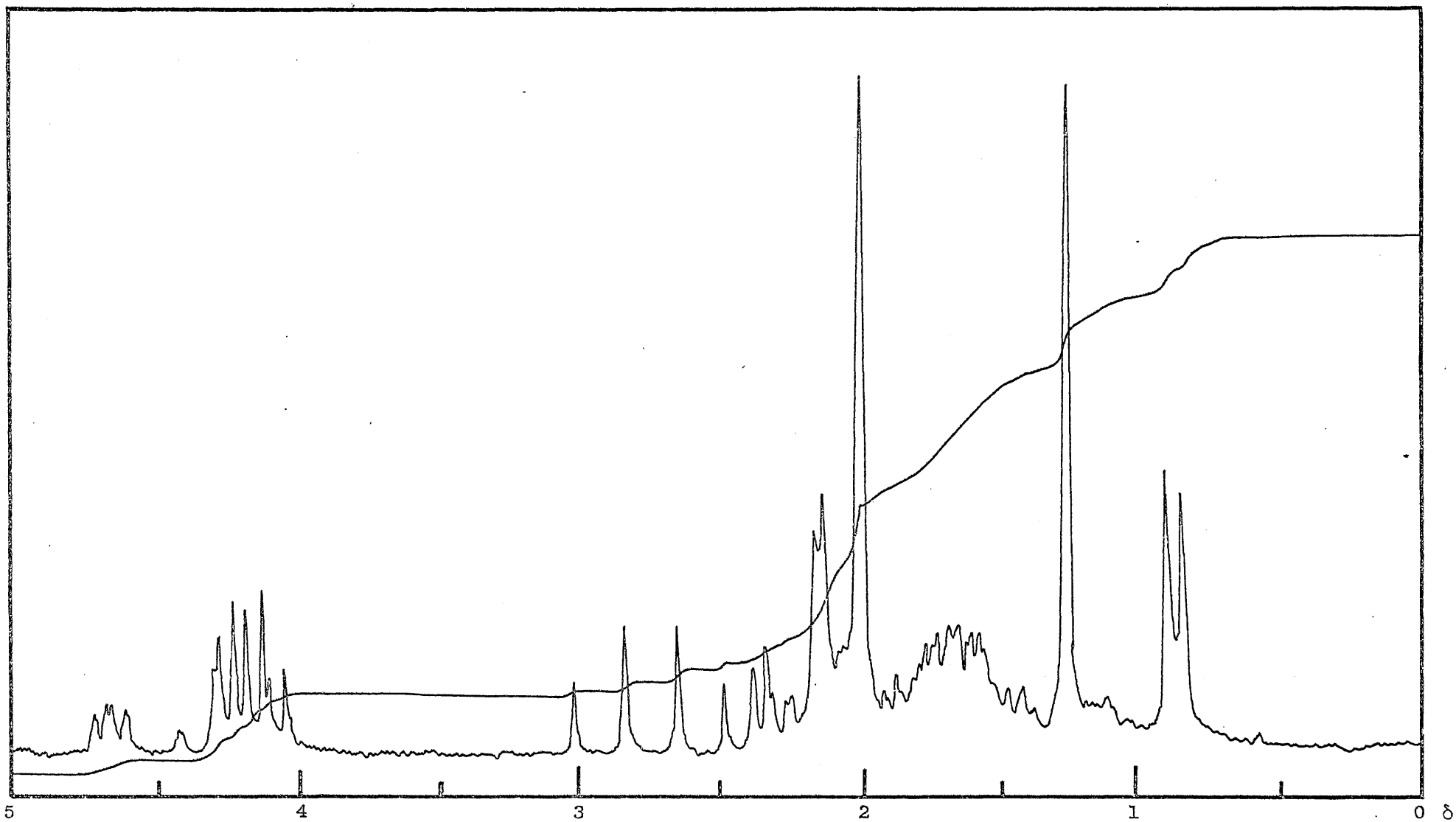
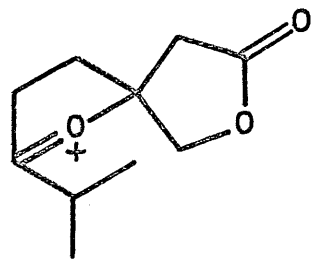


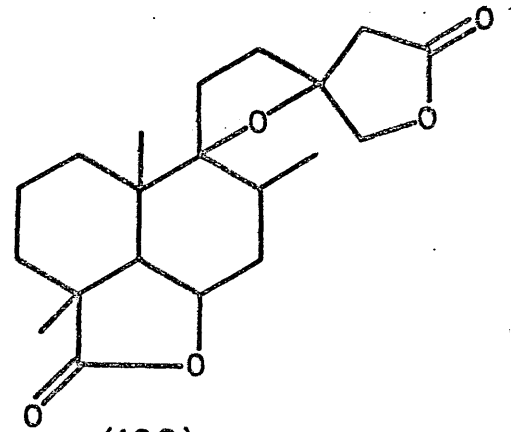
Figure 8: 100 MHz N.M.R. Spectrum of Leonitin (CDCl<sub>3</sub>)

The spectrum of compound X contained a tertiary methyl singlet at 1.10 (20-Me) whereas that of leonitin contained a sharp 3-proton singlet, characteristic of an acetate methyl group, at 2.02; the spectrum of compound X contained an AB quartet centred at 4.19 (J 9 Hz, 16-H<sub>2</sub>) whereas, in the spectrum of leonitin, there was a complicated 4-proton signal between 4.05 and 4.32, probably due to two AB quartets. These differences in the spectra are compatible with the 20-methyl group in compound X being replaced by a -CH<sub>2</sub>-O-CO-CH<sub>3</sub> group in leonitin, so that leonitin can be described as 20-acetoxy-X (104). The only other compounds containing a C-9, C-13 ether bridge which have been reported are grindelic acid (105),<sup>113</sup> lagochilin (106)<sup>114</sup> and two compounds (107)<sup>41</sup> and (108)<sup>92</sup> from horehound.

