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Mass Spectrometric Studies of Diterpenes

7. Aromatic Diterpenes

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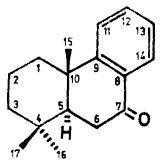
Examination of podocarpa-8,11,13-trien-7-ones and labelled derivatives shows that these compounds undergo essentially the same reactions on electron impact as the corresponding non-oxo compounds discussed earlier in this series.[1,2] A noticeable difference, however, is the formation from the oxo compounds of M−83 ions, which are characteristic of this group and which arise by elimination of ring A. New reactions giving rise to M−33 and M−103 species are also observed in the case where there is an isopropyl substituent in the aromatic ring in *peri*-position to the oxo-group. The mechanisms of these reactions are discussed in the light of high resolution studies and results obtained on examination of compounds labelled with deuterium in positions 2, 3, 5, 6, 16, 17, and 20. A summary of the influence on the fragmentation pattern of oxygenation at various positions in the podocarpa-8,11,13-triene framework is given.

Previous investigations in this series have shown that podocarpa-8,11,13-triene hydrocarbons and derivatives oxygenated only in the aromatic ring undergo unusual breakdown reactions, which are governed by the aromatic ring and lead to the formation of three characteristic indanyl ions.[1,2] Podocarpatrienes oxygenated at C(15), C(16), or C(17) follow essentially the same fragmentation routes and an oxygen function in these positions can be considered as a less important trigger of the fragmentation than the aromatic ring.[3] A greater influence on the fragmentation routes leading to the indanyl ions is, however, observed on nuclear oxygenation in ring A.[4] The effect of oxygenation at C(7) was thus of considerable interest, particularly as many such compounds are of natural occurrence and a detailed knowledge of their fragmentation reactions would be of great value in subsequent studies of plant material.

The present investigation was therefore undertaken and comprises a series of podocarpa-8,11,13-trien-7-ones, differing in aromatic substitution (*cf.* Table 1), and deuterium labelled derivatives (*cf.* Table 2). Some of the fragmentation reactions of podocarpa-8,11,13-trien-7-ones have recently been discussed briefly by Audier *et al.*[5]

Table 1. Podocarpa-8,11,13-triene-7-one derivatives examined.

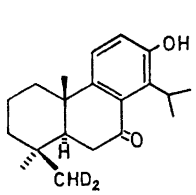


	Aromatic substituents		
	12	13	14
Sugiol	OH	iPr	
Nimbiol	OH	CH_3	
Sempervirol-7-one	iPr	OH	
12-Hydroxypodocarpa-8,11,13-trien-7-one	OH		
13-Isopropylpodocarpa-8,11,13-trien-7-one		iPr	
Totarol-7-one		OH	iPr
Totarol-7-one acetate		OAc	iPr

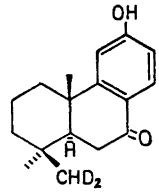
DEUTERIUM LABELLING AND HIGH RESOLUTION STUDIES

The deuterium labelling has, for reasons detailed below, been performed both on totarol-7-one (positions 2, 3, 5, 6, and 17) and on compounds lacking a 14-substituent (positions 2, 3, 6, 16, 17, and 20; *cf.* Table 2). The synthesis of these compounds with the exception of 17,17-d_2-totarol-7-one (I), 17,17-d_2-12-hydroxypodocarpa-8,11,13-trien-7-one (II), and 16,16-d_2-13-isopropylpodocarpa-8,11,13-trien-7-one (III), has been described earlier.[6]

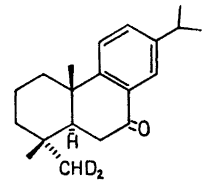
The first two compounds (I and II) were obtained from the corresponding deuterated phenols [6] by acetylation, chromic acid oxidation, and saponification. The third compound (III), lacking oxygen substituents in the aromatic ring, was prepared in one step by chromic acid oxidation of the corresponding deuterated hydrocarbon.[6]



I



II



III

High resolution studies have been carried out on totarol-7-one and sugiol. These show that both the carbonyl oxygen and the phenolic oxygen are retained in all ions of importance formed in the fragmentation of sugiol, while totarol-7-one gives rise to two ions, M − 33 and M − 103, which contain one oxygen only.

Table 2. Mass number of important peaks (m/e) in the spectra of podocarpa-8,11,13-trien-7-one derivatives and shifts observed on deuteration of these compounds. (Reported shifts are corrected for isotopic inhomogeneity and natural occurrence of ^{13}C).

