

Mass Spectrometric Studies of Diterpenes

6. Aromatic Diterpenes

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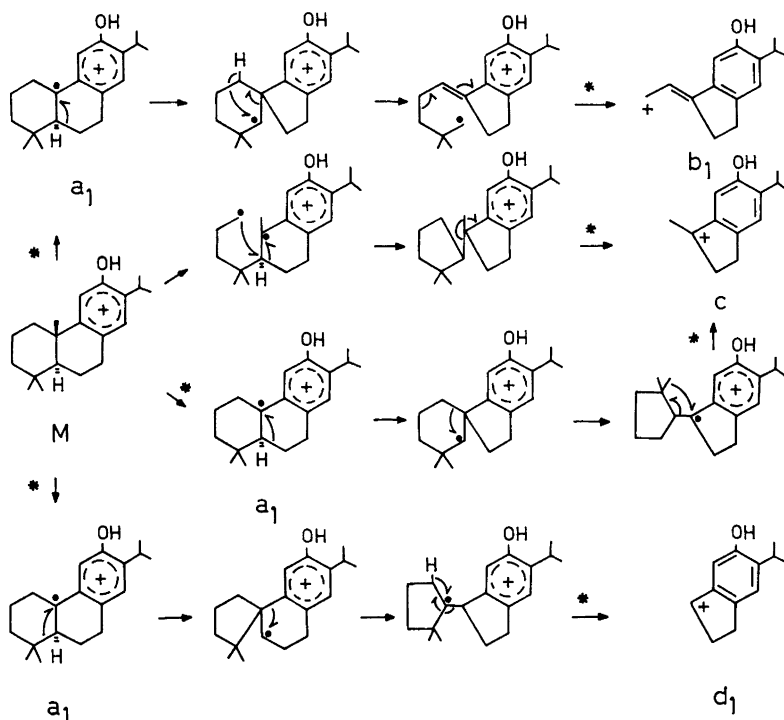
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Mass spectra of podocarpa-8,11,13-triene esters, aldehydes, and alcohols with the oxygenated substituent at C(10) differ little from those of the corresponding hydrocarbons, discussed earlier in this series since the most important intermediate, formed on extrusion of the 10-substituent, is the same. When the oxygenated group is at C(4) the mass spectrum is affected significantly only when the oxygenated group is COOCH₃. The reactions involving loss of H₂O from the alcohols, CO from the aldehydes, and CH₃OH and HCOOCH₃ from the esters, which are of wider interest, have been elucidated using deuterium labelling at 1, 2, 3, 5, 6, 7, 16, 17, and 20.

Earlier publications in this series have discussed the basic fragmentation reactions of compounds with a podocarpa-8,11,13-triene ring system^{1,2} and the effects of nuclear oxygenation in ring A.³ The present investigation extends these studies to podocarpa-8,11,13-trienes oxygenated at C(15), C(16), and C(17).^{*} The main fragmentation reactions of podocarpa-8,11,13-trienes are unusual, but the results of extensive deuterium labelling allow them to be accounted for by a novel set of closely related rearrangements.¹ The three main peaks in the central part of the spectra of podocarpa-8,11,13-triene hydrocarbons and derivatives oxygenated in the aromatic ring are mainly due to ions *b*₁, *c*, and *d*₁ as illustrated below for the case of ferruginol. Ion *a*₁, formed by elimination of the 10-substituent from the molecular ion, is the most important intermediate in the fragmentation.^{**}

* Part of the results discussed here and those dealt with in earlier publications in this series have been presented at the EUCHEM meeting in Sarlat, 8-12 September 1965.

** In the further discussion no attempt is made below to show neutral fragments or resonance forms of ions. Ions assumed to arise in the same manner are designated by the same letter irrespective of their different substitution pattern. When ions of different structures contribute to the same peak the same letter is used but the subscript is different, while when alternative structures are considered for an ion these are designated by the same letter but distinguished by one dash, two dashes, etc. Transitions for which metastable ions are observed are indicated by an asterisk in the diagrams.



Scheme 1.

Since then, work by Audier, Bory, Fetizon and Anh⁴ has shown that tricyclic diterpenes with a 8,9-double bond also give rise to three prominent peaks which are satisfactorily explained by ions formed in an analogous manner. Moreover, a rearrangement closely related to the above $a_1 \rightarrow d_1$ transformation has on the basis of a study of a large number of labelled derivatives recently been invoked by Goldsmith, Becher, Sample and Djerassi⁵ to account for the formation of the important m/e 112 ion in the fragmentation of 5α -androstan-3-one oxime. It thus seems that these rearrangements proposed initially to explain the formation of ions b_1 , c , and d_1 are of more common occurrence and utility than might at first have been expected.*

The present study is a continuation of a systematic investigation of diterpene mass spectra. It describes the main fragmentation reactions of podocarpa-8,11,13-trienes oxygenated at C(15), C(16), and C(17) based on studies of the compounds given in Table 1 and on results for the labelled derivatives shown in Tables 2–4. Recently, Audier, Bory, Defaye, Fetizon and Moreau have examined seven podocarpatrienes, oxygenated at C(15) or C(17), but only given a brief comment on their fragmentation, as no labelling or high-resolution studies were undertaken.⁷

* It is of interest that the base peak, M-179, in baurene derivatives⁶ can be accounted for by a similar process.

Table 1. Podocarpa-8,11,13-triene derivatives examined.

	R ₁	R ₂	R ₃	Aromatic substituents				A/B ring junction
				11	12	13	14	
1. Ferruginol-15-ol	CH ₂ OH	CH ₃	CH ₃		OH	iPr		<i>trans</i>
2. Ferruginol-15-al	CHO	CH ₃	CH ₃		OH	iPr		<i>trans</i>
3. Ethyl ferruginol-15-oate	COOEt	CH ₃	CH ₃		OH	iPr		<i>trans</i>
4. Carnosic acid dimethyl ether	COOH	CH ₃	CH ₃	OCH ₃	OCH ₃	iPr		<i>trans</i>
5. Deoxypodocarpol	CH ₃	CH ₂ OH	CH ₃					<i>trans</i>
6. Podocarpol	CH ₃	CH ₂ OH	CH ₃		OH			<i>trans</i>
7. 12-O-Methylpodocarpol	CH ₃	CH ₂ OH	CH ₃		OCH ₃			<i>trans</i>
8. 14-Methoxy-12-deoxy-enantio-podocarpol	CH ₃	CH ₂ OH	CH ₃				OCH ₃	<i>trans</i>
9. 4-Epidehydroabietol	CH ₃	CH ₂ OH	CH ₃			iPr		<i>trans</i>
10. 12-O-Methylferruginol-17-ol	CH ₃	CH ₂ OH	CH ₃		OCH ₃	iPr		<i>trans</i>
11. Totarol-17-ol	CH ₃	CH ₂ OH	CH ₃			OH	iPr	<i>trans</i>
12. Dehydroabietol	CH ₃	CH ₃	CH ₂ OH			iPr		<i>trans</i>
13. Deoxypodocarpal	CH ₃	CHO	CH ₃					<i>trans</i>
14. Podocarpal	CH ₃	CHO	CH ₃		OH			<i>trans</i>
15. 12-O-Methylpodocarpal	CH ₃	CHO	CH ₃		OCH ₃			<i>trans</i>
16. 14-Methoxy-12-deoxy-enantio-podocarpal	CH ₃	CHO	CH ₃				OCH ₃	<i>trans</i>
17. 4-Epidehydroabietal	CH ₃	CHO	CH ₃			iPr		<i>trans</i>
18. 12-O-Methylferruginol-17-al	CH ₃	CHO	CH ₃		OCH ₃	iPr		<i>trans</i>
19. Totarol-17-al	CH ₃	CHO	CH ₃			OH	iPr	<i>trans</i>
20. Dehydroabietal	CH ₃	CH ₃	CHO			iPr		<i>trans</i>
21. Methyl deoxypodocarpate	CH ₃	COOCH ₃	CH ₃					<i>trans</i>
22. Methyl deisopropyldehydroabietate	CH ₃	CH ₃	COOCH ₃					<i>trans</i>
23. Methyl 5β-deoxypodocarpate	CH ₃	COOCH ₃	CH ₃					<i>cis</i>
24. Methyl 5β-deisopropyldehydroabietate	CH ₃	CH ₃	COOCH ₃					<i>cis</i>
25. Methyl podocarpate	CH ₃	COOCH ₃	CH ₃		OH			<i>trans</i>
26. Methyl podocarpate acetate	CH ₃	COOCH ₃	CH ₃		OAc			<i>trans</i>
27. Methyl 12-O-methylpodocarpate	CH ₃	COOCH ₃	CH ₃				OCH ₃	<i>trans</i>
28. Methyl 14-methoxy-12-deoxy-enantio-podocarpate	CH ₃	COOCH ₃	CH ₃				OCH ₃	<i>trans</i>
29. Methyl callitrisate	CH ₃	COOCH ₃	CH ₃			iPr		<i>trans</i>
30. Methyl 12-O-methylferruginol-17-oate	CH ₃	COOCH ₃	CH ₃		OCH ₃	iPr		<i>trans</i>
31. Methyl 12-O-methyl-18,19-dehydroferruginol-17-oate	CH ₃	COOCH ₃	CH ₃		OCH ₃	isopropenyl		<i>trans</i>
32. Methyl totarol-17-oate	CH ₃	COOCH ₃	CH ₃		OH	iPr		<i>trans</i>
33. Methyl dehydroabietate	CH ₃	CH ₃	COOCH ₃			iPr		<i>trans</i>