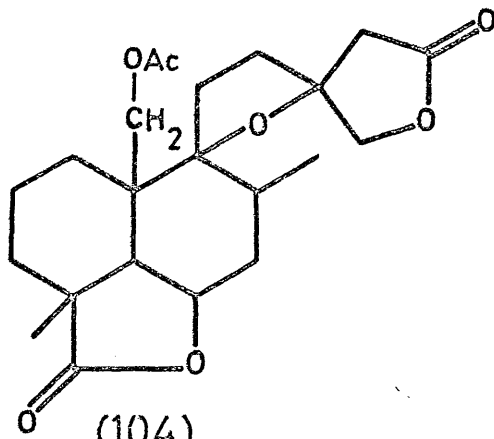
Treatment of leonitin with alcoholic potassium hydroxide afforded a compound C<sub>20</sub>H<sub>28</sub>O<sub>6</sub>, corresponding to the removal of C<sub>2</sub>H<sub>2</sub>O. However, this reaction did not only involve the simple cleavage of an acetate group since the infrared spectrum of the product contained peaks at 3650 (hydroxy-group), 1779 (γ-lactone) and 1715 (δ-lactone) cm<sup>-1</sup>. Acetylation of saponified leonitin did not afford the parent ester. Saponified leonitin crystallised slowly from aqueous ethanol and the melting point varied between 224° and 238°. Quantitative alkaline hydrolysis showed that 2.85 moles of alkali were consumed per mole of leonitin, indicating that ester cleavage and opening of both lactone rings occurred. On acidification, ring closure of the γ-hydroxy-acid took place normally between C-15 and C-16, but the carboxy-group at C-19 underwent ring closure with the primary hydroxy-group at C-20 (and not the secondary hydroxy-group at C-6),



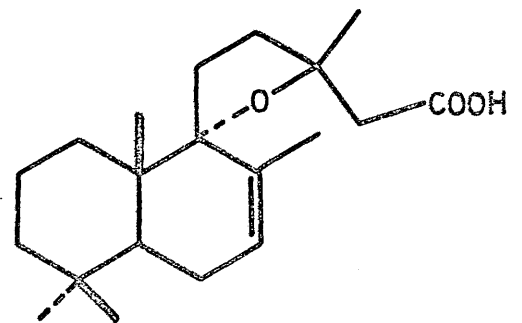
(102)



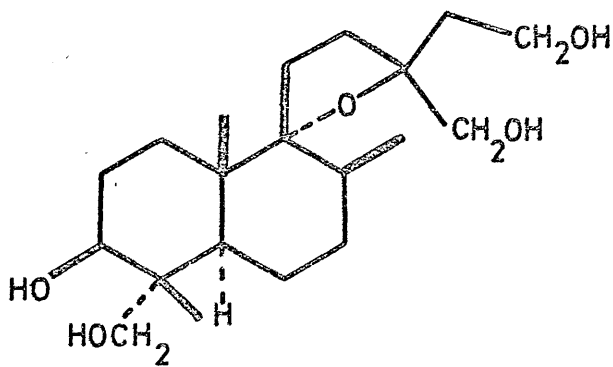
(103)



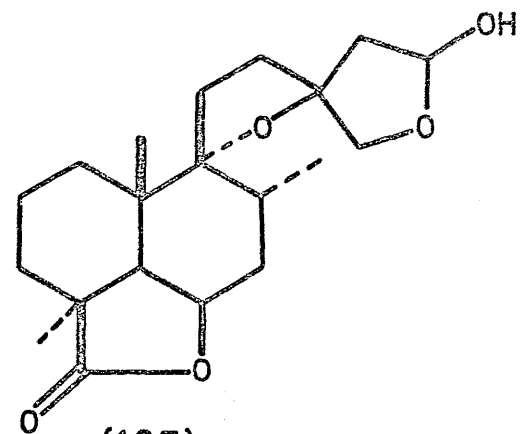
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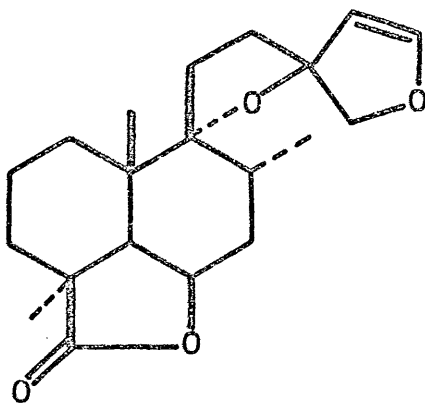
(105)



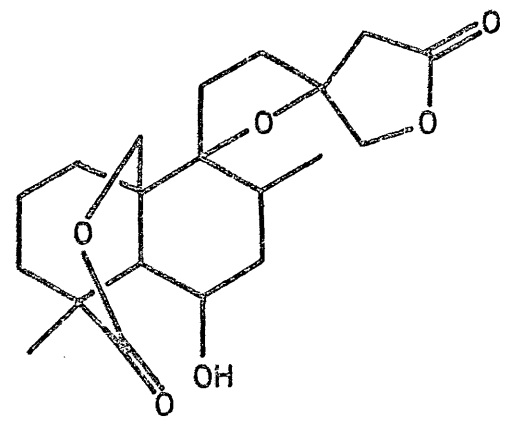
(106)



(107)



(108)