	M−15 a %	M−33 b %	M−43 c %	M−57 d %	M−71 e %	M−83 f %	M−85 g %	M−97 h %	M−103 i %	M−111 k %	69 m %
Totarol-7-one	285	267	257	243	229	217	215	203	197	189	69
2,2,3,3-d_4-Totarol-7-one	289(100)	271(100)	257 (50) 261 (40)	247(90)	229(10) 231(30) 232(20) 233(40)	218 (80)	217(70)	203 (90)	197(45) 198(40) 199(10)	189 (80)	72 (80)
5-d_1-Totarol-7-one	286(100)	267 (60) 268 (40)	257 (40) 260 (60)	244(90)	230(70)	218(100)	215(65) 216(35)	203 (90)	197(60) 198(40)	189 (35) 190 (65)	69(100)
6-6-d_2-Totarol-7-one	287(100)	268 (10) 269 (90)	259(100)	243(15) 245(80)	229(40) 230(15) 231(45)	219 (90)	216(15) 217(85)	205(100)	197(15) 198(50) 199(35)	191 (90)	69(100)
17,17-d_2-Totarol-7-one	287(90)	269(100)	259 (80)	245(95)	229(60) 231(40)	217 (70) 219 (30)	215(90) 217(10)	203 (65) 205 (35)	197(85) 199(15)	189 (65) 190 (10) 191 (25)	71(100)
Sugiol	285	—	257	243	229	217	215	203	—	189	69
2,2,3,3-d_4-Sugiol	289(100)	—	257 (35) 261 (65)	247(90)	229(20) 231(20) 232(20) 233(40)	218 (90)	215(30) 216(20) 217(40)	203(100)	—	189 (80)	72 (90)
6,6-d_2-Sugiol	287(100)	—	258 (40) 259 (45)	243(45) 245(55)	229(35) 231(65)	219(100)	217(70)	205(100)	—	191(100)	69(100)
20-d_1-Sugiol	286 (75)	—	258 (85)	244(65)	230(90)	218(100)	216(95)	204(100)	—	190(100)	69(100)
13-Isopropylpodocarpa-8,11,13-trien-7-one	269	—	241	227	213	201	199	187	—	173	69
16,16-d_2-13-Isopropylpodocarpa-8,11,13-trien-7-one	271(100)	—	243 (90)	229(95)	213(60) 215(40)	201 (80) 203(20)	199(80) 201(20)	187 (65) 189 (35)	—	173 (90)	71 (90)
12-Hydroxypodocarpa-8,11,13-trien-7-one	243	—	215	201	187	175	173	161	—	147	69
17,17-d_2-12-Hydroxypodocarpa-8,11,13-trien-7-one	245(100)	—	217 (90)	201(40) 203(60)	187(70) 189(20)	175 (80)	173(80)	161 (80) 163 (20)		147 (90)	71 (80)

DISCUSSION OF SPECTRA

Substitutional differences in the aromatic ring do not alter the main fragmentation reactions but give rise to some intensity variations in the present series, except in the case of totarol-7-one. This compound exhibits two additional peaks, M − 33 and M − 103, which are prominent and readily associated with the isopropyl substituent occurring in peri-position to the 7-oxo group.

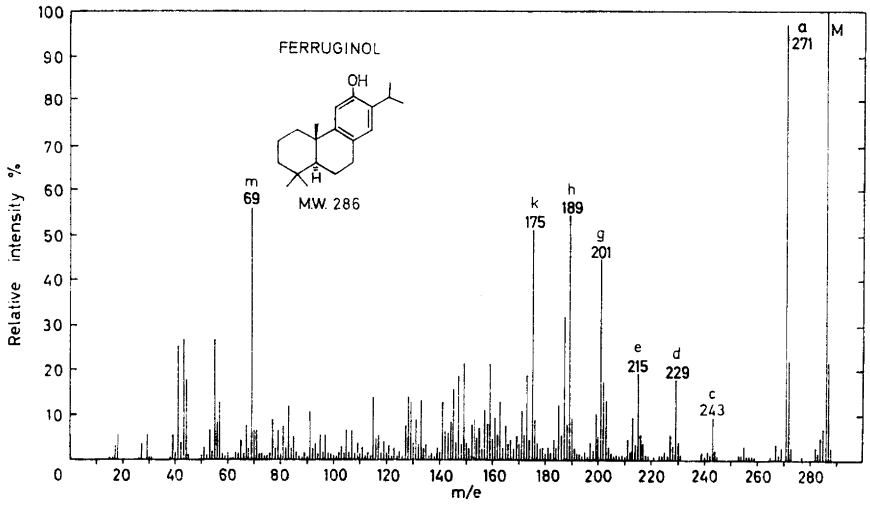


Fig. 1.