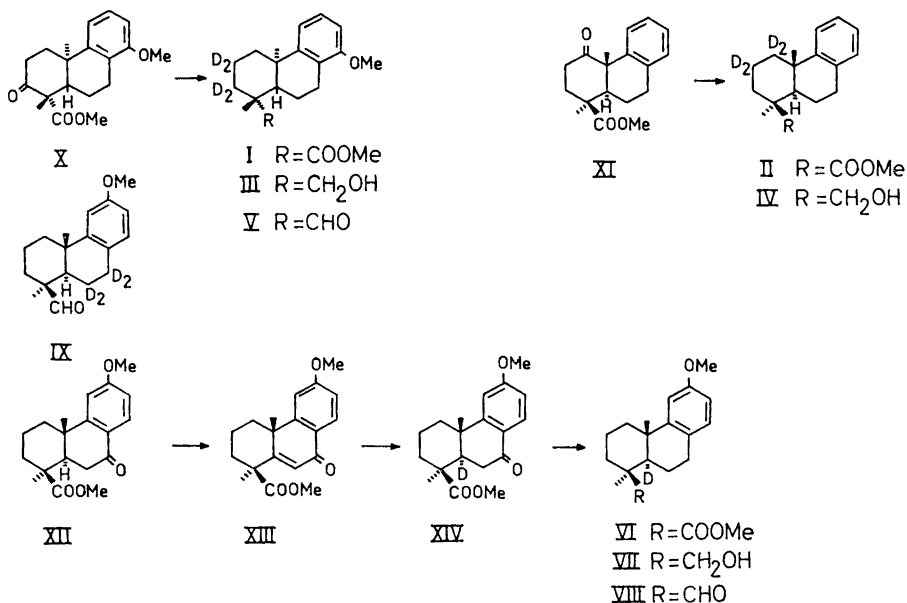
DEUTERIUM LABELLING

The deuterium labelling has, for reasons discussed below, been restricted to compounds with the oxygenated substituent attached to C(4). The synthesis of many of these compounds has been discussed elsewhere⁸ and only the preparation of the derivatives I—IX will be described here.

Clemmensen reduction of the keto esters X and XI under conditions described previously⁹ gave the corresponding 2,2,3,3-*d*₄- and 1,1,2,2-*d*₄-derivatives I and II. These on reduction with lithium tetrahydroaluminate gave the alcohols III and IV, of which III was converted to the aldehyde V by oxidation with chromic acid in acetone.

Conversion of methyl 12-*O*-methylpodocarpate to the corresponding 5 α -*d*₁-derivative VI was performed as shown below. Chromic acid oxidation of the former compound gave the ketone XII, which on treatment with selenium dioxide in acetic acid gave the corresponding Δ_5 -derivative XIII. This was reduced catalytically in a deuterium atmosphere and the product converted to the 5 α -*d*₁-ketone XIV by base-catalysed exchange. Clemmensen reduction of this intermediate furnished the desired ester VI, which was readily transformed to the alcohol VII and the aldehyde VIII as above.

The aldehyde IX was prepared from the previously synthesized 12-*O*-methyl-6,6,7,7-*d*₄-podocarpol by oxidation with chromic acid in acetone.



Scheme 2.

DISCUSSION OF SPECTRA

Substitutional differences in the aromatic ring have little influence on the main fragmentation reactions, but give rise to some variations in intensity, especially with respect to the abundance of the molecular ion and ion a_1 . Consistent with previous results in this series 12- and 14-oxygen substituents stabilise the molecular ion, while a 13-oxygen substituent stabilises ion a_1 .

Methylation or acetylation of the phenolic hydroxyl group, which simplifies the separation of the phenols and hence also the direct recording of spectra of the compounds emerging from a gas chromatograph, has little influence on the main fragmentation processes. As in the compounds studied previously methylation of the phenolic hydroxyl group leads to a shift of all peaks in the central and upper part of the spectra by 14 mass units, while acetylation of this group leaves the spectrum virtually unchanged because of the ready elimination of the acetate group as ketene.

An aromatic isopropyl substituent normally lowers the intensity of the peaks due to ions b_1 , c , and d_1 and increases the intensity of the peaks at lower mass numbers since the elimination of the isopropyl group as propene competes with other reactions. In contrast to the results obtained for the compounds studied previously, the $M-15$ peak in the case of the 16- and 17-hydroxy-derivatives and the corresponding esters is partly due to a species (a_2 , *vide infra*) formed by loss of a methyl group from the isopropyl group; in the case of the corresponding axial aldehydes the $M-15$ peak is exclusively due to this species (a_2).

The fragmentation of podocarpa-8,11,13-triene hydrocarbons and phenols was discussed on the basis of the assumption that the charge was localized to the aromatic moiety of the molecular ion and that subsequent reactions, initiated by α -cleavage (for nomenclature, *cf.* Ref. 10), were triggered by the locus of electron deficiency. Support for this view comes from a very recent study which shows that fragment ions with an even number of electrons do not undergo quasi photochemical or quasi pyrolytic eliminations even when the energy requirements for these are quite low.¹¹

In the present series of compounds alternative sites of the charge become possible due to the presence of an oxygenated substituent at C(4) or C(10). However, for reasons detailed below these are evidently less important triggers of fragmentation.

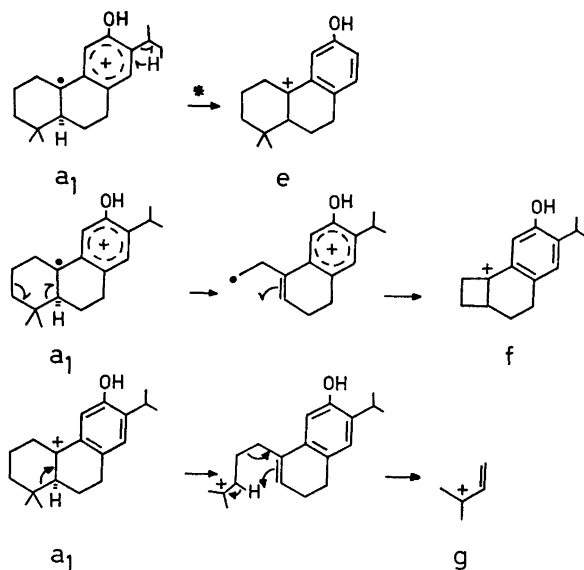
The introduction of an oxygen function at C(16) or C(17) has a greater influence on the fragmentation than has oxidation of C(15). The latter compounds will therefore be discussed together below, while the former are more conveniently dealt with as three separate groups divided according to the stage of oxidation.

Differences in the spectra within each group due to differences in the aromatic substitution follow a regular pattern, as discussed above. Only two representative spectra from each group, in all cases of abietane derivatives, are therefore given in full.