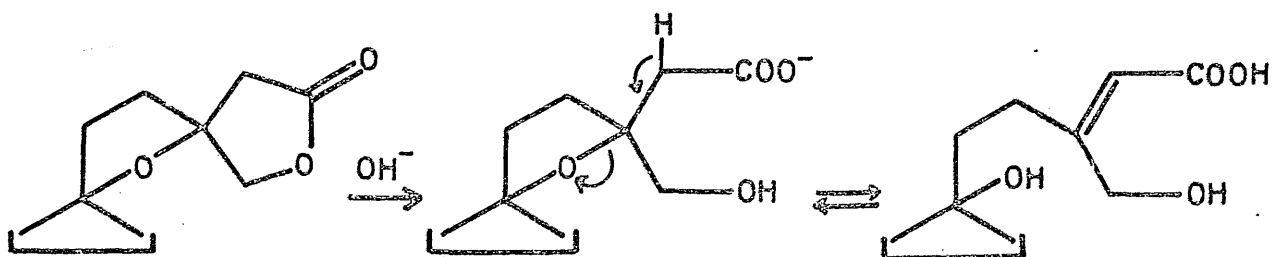


(109)

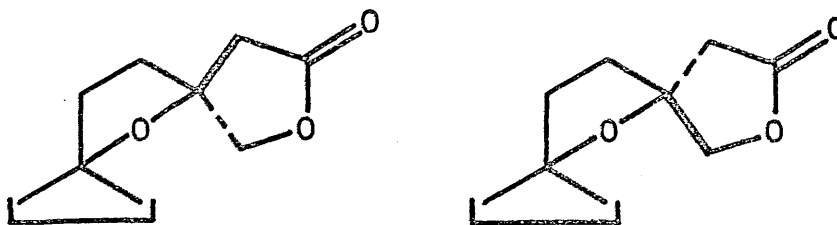
giving rise to compound (109) with a  $\delta$ -lactone system similar to that in dubiin.

The 100 MHz n.m.r. spectrum of saponified leonitin exhibited a curious "doubling-up effect", i. e. singlets appeared as doublets, doublets as quartets, etc. This effect was not observed in all of the peaks (notably the 18-Me peak was unaffected) and the magnitude of the splitting varied with different peaks. The effect was originally thought to be due to the  $\delta$ -lactone existing, in solution, as a mixture of half-boat and half-chair conformers.<sup>115</sup> This phenomenon has been observed with isabelin (110)<sup>116</sup> and neolinderalactone (111).<sup>117</sup> Consequently, n.m.r. spectra were run at various temperatures between 25° and 110° (as was done, for example, with isabelin) since, if this effect was the result of a mixture of conformers, it would be expected that at increased temperature the compound would exist predominantly in the high energy form with a resultant simplification of the spectrum. This expected simplification was not observed which meant that the splitting was not due to a mixture of conformers. This conclusion was supported by the fact that this splitting was not observed in the spectrum of dubiin which also contains this  $\delta$ -lactone system, and also that the 18-Me group in leonitin was not affected while the 17-Me group was strongly affected. Because of the close proximity of the 18-Me group to the  $\delta$ -lactone, a mixture of conformers would affect this group to a greater extent than the 17-Me group.

It was thus thought that the "doubling-up effect" could possibly arise as a result of the molecule, in the presence of alkali, undergoing a rearrangement of the following type:



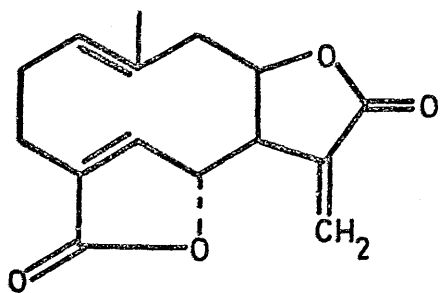
Acidification would then lead to ring closure in two possible forms, giving a mixture of epimers at C-13, i. e.



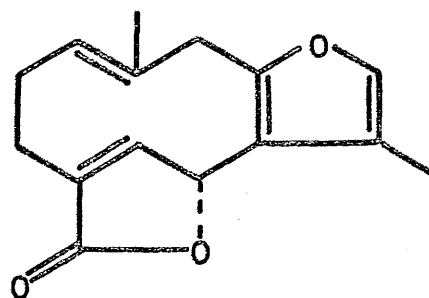
Thus if the "doubling-up effect" was a result of this type of rearrangement, it would be expected that the product from alkali treatment of compound X would also exhibit this phenomenon.

To test this hypothesis, compound X was treated with alkali under the same conditions. The product crystallised with difficulty, m. p. 207-220°, but t. l. c. in ethyl acetate showed a single spot corresponding to that of starting material. However, the 100 MHz n. m. r. spectrum (DMSO- $d_6$ ) of this product was identical with that of compound X. Therefore the reason for this effect is not known.

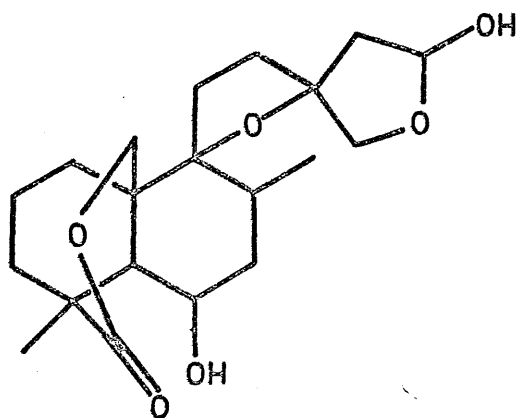
Several attempts were made to remove the acetate group from leonitin without the formation of the  $\gamma, \delta$ -dilactone but all proved fruitless. Partial hydrolysis could not be achieved using sodium bicarbonate, potassium carbonate or hydrochloric acid in various concentrations and for different lengths of time, both at room temperature and under reflux; either unchanged starting material or saponified leonitin were obtained, the products being identified by means of their infrared spectra. Hydrolysis experiments on leonitin did not follow the same pattern as those on marrubiin; treatment of leonitin with