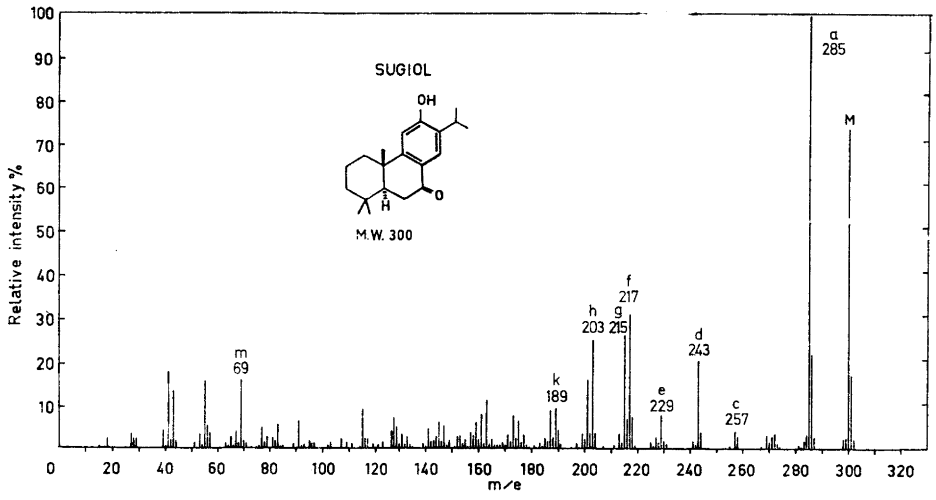


Fig. 2.

When compared with the corresponding non-oxo derivatives, the podocarpatrien-7-ones all show an additional prominent peak, M − 83, which is characteristic of the group. In other respects the spectra of the two series of compounds are quite similar and significant peaks at M − 15, M − 43, M − 57, M − 71, M − 85, M − 97, M − 111, and *m/e* 69 are in common as is evident from the spectra of ferruginol, sugiol, and totarol-7-one.