Compounds oxygenated at C(15). Ion a_1 has previously been shown^{1,2} to be the most important intermediate in the fragmentation of ferruginol and the ions formed *via* this intermediate contribute to all significant peaks

except the M-43 peak (the corresponding ion is formed from the molecular ion by elimination of a C(1)-C(3) fragment and the 5 α -hydrogen). Since oxygenation of C(15) would facilitate the elimination of the 10-substituent and increase the importance of ion a_1 the 15-oxygenated ferruginol derivatives 1-3 would be expected to give spectra which are nearly identical with that of ferruginol except for differences with respect to the M and M-43 peaks and minor intensity variations. This is indeed the case (*cf.* Figs. 1-2) and the abundant species may hence be formulated as ions a_1 , b_1 , c , d_1 , e , f , and g depicted in schemes 1 and 3.

Carnosic acid dimethyl ether (4), which contains further oxygen substituents in the aromatic ring and represents the only other 15-oxygenated derivative examined by us, exhibits essentially the same fragmentation pattern. Audier *et al.*⁷ on examining carnosic acid methyl ester diacetate and the corresponding 7-oxo derivative found that these compounds give a very strong M-42-42-60 peak. Although the loss of the C-10 substituent together with hydrogen occurs to some extent in the ester 3 and the acid 4 but not in the 15-hydroxy- and 15-oxo-derivatives, the reaction is of minor importance in these compounds and the intensity of the corresponding peak is only about 30% of that of the ion a peak.



Scheme 3.

Alcohols. The introduction of a hydroxyl group at C(16) or C(17) opens some new fragmentation routes, but affects the main reactions comparatively little, noticeably less than hydroxylation at C(3). The three indanyl ions b_1 , c , and d_1 remain the main contributors to the three prominent peaks in the

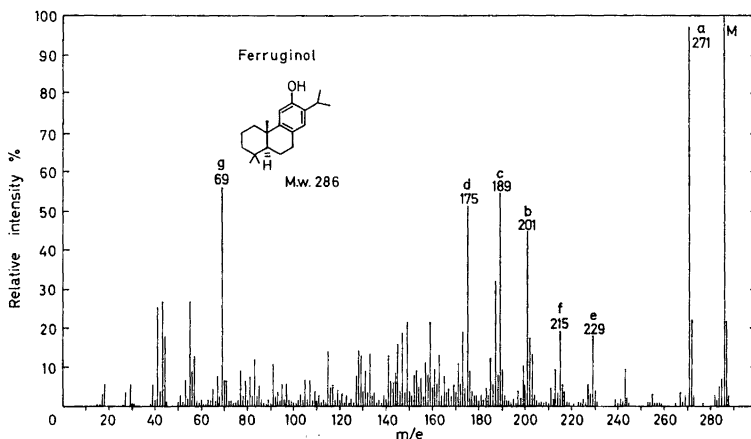


Fig. 1. Ferruginol.

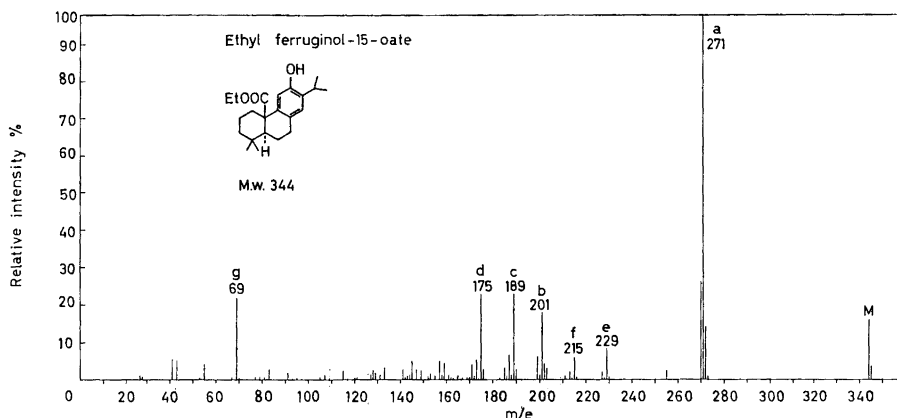


Fig. 2. Ethyl ferruginol-15-oate.

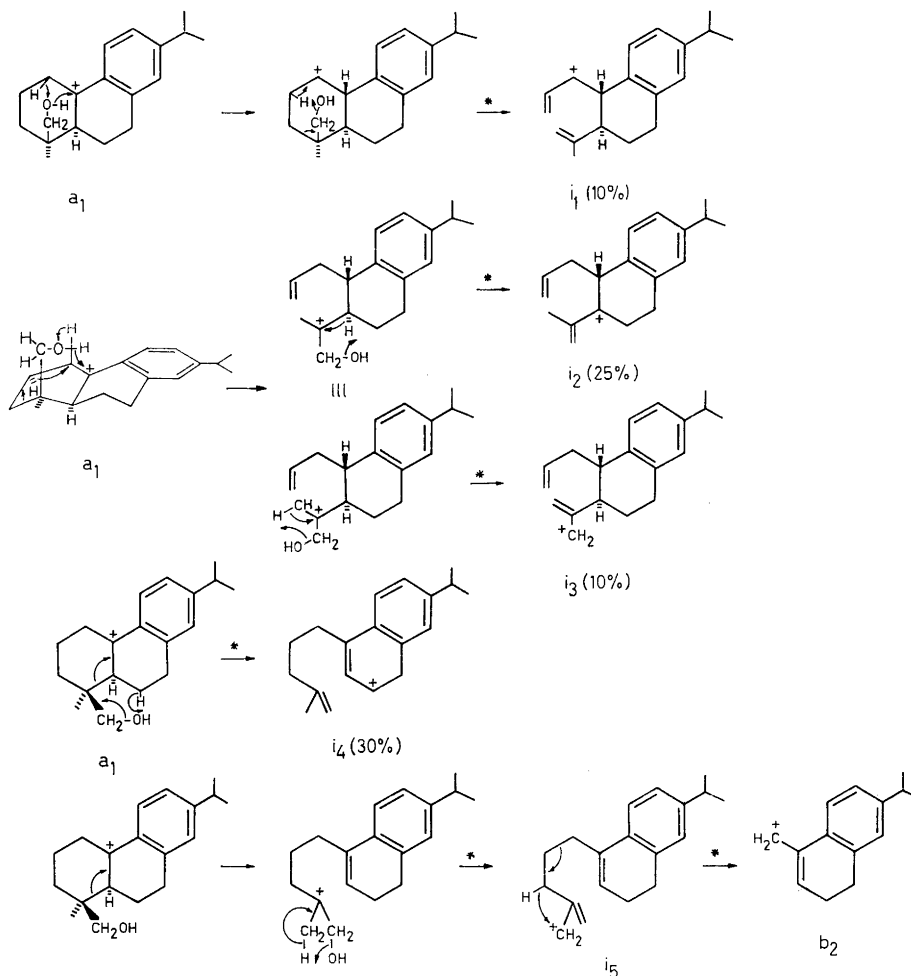
central part of the spectra, although their importance, especially that of b_1 , is somewhat reduced, as shown by the results given in Table 2.

The most important reaction associated with the presence of the hydroxyl group is the dehydration of ion a_1 giving M-33 species. The labelling results show that this reaction is quite complex, although for reasons mentioned above it should be triggered by the charge carrying moiety only.

The hydroxyl hydrogen is partly retained by the larger fragment on dehydration of ion a_1 ; 50 % when the carbinol group is axial and 15 % when it is equatorial. Similar retention (15 %) has been observed in the dehydration of the cyclohexanol molecular ion and here accounted for as a transfer of the hydroxyl hydrogen to the 2-position subsequent to the cleavage of the 1,2-

bond.¹² As this reaction in the present case would lead to the elimination of the carbinol group the transfer must here occur by some other route. Since direct abstraction of the hydroxyl hydrogen giving the $-\text{CH}_2\text{O}^+$ grouping would require even more energy than the formation of a primary, non-conjugated carbonium ion it is improbable. The transfer therefore is more likely to occur either by concerted shift of the hydroxyl hydrogen to the positively charged center and an acidic hydrogen on a vicinal carbon to the hydroxyl oxygen *via* a five-membered cyclic transition state or by an equilibration process involving protonation of the hydroxyl group by an acidic hydrogen on a carbon next to the charged center followed by protonation of the newly formed carbon-carbon double bond by the protonated hydroxyl group. The steric requirements of the concerted process should be quite strict, but, judging from Dreiding models, should be fairly readily satisfied in ion a_1 in view of the flexibility of rings A and B. As regards the stability and lifetime of a protonated hydroxyl group, it has most recently been shown that protonated ethanol (m/e 47) is an important species in the fragmentation of ethyl hexyl ether and is formed by transfer of two hydrogens from the hexyl unit to the oxygen and cleavage of the bond between the oxygen and the larger substituent.¹³ Although a strict choice between these alternatives cannot be made on the basis of the present evidence only the cyclic process will be considered below.