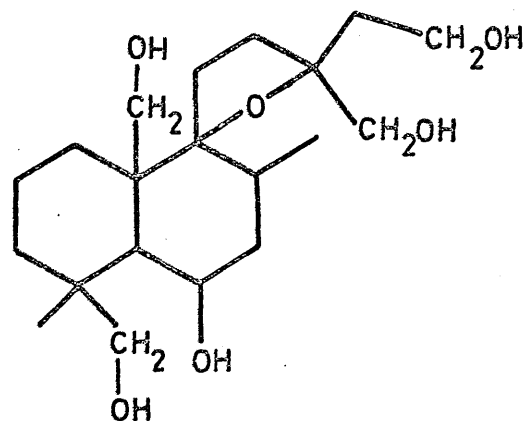
(110)



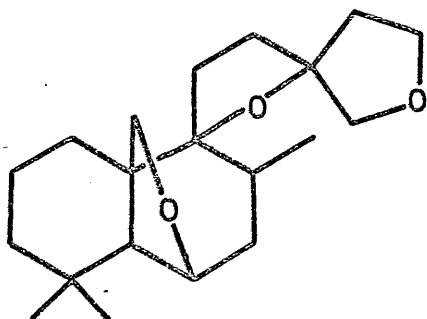
(111)



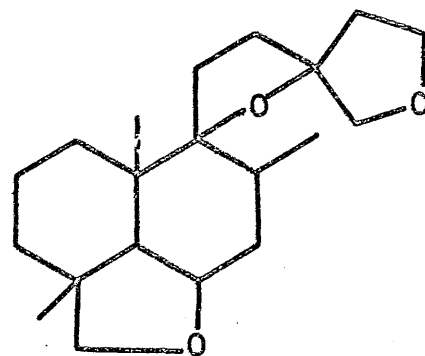
(112)



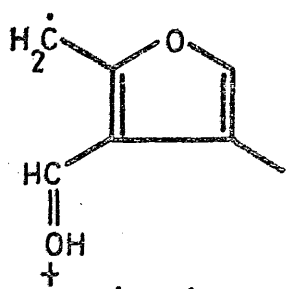
(113)



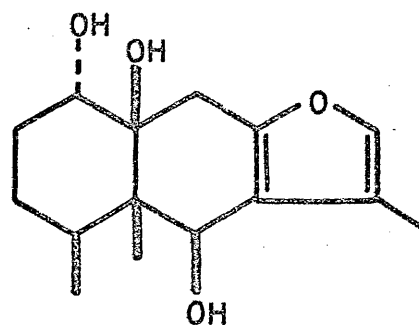
(114)



(115)



(116)



(117)

dilute sulphuric acid under reflux for 5 hours afforded saponified leonitin indicating that the C-4, C-6 lactone ring had been opened. On similar treatment of marrubiin, this lactone ring remained intact and starting material was recovered. The reason for this difference is not known.

Treatment of saponified leonitin with sodium borohydride at room temperature for 4 days afforded a crystalline compound  $C_{20}H_{30}O_6$ , whose infrared spectrum contained only one peak in the carbonyl region at  $1700\text{ cm}^{-1}$ , corresponding to a  $\delta$ -lactone. The band at  $1770\text{ cm}^{-1}$  due to the  $\gamma$ -lactone had disappeared indicating that this lactone had been reduced to the hemiacetal (112). McCrindle *et al.*,<sup>41</sup> on extraction of Marrubium vulgare, isolated a compound (107) with this system which they converted to marrubiin by treatment with tosyl chloride in pyridine under reflux. Similar treatment of the hemiacetal (112) should, therefore, lead to saponified dubiin by an analogous mechanism. However, treatment of the compound (112) with tosyl chloride under reflux afforded a gum which could not be induced to crystallise but which was shown by t.l.c. to be a mixture of two compounds in the approximate ratio of 5:1. The minor component was not isolated but had the same mobility as saponified dubiin in a number of different solvents. The major component was crystallised from ether and shown to be unchanged starting material.

Reduction of leonitin with lithium aluminium hydride yielded the pentaol  $C_{20}H_{36}O_6$  (113), which was tosylated and then reduced with lithium aluminium hydride to give a product which would not crystallise and was different (infrared spectrum) from the product obtained by an analogous pathway from compound X. The product (114) from

leonitin would be expected to be similar to that (91) obtained from dubiin under the same conditions, i. e. formation of a cyclic ether between C-6 and C-20. Tosylation of the lithium aluminium hydride reduction product of compound X afforded the expected triether (115), the mechanism being similar to that in which the ether (90) is formed from marrubiin.

### 3. 3 Euryopsol

The furanoeremophilane euryopsol,  $C_{15}H_{22}O_4$ , m. p. 174-175°, has been isolated pure from Euryops spathaceus DC. and Euryops tenuissimus (L.) DC. It has also been found to occur both by itself and together with euryopsonol (79)  $C_{15}H_{20}O_3$ , m. p. 230-231°, in the resin of Euryops floribundus, commonly known as "harpuisbos".

E. spathaceus is a small evergreen bush growing to about three feet in height with shiny needle-shaped leaves. The production of flowers apparently depends on the spring rainfall and the plants flowered only once during the course of this work, in the spring of 1970, a year of particularly good spring rains. On this occasion the plants were covered with a mass of small yellow flowers which allowed the plants to be identified positively for the first time. In the absence of flowers, the plants had tentatively been identified as E. tenuissimus; thus work carried out by Woolard<sup>88</sup> was done on E. spathaceus and not E. tenuissimus as stated.