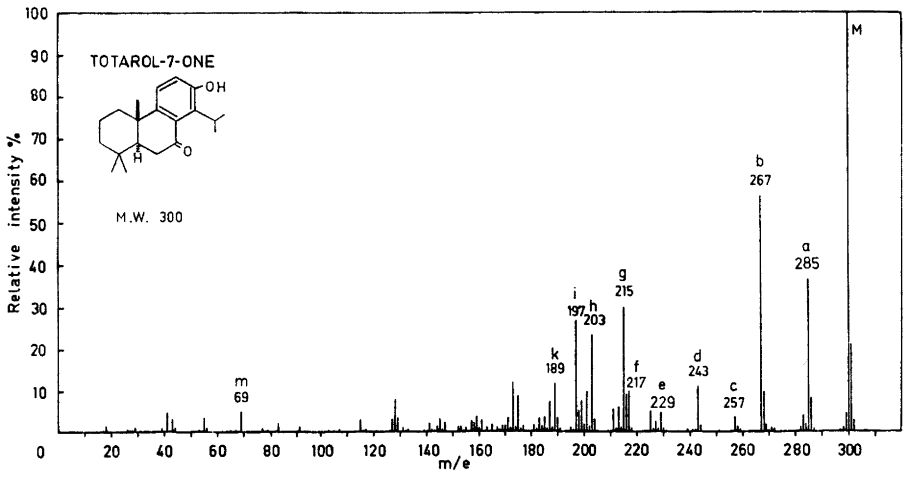
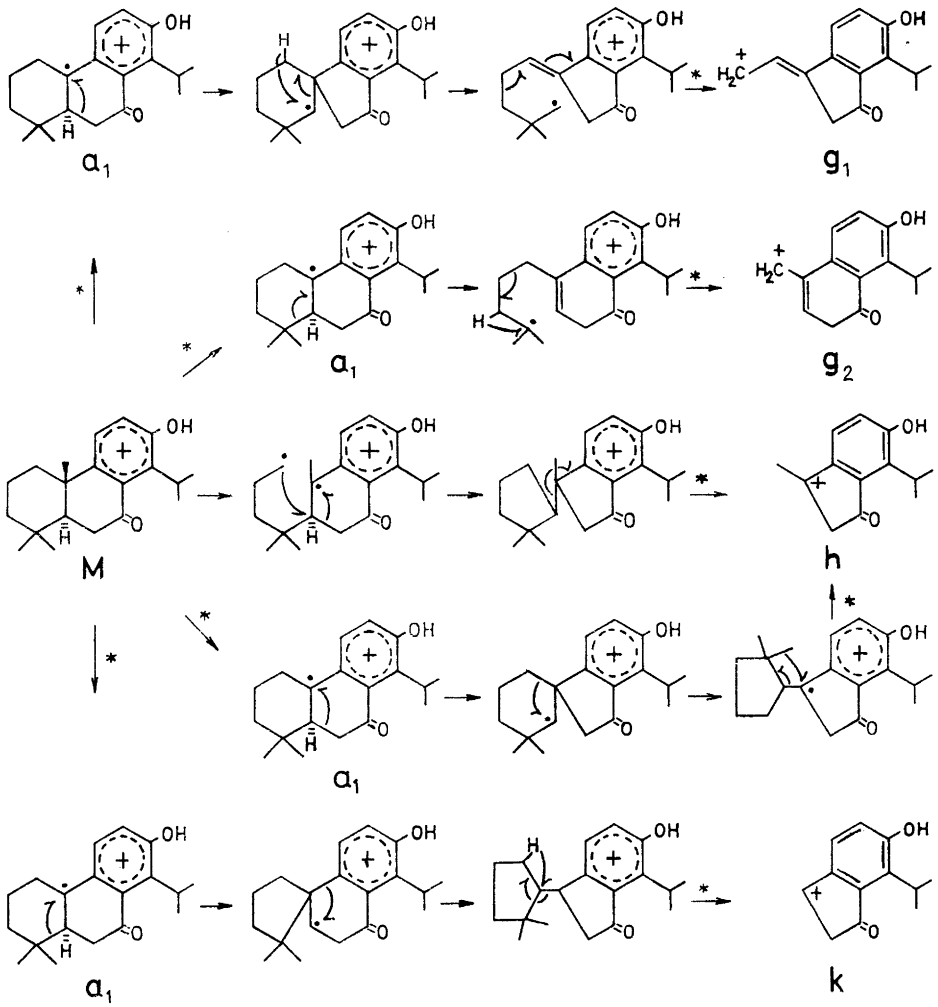


Fig. 3.

The labelling results (Table 2) indicate that the M − 15 peak comprises to an equal extent ions a_1* and a_2, while ion a_1 is the sole contributor in the case of the non-oxo compounds. Ion a_2 arises by loss of a methyl radical from the aromatic isopropyl substituent, while ion a_1 is formed by elimination of the angular methyl group at C(10). The latter ion is clearly more important and is an intermediate in the formation of the M − 85, M − 97, and M − 111 species which are of high abundance. The useful and readily rationalised pattern of intensity variation of the M and M − 15 peaks in the spectra of the compounds studied previously is not observed here. The reason for this is not fully understood as there are several factors which are likely to influence the ratio.

The three prominent peaks in the centre of the spectra, M − 85, M − 97, and M − 111, have been shown in the case of the non-oxo compounds to be due to a series of characteristic indanyl ions, formed by a novel set of closely related rearrangements. It is evident from an examination of the labelled compounds that the same rearrangements, which are visualised in Scheme 1, occur in the fragmentation of the 7-oxo compounds and lead to the formation of ions g_1, *h* and *k*.

* In the succeeding discussion no attempt is made 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. Transitions for which metastable ions are observed are indicated by an asterisk in the diagrams.

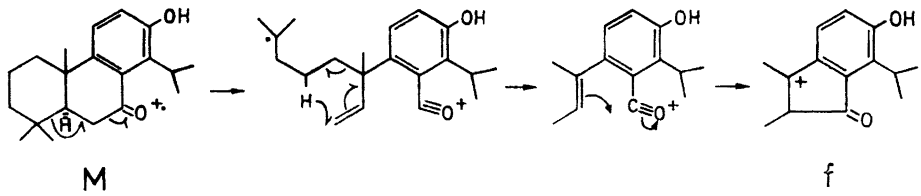


Scheme 1. Formation of the M−85, M−97, and M−111 ions from totarol-7-one.