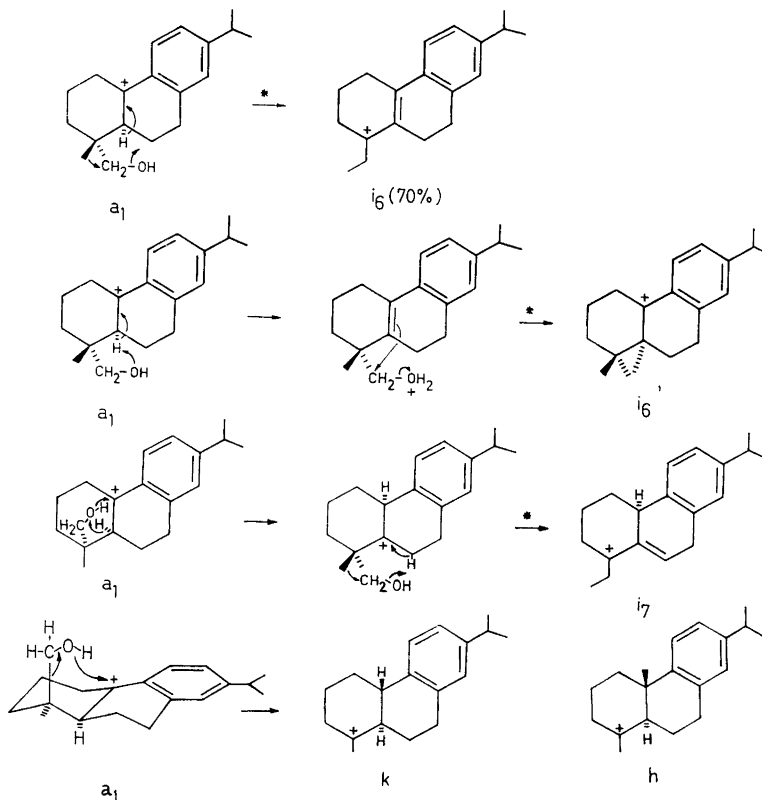
It is now worth considering possible structures for the M-33 species. When the carbinol group is axial the dehydration occurs in a less specific manner and deuterium is lost from positions 1 (~45 %), 2 (~10 %), 5 (~25 %) and 6 (~30 %), while the hydroxyl hydrogen is retained to the extent of 50 %. These results, on the premises discussed above, may be accounted for as outlined in scheme 4. The dominating reaction involves the transfer of the hydroxyl hydrogen to C(10) and simultaneous abstraction of the 1β -hydrogen, which when accompanied by a concurrent, sterically favoured shift of the 2α -hydrogen to C(1) and cleavage of the 3,4-bond gives a rearranged intermediate and when followed by transfer of the 2β -hydrogen to the hydroxyl oxygen with concurrent cleavage of the 3,4-bond and elimination of water gives ion i_1 . The intermediate on loss of water gives ion i_2 when the 5α -hydrogen is utilised, and ion i_3 when C(16) is the source of hydrogen. The fraction of the M-33 ions formed by loss of a ring B hydrogen is accounted for by ion i_4 . Although these ions account for all of the observed loss of deuterium on carbon and about 90 % of the deuterium retained they do not explain more than 75 % of the M-33 ion current. Since this discrepancy can hardly be ascribed to an isotope effect, as it would require both discrimination against deuterium on carbon and against hydrogen on oxygen, a further species formed by elimination of a 1δ -hydrogen must be taken into account. This can be formulated as ion i_5 . In agreement with observed metastable ions and shifts of the M-101 peak ion i_5 undergoes expected further fragmentation to give ion b_2 .



Scheme 4.

In the case of the equatorial epimer the label is preferentially lost from C(5) (70 %) and to a minor extent (15 %) from ring B, while the hydroxyl hydrogen here is retained to the extent of 15 %. The main species can therefore be formulated as the well stabilized ion i_6 when invoking methyl migration, or as ion i_6' , when invoking stabilisation by bond formation as previously postulated in the dehydration of cyclohexanol and 5α -cholestan- 3α -ol.¹² The minor product formed with loss of a ring B hydrogen may be accounted for, as in the case of the axial epimer, by ion i_4 . The retention of the hydroxyl hydrogen by the larger fragment can be explained by either of the two routes $a_1 \rightarrow i_1$ and $a_1 \rightarrow i_7$, which have in common the transfer of the hydroxyl hydrogen to C(10) but differ with respect to the sources of the two hydrogens lost as

water. Although these alternatives can not be distinguished on the basis of the present evidence it is worth mentioning that ions i_6 (i_6'), i_4 , and i_1 quantitatively account for the M—33 ion current, while ions i_6 (i_6') and i_7 only account for 70 % implying that ion i_5 may be formed to about the same extent as in the case of the axial epimer.



Of considerable interest in this context is the occurrence of the moderately intense M—45 peak in the spectra of the axial compounds since cleavage of the 4,17-bond would be expected to occur in competition with the shift of the 1β -hydrogen to the hydroxyl oxygen on transfer of the hydroxyl hydrogen to C(10). Consistent with expectation the M—45 peak is almost completely shifted (90 %) on replacement of the hydroxyl hydrogen by deuterium and its position essentially unaffected (90 %) on labelling of C(17). The corresponding species can therefore be formulated as ion k formed from ion α_1 by transfer of the hydroxyl hydrogen to C(10) accompanied by cleavage of the 4,17-bond and elimination of formaldehyde.

A further reaction due to the presence of the hydroxyl group is the loss of the carbinol group from the molecular ion with formation of $M-31$ species. This diagnostically valuable, but less favoured reaction can be depicted mainly as simple cleavage of the bond between the carbinol group and C(4), resulting from charge localisation to the carbinol oxygen and the product formulated as ion h for both the axial and equatorial isomers.

A discussion of the peaks occurring in the region between $M-45$ and $M-101$ will be omitted as these are of little diagnostic value. However, it should be mentioned that in the compounds with an aromatic isopropyl substituent there is a pronounced increase in the intensity of the $M-75$ peak, which is then the strongest peak in this region. As shown by metastable ions and lack of shift in the spectrum of the 20- d_1 -derivative this is due to ions (l_1-l_7), formed by elimination of the isopropyl group as propene from the $M-33$ species.

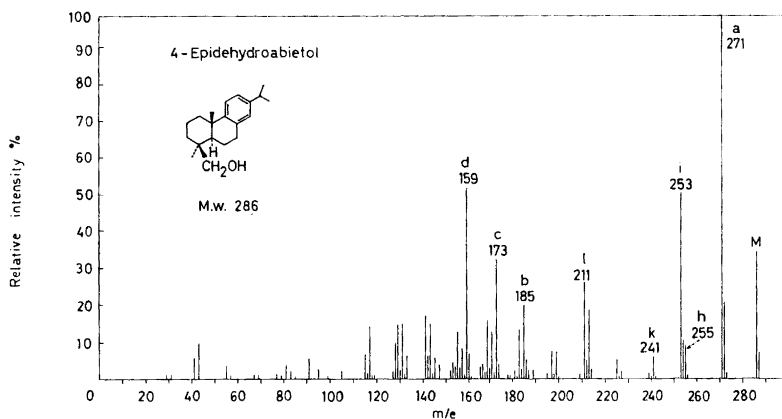


Fig. 3. 4-Epidehydroabietol.