E. tenuissimus is very similar in appearance to E. spathaceus, the leaves and stems being virtually identical. However, the small yellow flowers differ slightly from those of E. spathaceus and the plants

are slightly taller, growing to about 4-5 feet in height. Both plants are extremely resinous. E. spathaceus was collected at the side of the Grahamstown-Cradock road, 5 miles from Grahamstown; E. tenuissimus was collected on Sugar Loaf Hill on the outskirts of Grahamstown.

Extraction of both plants and subsequent saponification yielded pure euryopsol. Extractions were carried out on freshly picked stems, the leaves being separated and discarded. The stems were steeped in acetone at room temperature for 2-3 days. Filtration yielded a light brown extract which was evaporated to a dark brown sticky gum. Saponification of the gum with ethanolic potassium hydroxide, extraction with ether and crystallisation of the concentrated ethereal extract yielded very fine needles of euryopsol, m. p. 171-173°, raised on recrystallisation from ethanol to 174-175°.

Both the infrared (Figure 9) ( $\nu_{\max}$  (KBr) 885  $\text{cm}^{-1}$ ) and ultraviolet ( $\lambda_{\max}$  220 nm,  $\epsilon$  6,800) spectra of euryopsol indicated the presence of a furan ring.<sup>97</sup> This was confirmed by a positive Ehrlich test<sup>46</sup> (negative with euryopsonol due to the electron withdrawing effect of the C-9 keto-group). The infrared spectrum showed strong hydroxyl and no carbonyl absorption.

The mass spectrum of euryopsol (Figure 10) showed a molecular ion peak at  $m/e$  266 and peaks at  $m/e$  248 and 230 ( $M - 18 - 18$ ). The base peak at  $m/e$  124 is due to the fragment (116).

The 60 MHz n. m. r. spectrum (DMSO- $d_6$ ) of euryopsol (Figure 11) contained peaks at 0.68 (3H, doublet, J 5 Hz; doublet showed considerable "filling in"), 1.06 (3H, singlet), 1.94 (3H, doublet, J 1 Hz), 2.73