It may be noted that ion g_1 is more important in the fragmentation of totarol-7-one (65 %) than in that of sugiol (40 %). In the spectrum of the latter compound the M−85 peak can partly (30 %) be associated with ion g_2, formed from ion a_1 by elimination of a C(2)-C(4) fragment with substituents, while the contribution of this species is noticeably lower (15 %) for totarol-7-one. An apparent reason for the fairly high abundance of ion g_2 is that the presence of the 7-oxo group when enolised makes this species a highly stable naphthyl ion. The difference in abundance of this species in the fragmentation of sugiol and totarol-7-one can be ascribed to the position of the isopropyl substituent,

which in totarol-7-one permits additional competing reactions to take place and may provide some steric hindrance towards the enolisation process.

In contrast to the spectra of all other podocarpatrienes examined the 7-oxo compounds display a prominent M − 83 peak. Although less intense in the spectrum of totarol-7-one than in the spectra of the other ketones of the present series, it is evident from the labelling results that the peak can be ascribed to the same ion. As it is clear that the formation of this ion involves removal of the hydrogens at C(16) and C(17) together with three of the four hydrogens at C(2) and C(3), it may be concluded that the corresponding ion is formed by elimination of ring A. As it is specific to the 7-oxo compounds it seems likely that the reaction occurs under a more direct influence of the carbonyl group. Although several mechanisms meeting these requirements can be formulated only one is depicted in Scheme 2.

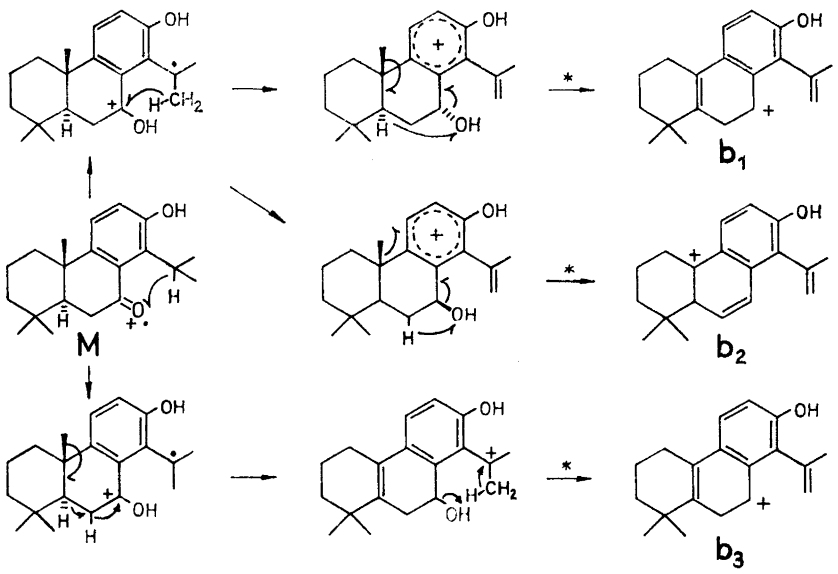


Scheme 2. Formation of the M − 83 ion from totarol-7-one.

Here, opening of the 6,7- and 4,5-bonds giving a 5,6-double bond, followed by transfer of a hydrogen from C(2) to C(6) by a McLafferty reaction with cleavage of the 1,10-bond, leads to ion *f*, which accounts for all results obtained.

It was mentioned previously that totarol-7-one exhibits two peaks, M − 33 and M − 103, not observed for the other podocarpa-8,11,13-trien-7-ones examined and that the formation of these species could be associated with the presence of an isopropyl substituent in *peri*-position to the oxo group. Examination of metastable ions shows that the M − 33 species are derived from ion *a* by elimination of water or by simultaneous loss of the methyl group at C(10) and water from the molecular ion. The deuteration results show that label is retained quantitatively at C(2), C(3), and C(17), but partly lost from C(5) (40 % d_1) and C(6) (90 % d_2). The hydrogens in ring A are thus not accessible in the dehydration reaction, which implies that it occurs without opening of a skeletal bond. The hydrogens required are thus taken from positions 5 and 6 and the isopropyl group. This indicates that the dehydration of totarol-7-one occurs in a complex way, which is consistent with previous studies in this series [3,4] and also with results obtained for many other compounds, *e.g.* monoterpenes [7] and steroids.[8]