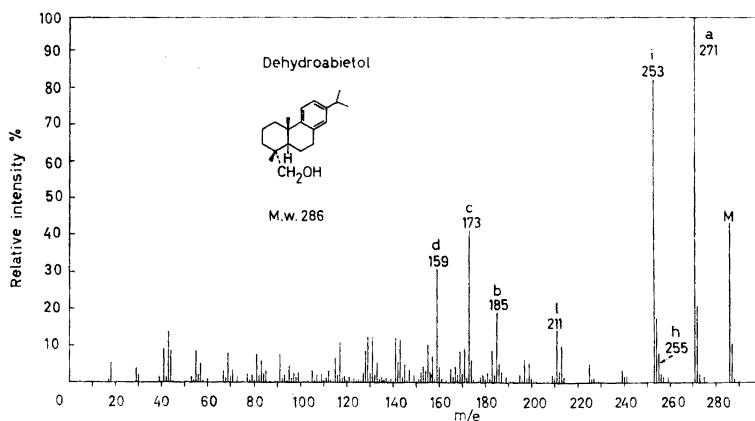


Fig. 4. Dehydroabietol.

Table 2. Shifts of the important peaks (m/e) in the mass spectra of deuterated podocarpa-8,11,13-triene-16- and -17-ol derivatives and corresponding non-deuterated compounds.

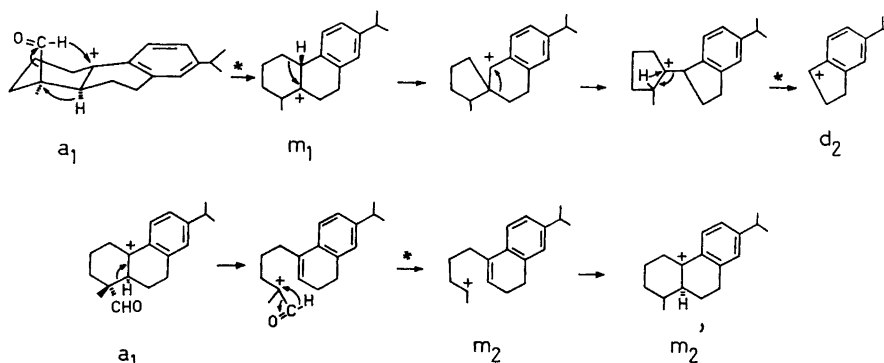
(Reported shifts are corrected for isotopic inhomogeneity and natural occurrence of ^{13}C)

	M-15 a (%)	M-31 h (%)	M-33 i (%)	M-45 k (%)	M-101 b (%)	M-113 c (%)	M-127 d (%)
Deoxypodocarpol	229	213	211	199	143	131	117
1,1,2,2- d_4 -Deoxypodocarpol	233 (100)	217 (100)	213 (10) 214 (35) 215 (55)	203 (70)	145 (30) 146 (35)	131 (90)	117 (70)
12- <i>O</i> -Methylpodocarpol	259	243	241	229	173	161	147
12- <i>O</i> -Methyl-5- d_1 -podocarpol	260 (100)	244 (90)	242 (75)	230 (95)	173 (60)	161 (75)	148 (50)
12- <i>O</i> -Methyl-6,6,7,7- d_4 -podocarpol	263 (90)	247 (100)	244 (30) 245 (60)	233 (100)	177 (70)	165 (75)	151 (70)
12- <i>O</i> -Methyl-17,17- d_2 -podocarpol	261 (100)	243 (100)	243 (90)	229 (70)	173 (80)	161 (85)	147 (90)
17- <i>O</i> - d_1 -12- <i>O</i> -Methylpodocarpol	260 (100)	243 (80)	241 (50)	230 (90)	173 (70)	161 (80)	147 (75)
14-Methoxy-12-deoxy- <i>enanti</i> -podocarpol	259	243	241	229	173	161	147
14-Methoxy-12-deoxy-2,3,3- d_4 - <i>enanti</i> -podocarpol	263 (100)	247 (100)	244 (10) 245 (90)	233 (90)	175 (50)	161 (90)	147 (75)
12- <i>O</i> -Methylferruginol-17-ol	301	285	283	271	215	203	189
12- <i>O</i> -Methyl-20- d_1 -ferruginol-17-ol	302 (75)	286 (70)	284 (90)	272 (90)	216 (80)	204 (90)	190 (100)
Totarol-17-ol	287	271	269	257	201	189	175
17,17- d_2 -Totarol-17-ol	289 (100)	271 (100)	271 (90)	257 (90)	201 (100)	189 (90)	175 (100)
Dehydroabietol	271	255	253	233	185	173	159
5- d_1 -Dehydroabietol	272 (100)	256 (100)	253 (70)	235 (50)	185 (50)	173 (70)	160 (60)
6,6,7,7- d_4 -Dehydroabietol	275 (100)	259 (100)	256 (15) 257 (85)	235 (15) 236 (85)	189 (75)	177 (80)	163 (80)
16,16- d_2 -Dehydroabietol	273 (100)	255 (100)	255 (100)	235 (100)	185 (100)	173 (90)	159 (90)
16- <i>O</i> - d_1 -Dehydroabietol	272 (100)	255 (100)	253 (85)	235 (85)	185 (80)	173 (90)	159 (70)

The spectra of the compounds with *A/B-trans* configuration epimeric at C(4) differ comparatively little in appearance and although these differences would serve to distinguish between epimers when run under identical conditions, they would not serve the purpose of configurational assignments. However, the large difference in retention of the label by the $M-33$ species (50 % axial $-CH_2OH$, 15 % equatorial $-CH_2OH$) on replacement of the hydroxyl hydrogen by deuterium offers a fast and convenient way of solving the latter problem; the only additional operation required is the addition of a drop of *O*-deuterio methanol to the sample in the direct inlet probe prior to the recording of the spectrum.

Aldehydes. The main effect of replacing one of the geminal methyl groups by an aldehyde group is that ion a_1 now undergoes facile loss of carbon monoxide (confirmed by accurate mass measurement), a reaction which significantly alters the appearance of the upper part of the spectrum. It leads to the occurrence of a prominent $M-43$ peak and in the spectra of the axial aldehydes to a considerable reduction in the intensity of the peak due to ion a_1 ; the prominent $M-15$ peak observed in the spectra of the axial aldehydes carrying an aromatic isopropyl substituent, as shown by the results obtained on labelling of C(20), is due almost exclusively to ion a_2 formed by elimination of a methyl radical from the isopropyl group. In the spectrum of the equatorial aldehyde, the $M-15$ peak is probably due both to ion a_1 and a_2 .

Although the $M-43$ species according to metastable ions are formed from ion a_1 both when the aldehyde group is axial and equatorial it is evident that the products are different as metastable ions, implying subsequent fragmentation, are only observed for the axial isomer. The product of this reaction is a $M-125$ species. The $M-125$ peak is almost completely shifted (90–100 %) on labelling of C(6), C(7), and C(20), but its position essentially unaffected (80–90 %) on labelling of C(1), C(2), and C(3), while partial shifts are observed on labelling of C(5) (40 %) and C(17) (50 %). These results suggest that the $M-125$ ion current is partly due to ion d_1 formed in the normal fashion from ion a_1 with retention of the 5α -hydrogen (40 %), and partly to a species formed from the $M-43$ intermediate with retention of the 17-hydrogen (50 %). The latter route, explaining the increased intensity of the $M-125$ peak, can



Scheme 6.

be depicted as shown above. Transfer of the aldehydic hydrogen to C(10) with concurrent shift of the 5α -hydrogen to C(4) and loss of carbon monoxide gives the M-43 intermediate ion m_1 , which on further fragmentation gives ion d_2 now with retention of the aldehydic hydrogen.

In the case of the equatorial aldehyde the larger distance between the aldehydic hydrogen and C(10) in ion a_1 makes a direct transfer less likely and the formation of the M-43 species may here be depicted as occurring by cleavage of the 4,5-bond and a shift of the aldehydic hydrogen to C(4) with elimination of carbon monoxide to give ion m_2 or the corresponding cyclised product ion m_2' . This is consistent with the absence of significant retention of the 16-hydrogen by any of the abundant ions of lower mass number.

Other peaks in the upper part of the spectrum are of low intensity. This is also true for the diagnostically valuable M-29 peak, which as in the case of the corresponding alcohols can be ascribed mainly to ion h .