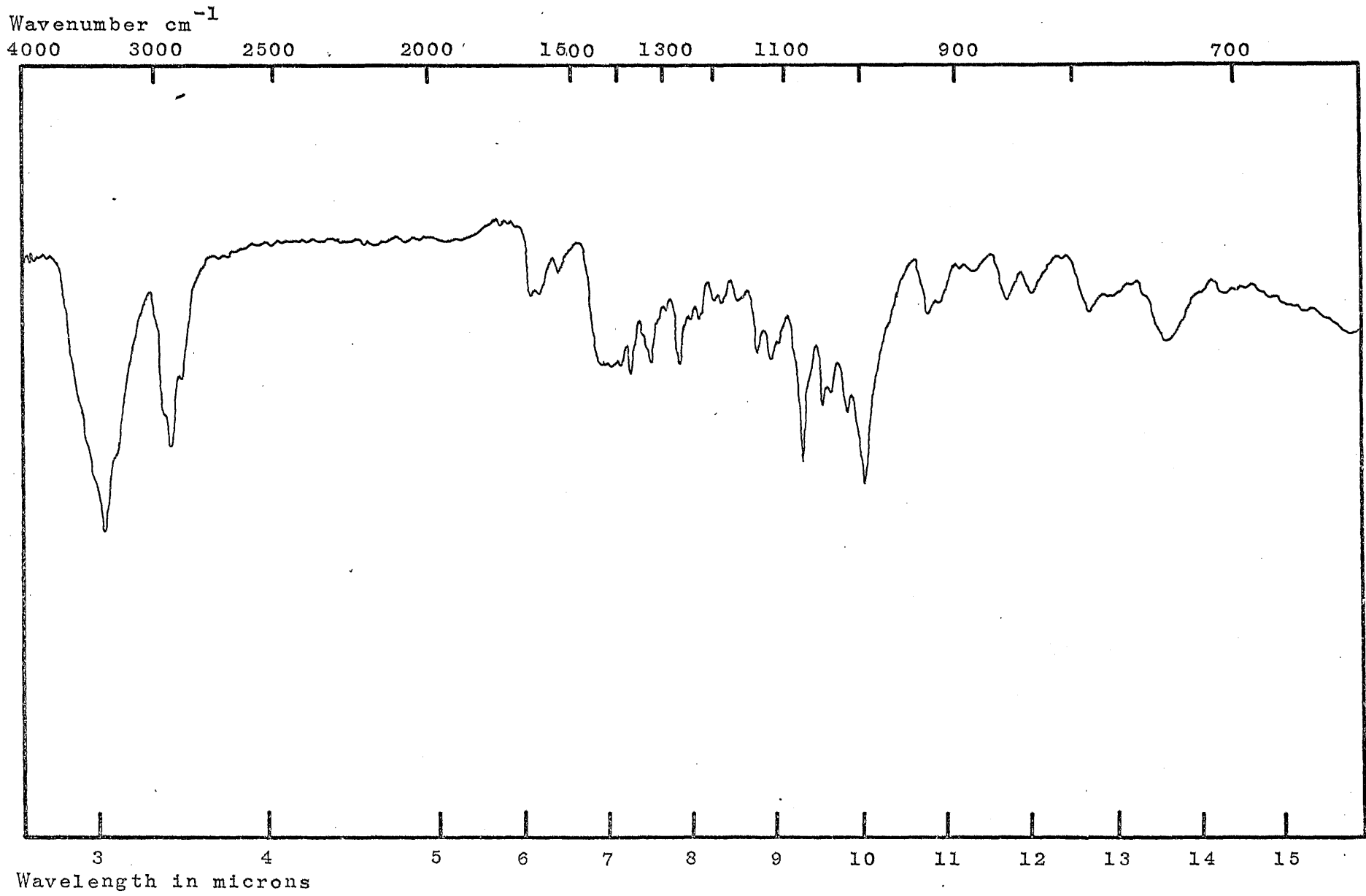


Figure 9: Infrared Spectrum of Euryopsol

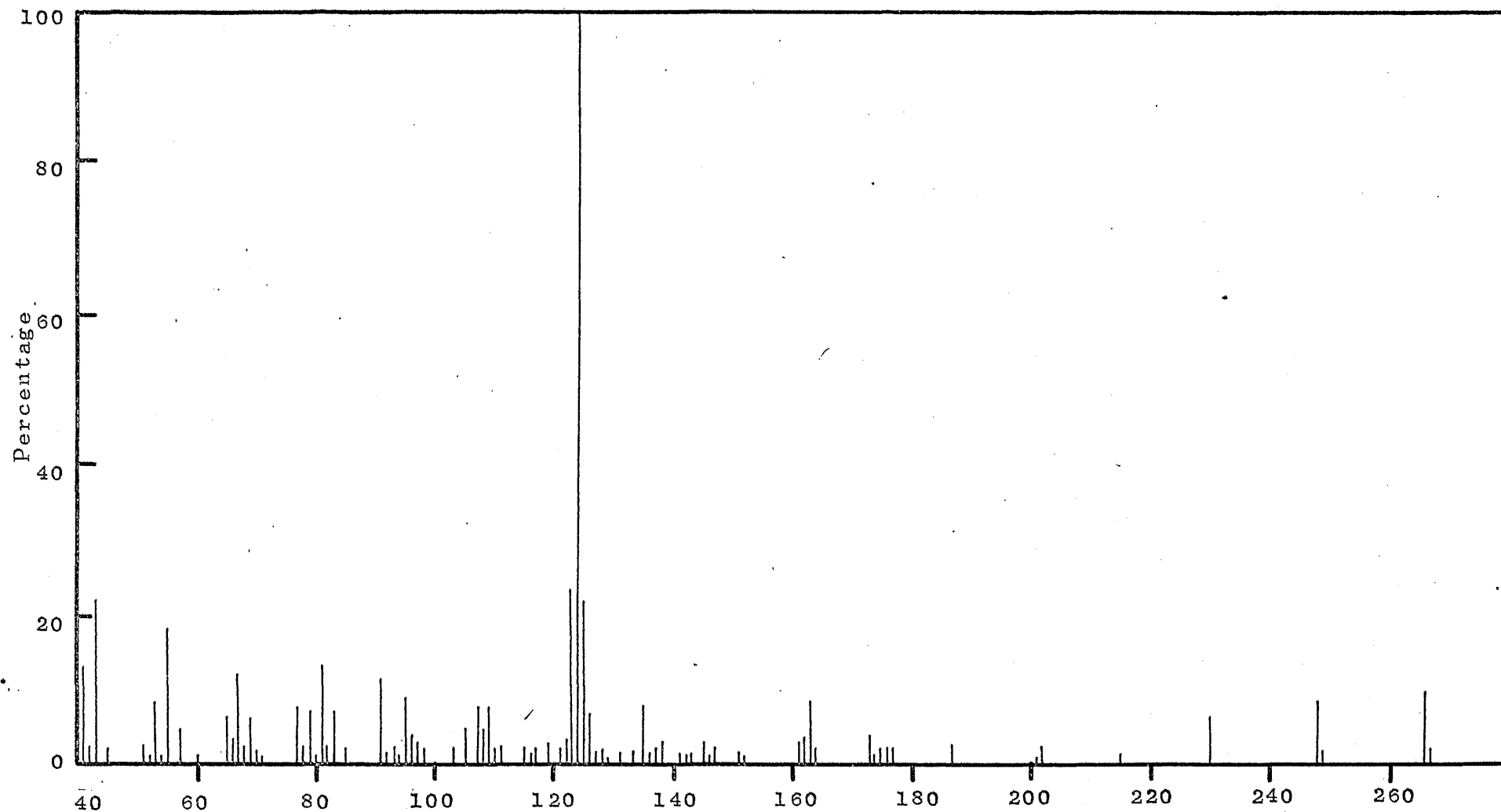


Figure 10: Mass Spectrum of Euryopsol

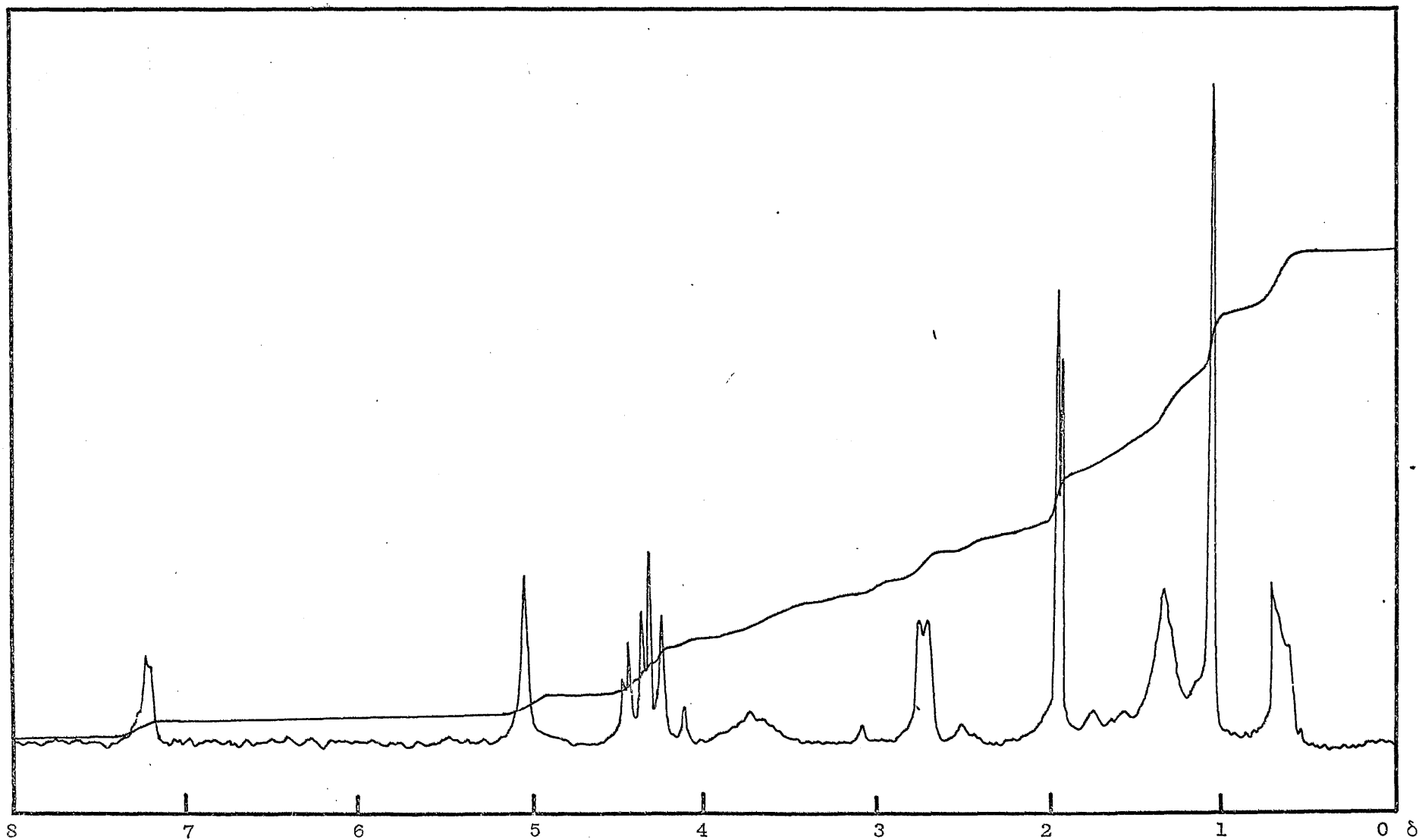


Figure 11: 60 MHz N.M.R. Spectrum of Euryopsol (DMSO-d<sub>6</sub>)

(2H, AB quartet, J 19 Hz), 3.65 (1H, multiplet), 4.18 (1H, doublet, J 8 Hz), 4.39 (1H, doublet, J 8 Hz), 4.43 (1H, doublet, J 5 Hz), 5.06 (1H, singlet) and 7.22 (1H, quartet, J 1 Hz). On addition of  $D_2O$ , the signals at 4.39, 4.43 and 5.06 disappeared, the doublet at 4.18 collapsed to a singlet and the multiplet at 3.65 sharpened slightly, showing that the molecule contains three hydroxy-groups, one of which is tertiary (signal at 5.06) and two of which are secondary (signals at 4.39 and 4.43).<sup>118</sup>

Oxidation of euryopsol with Sarett reagent<sup>30</sup> afforded a crystalline compound  $C_{15}H_{18}O_4$ , m. p. 150-152°, in poor yield. This would not form a crystalline 2,4-dinitrophenylhydrazone derivative and gave a negative Ehrlich test.<sup>46</sup> The ultraviolet spectrum contained bands at 270 ( $\epsilon$  3,150) and 208 nm ( $\epsilon$  15,200)<sup>119</sup> and the infrared spectrum contained peaks at 1672 and 1712  $cm^{-1}$ , indicating that the compound contains an  $\alpha,\beta,\gamma,\delta$ -unsaturated ketone. This is compatible with the presence of a 6-keto-group since it is known<sup>120-123</sup> that 6-oxofuranoeremophilanes have  $\lambda_{max}$  269 nm ( $\epsilon \sim 4,000$ ) and 9-oxofuranoeremophilanes have  $\lambda_{max}$  280-282 nm ( $\epsilon \sim 20,000$ ). Accordingly, euryopsol must have a secondary hydroxy-group at C-6.

Treatment of euryopsol with sodium periodate in various proportions resulted in the uptake of periodate, the amount of periodate absorbed per mole of euryopsol varying from 1.06 ( $\frac{1}{2}$  hour) to 2.20 (72 hours). The oily product, shown by t. l. c. to consist chiefly of one compound, could not be induced to crystallise or form any of the usual carbonyl derivatives, although its infrared spectrum contained carbonyl peaks at 1620 and 1720  $cm^{-1}$ .