It seems likely that the tertiary hydrogen of the isopropyl group is initially transferred to the carbonyl oxygen as depicted above. This may then be followed by migration of a primary hydrogen from the isopropyl group, again involving a six-membered ring intermediate, to give two different ions; one with an α-oriented hydroxyl group and one with the corresponding group β-oriented. The former undergoes dehydration by normal 1,3-elimination in-

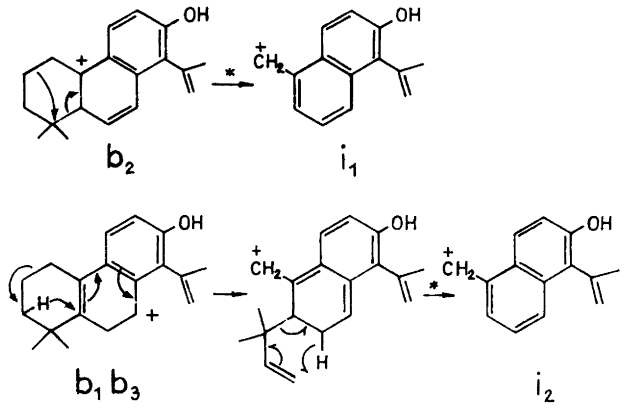


Scheme 3. Formation of the M − 33 ions from totarol-7-one.

volving the 5α-hydrogen and loss of the angular methyl group to give ion b_1, accounting for 60 % of the M − 33 ion current. The intermediate with the hydroxyl group β-oriented, which cannot undergo 1,3-elimination of water for steric reasons, suffers dehydration by the less usual 1,2-elimination and extrusion of the angular methyl group to give ion b_2. This accounts for about 10 % of the M − 33 ion current.

The remainder of the M − 33 ion current (30 %) must be due to an ion where the hydrogens of the water are derived exclusively from the isopropyl group. The formation of a species meeting these requirements, ion b_3, may occur as depicted in Scheme 3.

The M − 103 peak is of high abundance in the spectrum of totarol-7-one. The corresponding species are, according to the results obtained from metastable ions, derived from the M − 33 ions by loss of a C_5H_{10} unit. This is borne out by the high-resolution studies, which show that both parent and daughter ions contain one oxygen only. Since there are several species contributing to the M − 33 ion current, it may be expected that the M − 103 peak is not due to a single species and this is indeed the case. The labelling results imply that the M − 103 species are formed essentially *via* two different paths; one (40 %) leading to the formation of ion i_1 by simple cleavages of ion b_2 and the other (30 %) to an ion, i_2, derived from ion b_1 and b_3 by transfer of one of the four hydrogens at C(2) and C(3) to the larger fragment and back-transfer of a 6-hydrogen to the smaller, neutral fragment. These reactions are visualised in Scheme 4.

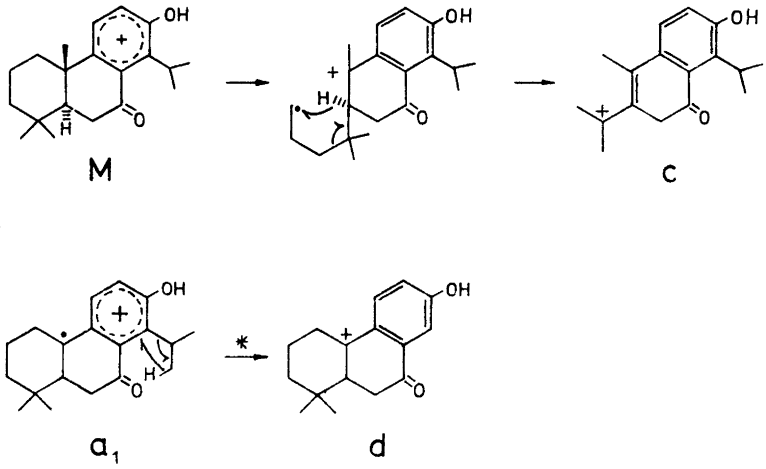


Scheme 4. Formation of the M−103 ions from totarol-7-one.

The upper part of the spectra contains in addition three peaks, M−43, M−57, and M−71, but these are of rather low intensity and for reasons detailed below, of limited diagnostic value. The deuteration results show that they are all due to several different ions, though the high resolution studies demonstrate that they all arise by elimination of hydrocarbon fragments.

The M−43 peak is due to a set of ions. All of these are evidently not the same in the case of totarol-7-one and sugiol, as there is a considerable difference with respect to the loss of label at C(6) in the two compounds. However, it seems probable that part of the ion current (35−40 %) in both cases is due to ion *c*; the corresponding species is also formed from the non-oxo derivatives.