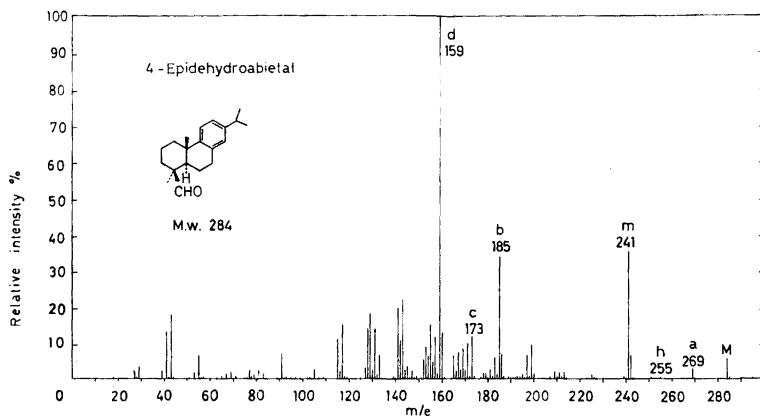


Fig. 5. 4-Epidehydroabietal.

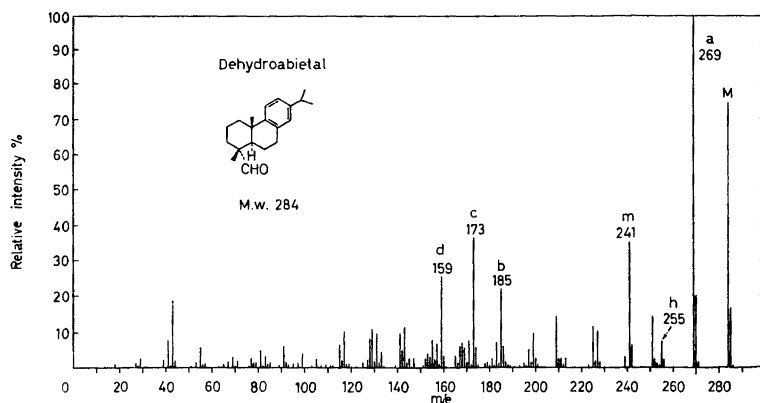


Fig. 6. Dehydroabietal.

Table 3. Shifts of the important peaks (*m/e*) in the mass spectra of deuterated podocarpa-8,11,13-triene-16- and 17-ol derivatives and corresponding non-deuterated compounds. (Reported shifts are corrected for isotopic inhomogeneity and natural occurrence of ¹³C).

	M-15 a (%)	M-29 h (%)	M-43 m (%)	M-99 b (%)	M-111 c (%)	M-125 d (%)
12-O-Methylpodocarpal	257	243	229	173	161	147
12-O-Methyl-5- <i>d</i> ₁ -podocarpal	258 (100)	244 (100)	230 (100)	173 (60)	161 (90)	147 (60)
12-O-Methyl-6,6,7,7- <i>d</i> ₄ -podocarpal	261 (100)	247 (70)	233 (95)	177 (70)	165 (80)	151 (90)
12-O-Methyl-17- <i>d</i> ₁ -podocarpal	258 (90)	243 (100)	230 (100)	173 (80)	161 (80)	148 (50)
14-Methoxy-12-deoxy-enantio-podocarpal	257	243	229	173	161	147
14-Methoxy-12-deoxy-2,2,3,3- <i>d</i> ₄ -enantio-podocarpal	261 (100)	247 (100)	233 (100)	175 (50)	161 (90)	147 (90)
12-O-Methylferruginol-17-ol	299	285	271	215	203	189
12-O-Methyl-20- <i>d</i> ₁ -ferruginol-17-ol	300 (50)	286 (50)	272 (100)	216 (90)	204 (90)	190 (100)
Dehydroabietal	269	255	241	185	173	159
5- <i>d</i> ₁ -Dehydroabietal	270 (100)	256 (70)	242 (100)	185 (60)	173 (70)	160 (65)
6,6,7,7- <i>d</i> ₄ -Dehydroabietal	273 (100)	259 (100)	245 (100)	189 (80)	177 (100)	163 (95)
16- <i>d</i> ₁ -Dehydroabietal	270 (100)	255 (80)	242 (100)	185 (90)	173 (95)	159 (80)

Table 4. Shifts of the important peaks (*m/e*) in the mass spectra of deuterated methyl podocarpa-8,11,13-triene-16- and -17-olate derivatives and corresponding non-deuterated compounds. (Reported shifts are corrected for isotopic inhomogeneity and natural occurrence of ¹³C).

	M-15 a (%)	M-47 n (%)	M-59 h (%)	M-75 o (%)	M-129 b (%)	M-131 q (%)	M-141 c (%)	M-155 d (%)
Methyl deoxypodocarpate	257	225	213	197	143	141	131	117
Methyl 1,1,2,2- <i>d</i> ₄ -deoxypodocarpate	261 (100)	228 (40)	217 (100)	200 (60)	145 (50)	143 (80)	131 (70)	117 (60)
Methyl 12-O-methyl-podocarpate	287	255	243	227	173	171	161	147
Methyl 12-O-methyl-5- <i>d</i> ₁ -podocarpate	288 (95)	256 (100)	244 (95)	228 (95)	174 (55)	172 (60)	161 (85)	148 (60)
Methyl 12-O-methyl-6,6,7,7- <i>d</i> ₄ -podocarpate	291 (100)	258 (60)	247 (80)	230 (10)	177 (60)	173 (60)	165 (60)	151 (70)
Methyl 12-O-methyl-7- <i>d</i> ₁ -podocarpate	288 (100)	256 (100)	244 (100)	228 (100)	174 (100)	172 (80)	162 (80)	148 (90)
Methyl 14-methoxy-12-deoxy-enantio-podocarpate	287	255	243	227	173	171	161	147
Methyl 14-methoxy-12-deoxy-2,2,3,3- <i>d</i> ₄ -enantio-podocarpate	291 (100)	259 (100)	247 (100)	231 (100)	173 (50)	171 (50)	161 (100)	147 (80)
Methyl 12-O-methylferruginol-17-olate	329	297	285	269	215	213	203	189
Methyl 12-O-methyl-20- <i>d</i> ₁ -ferruginol-17-olate	330 (75)	298 (100)	286 (95)	270 (100)	216 (100)	214 (100)	204 (100)	190 (90)
Methyl dehydroabietate	299	267	255	239	185	183	173	159
Methyl 5- <i>d</i> ₁ -dehydroabietate	300 (100)	268 (50)	256 (100)	240 (50)	186 (70)	184 (100)	173 (60)	160 (90)
Methyl 6,6,7,7- <i>d</i> ₄ -dehydroabietate	303 (100)	271 (100)	259 (100)	243 (100)	189 (90)	185 (90)	177 (100)	163 (100)

In the central part of the spectra there are three prominent peaks, M-99, M-111, and M-125, the intensities of which in the equatorial aldehyde are close to those observed for the corresponding hydrocarbon. In the axial aldehydes however, the intensity of the M-125 peak is increased and when the compound carries an aromatic isopropyl substituent that of the M-111 peak reduced. The main contributors to these peaks can on the basis of the available evidence (Table 3) again be ascribed to the indanyl ions b_1 , c , and d . It is clear however, that the contribution by ion b_1 to the M-99 peak is reduced and as discussed above that ion d , when the aldehyde group is axial, is partly formed by a new route.