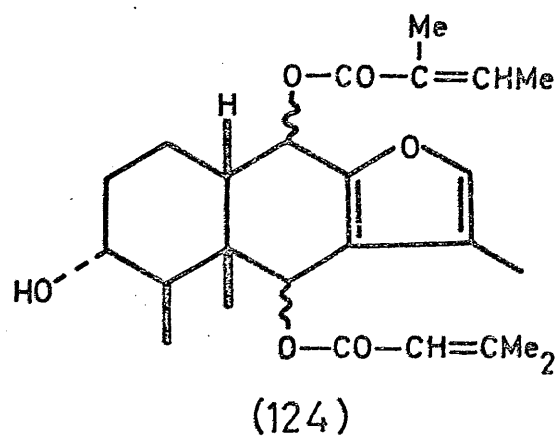
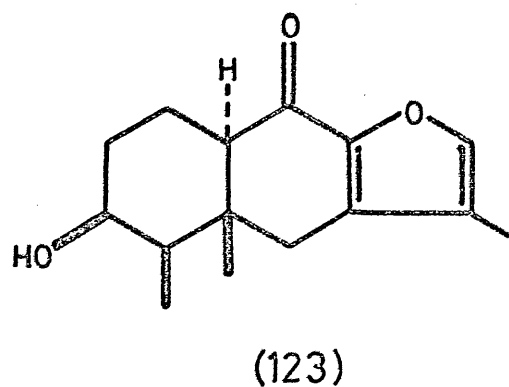
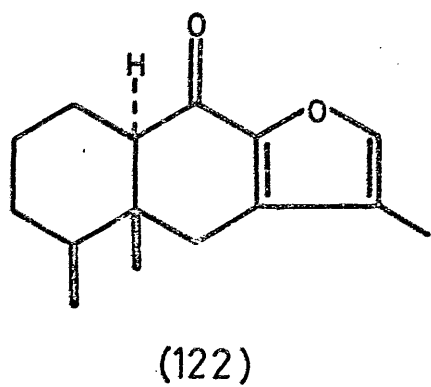
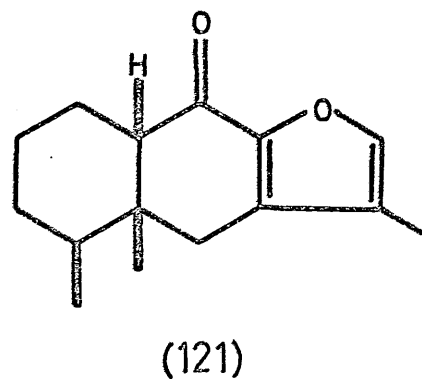
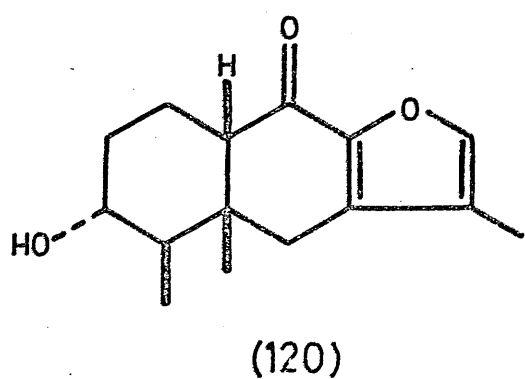
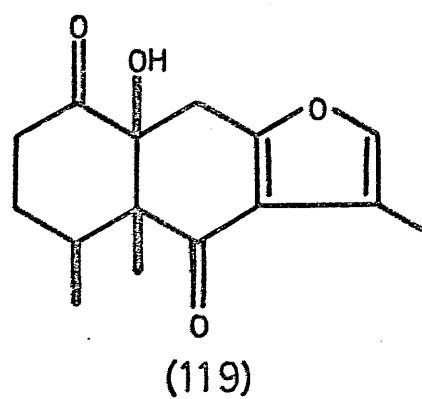
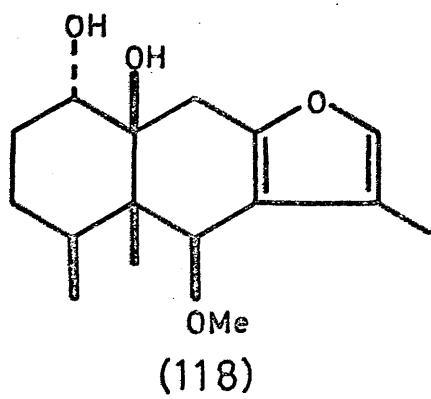
Manganese dioxide oxidation of euryopsol gave, in low yield, a gum which could not be crystallised or sublimed. T.l.c. showed it to be a mixture of two compounds in approximately equal proportions.

The structure of euryopsol (117) was deduced by Williams and Wilson from the results of deuterium exchange in the presence of alkali, coupled with extensive n. m. r. work employing solvent induced shifts. On treatment of euryopsol with methanolic hydrochloric acid, they were able to prepare the monomethyl ether (118). The product from the Sarett's oxidation of euryopsol is therefore (119). This work has been published.<sup>118</sup>

Euryopsonol,  $C_{15}H_{20}O_3$ , m. p. 230-231<sup>o</sup>, a closely related compound, has been isolated from the unsaponifiable fraction of the resin of E. floribundus.<sup>124</sup> Rivett and Woolard<sup>120</sup> proposed a cis-fused structure (120) since it was converted to 9-oxofuranoeremophilane considered<sup>125, 126</sup> to contain a cis-fused system as shown in (121). However, cis-fused ketones are readily isomerised by alkali to the thermodynamically more stable trans-fused compounds. It is, therefore, not surprising that Novotny et al.<sup>127</sup> have since shown that 9-oxofuranoeremophilane is, in fact, the trans-fused ketone (122) by preparation of authentic (121) and conversion by alkali to (122). Thus the originally proposed structure of euryopsonol must now be amended to (79).

Euryopsonol and its C-3 epimer, epieuryopsonol (123) have been compared with degradation products of kablicin (124) obtained from Petasites kablikianus.<sup>128</sup>

Since euryopsonol is isolated from the resin of E. floribundus under strongly alkaline conditions, it almost certainly occurs in the plant as the cis-fused isomer and the angelate ester. Alkali treatment



results in the removal of the angelate group and conversion to the more stable trans-form. Isolation of the cis-fused ester from the unsaponified extract of E. floribundus was therefore attempted. Thus, two samples of E. floribundus, kindly collected by Mr P. J. Steynberg on the farm "Sondagsrivierhoek" near Graaff-Reinet, were extracted as before. However, these yielded almost pure euryopsol containing less than 1% of euryopsonol, estimated according to the ultraviolet spectrum. This was surprising since previous extractions, by Woolard, of material from the same source yielded either pure euryopsonol or mixtures of euryopsonol (major fraction) and euryopsol. The reason for this variation is not known.

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