It is evident from the deuteration studies that label is retained nearly quantitatively at all positions of totarol-7-one on formation of the M−57

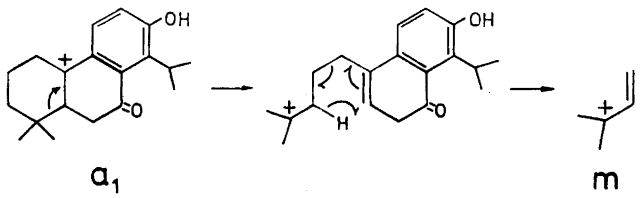


Scheme 5. Formation of the M−43 and M−57 ions from totarol-7-one.

species, while it is partly lost (45 % d_0) from the 6-position when these species arise from sugiol. Although it is clear that not all M − 57 ions are the same when formed from sugiol and totarol-7-one, it may be concluded from available results that there is also a common species, ion *d*, formed from ion a_1 by loss of the isopropyl group as propene. In the case of sugiol this represents about 45 % of the total M − 57 ion current, but considerably more (80 %) in the case of totarol-7-one.

The labelling results for the M − 71 peak are more complex and difficult to rationalise and it seems clear that there is no dominating species formed.

In the lower part of the spectra of all podocarpa-8,11,13-trien-7-ones there is a prominent peak at *m/e* 69, which is also observed in the spectra of the non-oxo compounds. The labelling results show that in both series of compounds it can be ascribed to ion *m*, formed from ion a_1 by cleavage of the 4,5-bond and a subsequent McLafferty reaction involving transfer of a hydrogen from C(3) to C(5) with rupture of the 1,2-bond as depicted in Scheme 6.



Scheme 6. Formation of the *m/e* 69 ion from totarol-7-one.

CONCLUDING REMARKS

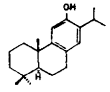
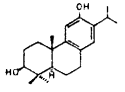
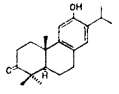
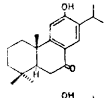
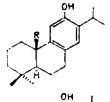
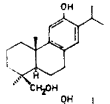
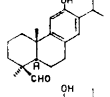
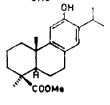
7-Oxo-podocarpa-8,11,13-trienes fragment in essentially the same manner as the corresponding non-oxo compounds, but give in addition an abundant and diagnostically important M − 83 species. The influence of the 7-oxo group on the fragmentation is thus not very pronounced. A stronger effect, however, is observed when the steric conditions are such that the oxo group can interact with an aromatic substituent in *peri* position and a fair part of the total ion current consists of M − 33 and M − 103 ions in totarol-7-one.

With the now accumulated knowledge on the fragmentation of podocarpatrienes,[1–4] it is possible to conclude that mass spectrometry constitutes a powerful tool in the structure elucidation of this group of compounds. An account of the most important species formed in the fragmentation of these compounds, showing the influence of oxygenation at various positions, is given in Table 3.

EXPERIMENTAL

The low resolution mass spectra were obtained on an LKB 9000 instrument using the direct inlet system. The temperature of the inlet system was kept at minimum (50–150°) and that of the ion source at 290°. The electron energy used was 70 eV. The high resolution measurements were performed on an Atlas SM 1 instrument using the direct inlet system (inlet temperature 60–250°; ion source temperature 250°; electron energy

ble 3. **Summation of mass numbers and structures of important fragments derived from representative podocarpa-8,11,13-trienes on electron impact.**

npounds		Mass numbers of ions A[a]	B	C[b]	D[c]	E	F	G	H	I	K	L	N	O	P
RUGINOL 86		**M**−15 271						**M**−85 201			**M**−97 189		**M**−111 175		69
KIOL 02		**M**−15 287		**M**−33 269					**M**−101 201	**M**−103 199		**M**−115 187	**M**−127 175		
KIONE 00		**M**−15 285						**M**−99 201		**M**−101 199		**M**−113 187	**M**−125 175	125	
OL 00		**M**−15 285		[d]			**M**−83 217	**M**−85 215	**M**−85 215		**M**−97 203		**M**−111 189		69
UGINOL-15-OL H_2OH 02		[e] **M**−R 271						**M**−R−70 201			**M**−R−82 189		**M**−R−96 175		69
UGINOL-17-OL 02		[f] **M**−15 **M**−31 **M**−33 287 271 269						**M**−101 201	**M**−101 201		**M**−113 189		**M**−127 175		
UGINOL-17-AL 00		[f] **M**−15 **M**−29 **M**−43 285 271 257						**M**−99 201			**M**−111 189		**M**−125 175		
YL FERRUGINOL-17-OATE 30		[f] **M**−15 **M**−59 **M**−47 **M**−75 315 271 283 255							**M**−129 201	**M**−131 199	**M**−141 189		**M**−155 175		

[a] The M−15 peak in the spectra of some podocarpa-8,11,13-trienes is partly due to a species formed by elimination of a methyl radical from the isopropyl substituent.