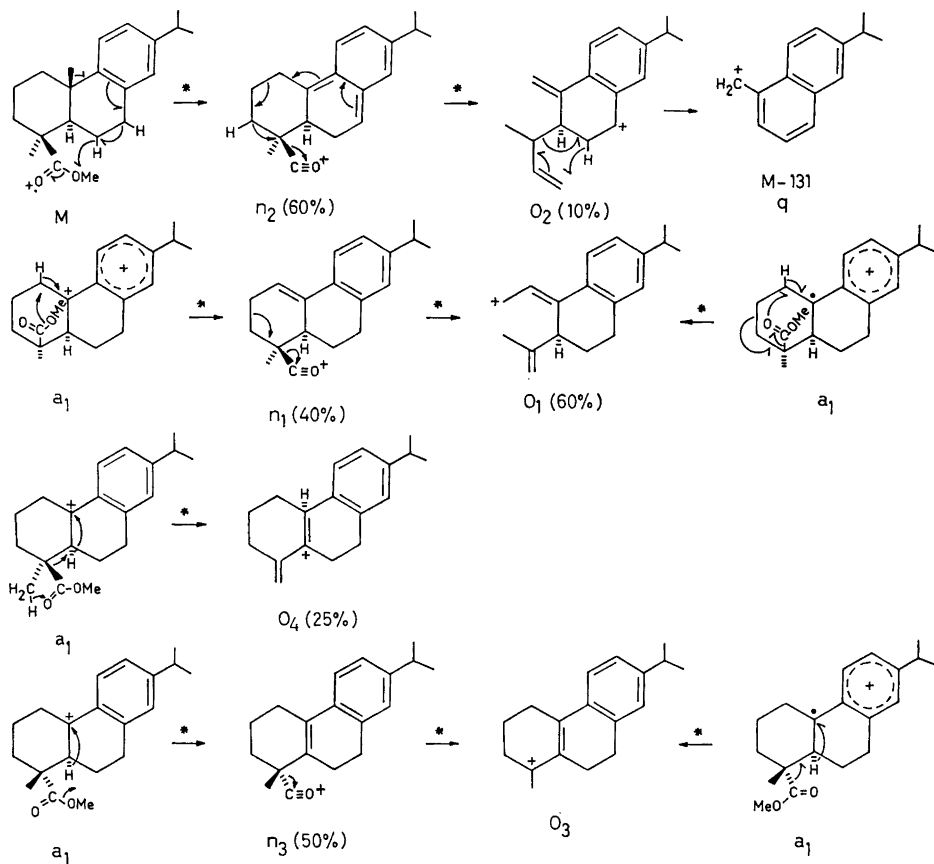
Methyl esters. Substitution of one of the geminal methyl groups by a carbomethoxy group influences the fragmentation pattern to a noticeably larger extent than the corresponding substitution by a carbinol or aldehyde group. The presence of this group causes the appearance of a very strong M-75 peak and significant, but less intense M-47 and M-59 peaks; the peaks in the central and lower part of the spectra are of reduced intensity.

The M-59 species can be ascribed mainly to ion h , formed from the molecular ion by extrusion of the carbomethoxy group when this is ionised. The formation of the M-47 ($\text{CH}_3 + \text{CH}_3\text{OH}$) and M-75 ($\text{CH}_3 + \text{HCOOCH}_3$) species is more involved and clearly dependent on the orientation of the ester group (*cf.* Scheme 7). In view of this dependence, which in the A/B-*trans* series is evident from the labelling results and in the A/B-*cis* series (compounds 23 and 24) from the difference in the fragmentation pattern, it may be concluded that these elimination reactions occur mainly without prior cleavage of a skeletal bond. The obvious reason why the 4α -epimer of the A/B-*cis* series in contrast to the 4β -epimer and the A/B-*trans* fused compounds, should give rise to prominent M-32 and M-60 peaks, while in other respects be very similar, is that the oxygens of the ester group can most readily get within bond forming distance of the benzylic 7α -hydrogen.

In the case of the axial esters the formation of the M-47 species involves loss of a hydrogen derived partly (60%) from ring B and partly (40%) from C(1). According to metastable ions these species originate both from the molecular ion and ion a_1 and undergo subsequent loss of carbon monoxide to give M-75 ions, which in turn eliminate a C_4H_8 fragment, derived from C(2)-C(4) and a ring B hydrogen, to give the M-131 species. The M-47 species can therefore be formulated as ions n_1 and n_2 , accounting for, respectively, 40 and 60 % of the M-47 ion current.* The M-75 species formed via these ions as intermediates can be depicted as ions o_1 and o_2 , of which the former accounting for 60 % of the M-75 ion current evidently also originates from ion a_1 . Ion o_2 , intermediate in the formation of the M-131 species and formulated as the highly stabilized ion q only accounts for about 10 % of the M-75 ion current. This leaves about 30 % of the M-75 ion current unaccounted for provided that, as in the case of the M-47 species, there is

* It has been shown for 6-phenylhexanoic acid methyl ester with the aid of deuterium labelling that the elimination of MeOH and HCOOMe do not occur in the fashion typical of fatty acid methyl esters but involve a benzylic hydrogen quite specifically which was assigned to the activating effect of the aromatic group.¹⁴ The charge in these reactions was assumed to be located on the ester group, but as shown by the above results this is not necessary.

no isotope effect. In view of that C(16) in ion a_1 is the only remaining possible source of hydrogen the corresponding species may be formulated as ion o_4 .

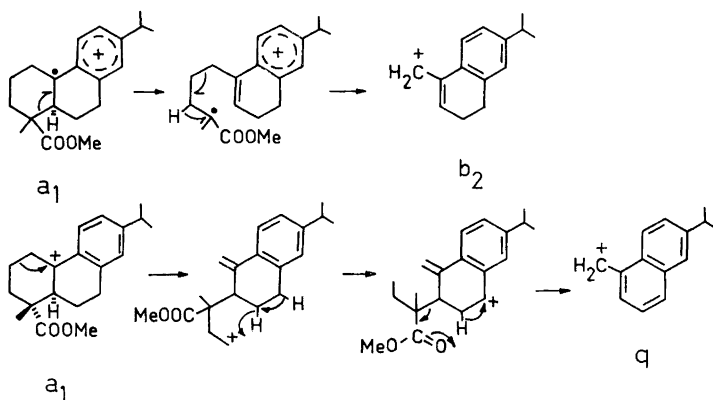


Scheme 7.

In the case of the equatorial esters the formation of the M-47 and M-75 species involves mainly (50 %) the 5α -hydrogen and the corresponding charged fragments can be formulated as ions n_3 and o_3 . The latter according to metastable ions is formed both from ion a_1 and ion n_3 . In view of the results obtained for the axial esters it is highly probable that the larger part of the remaining contribution to the M-47 and M-75 peaks are due to ions n_1 and o_1 , respectively. This is consistent with the 1α -hydrogen and the oxygens of the α -oriented carbomethoxy group being readily able to come within bond-forming distance of one another in ion a_1 and with complete retention of the 6- and 7-hydrogens.

The significant M-141 and M-155 peaks in the central part of the spectra can on the basis of the labelling results be ascribed mainly to the indanyl

ions c and d_1 for both the equatorial and axial esters and this is born out by observed metastable ions which imply that the former originates from the molecular ion and the latter from ion a_1 .



Scheme 8.

The main contributor to the $M-129$ peak can from the labelling results alone be ascribed to a species formed from ion a_1 by loss of a $C(2)-C(4)$ fragment and the corresponding reaction giving ion b_2 depicted as shown above. Ion q has already been identified above as the main contributor to the $M-131$ peak in the spectra of the axial esters. This is evidently also the case for the equatorial esters, although the route of formation must be somewhat different as the labelling results show that ion o_2 can no longer be an intermediate. The process may be visualized as shown in scheme 8, which in fact only differs from that discussed above in that the 6-hydrogen is transferred to $C(2)$ in the first rather than in the second step.

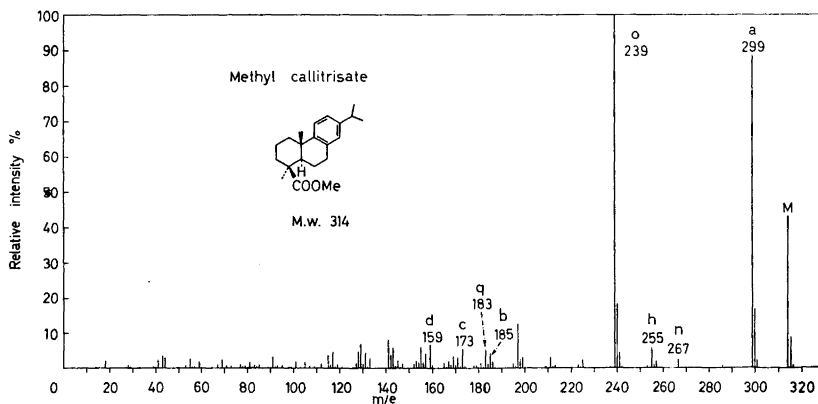


Fig. 7. Methyl callitrisate.

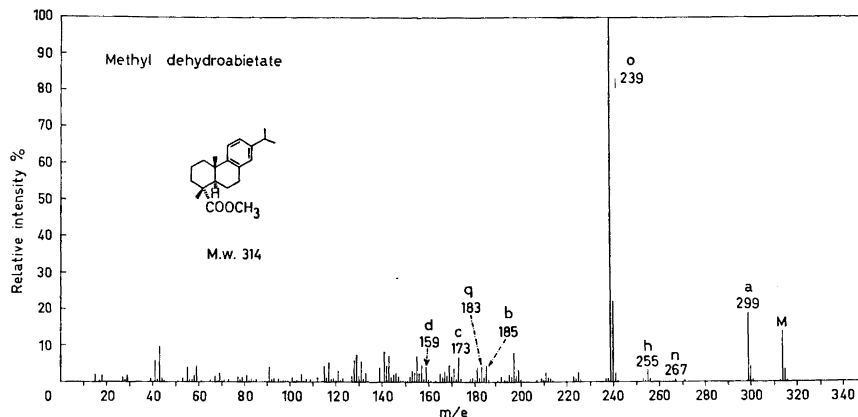


Fig. 8. Methyl dehydroabietate.

Concluding remarks. The central parts of the spectra are not affected significantly by oxygenation of the methyl groups. The upper parts of the spectra of compounds oxygenated at C(16) and C(17), however, contain peaks due to ions formed by loss of simple fragments such as water, methanol, and methyl formate. As there were no previous studies on the course of such eliminations in similar systems extensive deuterium labelling was undertaken to elucidate the mechanisms. Although complex, the results indicate that there are mainly two factors which determine the selection of hydrogen involved, namely the sterical accessibility and the acidity of the hydrogen.

EXPERIMENTAL

The mass spectra were obtained on an LKB 9000 instrument, equipped with a peak matcher 9020, using either the direct or gas-chromatographic inlet system. The temperature of the inlet system was kept at minimum (50–225°) and that of the ion source at 290–310°. The electron energy used was 70 eV.

Methyl 14-methoxy-12-deoxy-2,2,3,3-d₄-enantio-podocarpate I. A mixture obtained by treating acetyl chloride (10 ml) with deuterium oxide (5 ml) at 10–20° was added to methyl 14-methoxy-12-deoxy-3-keto-*enantio-podocarpate* (180 mg) X and dry amalgamated zinc filings. The reaction mixture was kept at reflux temperature until no starting material remained (1 1/2 h). After dilution with water, extraction with ether and chromatography on silica gel the reduction product (70 mg) was boiled under reflux with conc. hydrochloric acid (6 ml), acetic acid (9 ml), and amalgamated zinc filings for 5 h. Work up and chromatography on silica gel furnished methyl 14-methoxy-12-deoxy-2,2,3,3-d₄-*enantio-podocarpate I* (60 mg), m.p. 119–120.5°. Isotopic composition: 82% d₄, 8% d₃, 7% d₂, 2% d₁, 1% d₀.

Methyl 12-O-methyl-5-d₁-podocarpate VI. Methyl 12-O-methyl podocarpate (2 g) in acetic acid (30 ml) was heated with chromium trioxide (1.5 g) in acetic acid (10 ml) for 1 h at 60°. The reaction mixture was diluted with water followed by extraction with ether. Recrystallisation from ethanol gave methyl 12-O-methyl-7-keto-podocarpate XII (1.7 g), m.p. 123–125°.

The keto ester (1.7 g) in acetic acid (30 ml) was boiled under reflux with selenium dioxide (1 g) for 1 h. Work up as above and chromatography on silica gel gave methyl

12-*O*-methyl-5-en-7-on-podocarpate XIII (1.5 g), which on recrystallisation from a benzene-ethanol mixture had m.p. 177–179°.

Part of this material (1 g) was dissolved in deuterioethanol (24 ml) and deuterioacetic acid (8 ml) and stirred with palladium on charcoal (10 %) under a deuterium atmosphere at room temperature for 4 h. The catalyst was filtered off and the solvents removed. The product obtained was added to a solution of aqueous potassium hydroxide (45 %, 5 ml) in ethanol (20 ml) and kept at reflux temperature for 2 h under nitrogen. Dilution with water, acidification with sulphuric acid, extraction with ether and chromatography on silica gel gave methyl 12-*O*-methyl-5-*d*₁-7-keto-podocarpate XIV (330 mg) m.p. 121–124°.

Part of this ketone (160 mg), acetic acid (10 ml), conc. hydrochloric acid (5 ml), and amalgamated zinc filings were boiled under reflux for 1/2 h. Work up and chromatography on silica gel gave methyl 12-*O*-methyl-5-*d*₁-podocarpate VI (120 mg), m.p. 126–128°. The isotopic composition of the deuterio ester was 85 % *d*₁, 13 % *d*₀ and 2 % *d*₂.

14-Methoxy-12-deoxy-2,2,3,3-*d*₄-enantiopodocarpol III and 12-*O*-methyl-5-*d*₁-podocarpol VII. Methyl 14-methoxy-12-deoxy-2,2,3,3-*d*₄-enantiopodocarpate (50 mg) in ether (20 ml) and lithium tetrahydroaluminate were boiled under reflux for 2 h. Excess reagent was destroyed with ethyl acetate and the mixture was diluted with aqueous sulphuric acid and extracted with ether. Chromatography on silica gel gave 14-methoxy-12-deoxy-2,2,3,3-*d*₄-enantiopodocarpol III (30 mg), m.p., 156–158°.

Methyl 12-*O*-methyl-5-*d*₁-podocarpate (60 mg) was reduced in the same way and gave 12-*O*-methyl-5-*d*₁-podocarpol VII (58 mg), m.p. 89–91°.

14-Methoxy-12-deoxy-2,2,3,3-*d*₄-enantiopodocarpal V, 12-*O*-methyl-5-*d*₁-podocarpal VIII and 12-*O*-methyl-6,6,7,7-*d*₄-podocarpal IX. 14-Methoxy-12-deoxy-2,2,3,3-*d*₄-enantiopodocarpol (25 mg) in acetone (10 ml) was stirred with chromium trioxide (16 mg) in dilute sulphuric acid (10 %, 0.8 ml) for 30 min. Work up and chromatography on silica gel gave 14-methoxy-12-deoxy-2,2,3,3-*d*₄-enantiopodocarpal V (22 mg), m.p. 113–114.5°.

12-*O*-Methyl-5-*d*₁-podocarpal VIII, m.p. 128–131°, and 12-*O*-methyl-6,6,7,7-*d*₄-podocarpal IX, m.p. 129–131°, were obtained in a similar manner from 12-*O*-methyl-5-*d*₁-podocarpol and 12-*O*-methyl-6,6,7,7-*d*₄-podocarpol, respectively. The isotopic composition of the 6,6,7,7-*d*₄-derivative was 68 % *d*₄, 17 % *d*₃, 9 % *d*₀, 3 % *d*₁, 3 % *d*₂.

Methyl 1,1,2,2-*d*₄-deoxypodocarpate II and 1,1,2,2-*d*₄-deoxypodocarpol IV. Methyl 1-keto-deoxypodocarpate XI (1 mg) was reduced for 1 h under the same conditions as described for the 2,2,3,3-*d*₄-derivative. The reaction mixture was worked up. The small amount of material available admitted no further purification and no back-exchange. A mass spectrum of methyl 1,1,2,2-*d*₄-deoxy-podocarpate was therefore recorded using the gas chromatographic inlet system of the mass spectrometer (XE-60 column, 150°). Isotopic composition: 64 % *d*₄, 15 % *d*₃, 7 % *d*₂, 6 % *d*₀, 5 % *d*₅, 3 % *d*₁. The crude ester was reduced with lithium tetrahydroaluminate in ether for 2 h and was worked up in the usual way. A mass spectrum of 1,1,2,2-*d*₄-deoxypodocarpol was again obtained using the gas chromatographic inlet system (Apiezon L column, 225°).

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