[b] These fragments are formed by loss of a methyl radical and water or, in the case of the methyl ester of a methyl radical and methanol but they are not visualized because of the complexity of these reactions. When an aromatic isopropyl substituent is present, subsequent fragmentation of the M−33 species to M−75 species is observed.

[c] M−43 peaks are also observed in the spectra of a number of other podocarpa-8,11,13-trienes, however, these are less significant and due to species formed in a different manner.

[d] Totarol-7-one, with the isopropyl substituent in the 14-position, gives rise to a significant M−33 species which undergoes further fragmentation to give an abundant M−103 ion.

[e] The same fragmentation is also observed for ferruginol-15-al (R=CHO) and ethyl ferruginol-15-oate (R=COOEt).

[f] Essentially the same fragmentation is also observed for the corresponding 4-epimers.

70 eV; resolution = 10 000). The following values were obtained for totarol-7-one: 300.2096 (M, $C_{20}H_{28}O_2$ = 300.2089), 285.1873 (M−15, $C_{19}H_{25}O_2$ = 285.1854), 267.1794 (M−33, $C_{19}H_{23}O$ = 267.1749), 257.1545 (M−43, $C_{17}H_{21}O_2$ = 257.1541), 243.1399 (M−57, $C_{16}H_{19}O_2$ = 243.1385), 229.1229 (M−71, $C_{15}H_{17}O_2$ = 229.1228), 217.1215 (M−83, $C_{14}H_{17}O_2$ = 217.1228), 215.1104 (M−85, $C_{14}H_{15}O_2$ = 215.1072), 203.1097 (M−97, $C_{13}H_{15}O_2$ = 203.1072), 197.0967 (M−103, $C_{14}H_{13}O$ = 197.0966), 189.0912 (M−111, $C_{12}H_{13}O_2$ = 189.0915). The corresponding values for sugiol were: 300.2102 (M, $C_{20}H_{28}O_2$ = 300.2089), 285.1815 (M−15, $C_{19}H_{25}O_2$ = 285.1854), 257.1528 (M−43, $C_{17}H_{21}O_2$ = 257.1541), 243.1395 (M−57, $C_{16}H_{19}O_2$ = 243.1385), 229.1207 (M−71, $C_{15}H_{17}O_2$ = 229.1228), 217.1228 (M−83, $C_{14}H_{17}O_2$ = 217.1228), 215.1101 (M−85, $C_{14}H_{15}O_2$ = 215.1072), 203.1090 (M−97, $C_{13}H_{15}O_2$ = 203.1072), 189.0918 (M−111, $C_{12}H_{13}O_2$ = 189.0915).

17,17-d_2-*Totarol-7-one(I) and 17,17*-d_2-*12-hydroxypodocarpa-8,11,13-trien-7-one (II).* 17,17-d_2-Totarol (3 mg) on treatment with acetic anhydride (0.6 ml) and pyridine (1.2 ml) at 100° for 2 h gave, after work up, 17,17-$d_2$totarol acetate (3 mg). The acetate (3 mg) in acetic acid (2 ml) was treated with chromium trioxide (2 mg) in water (0.5 ml) for 1 h at 60°. Dilution with water followed by extraction with ether gave the 7-one acetate, which was refluxed with ethanol (2 ml) and aqueous potassium hydroxide (45 %, 0.3 ml) for 2 h under nitrogen. Acidification with dilute sulphuric acid and extraction with ether gave 17,17-d_2-totarol-7-one (I) which was purified by preparative TLC. Isotopic composition: 52 % d_2, 31 % d_3, 11 % d_1, 4 % d_0, 2 % d_4. 17,17-d_2-12-Hydroxypodocarpa-8,11,13-trien-7-one (II) was obtained in a similar manner from 17,17-d_2-12-hydroxypodocarpa-8,11,13-triene. Isotopic composition: 91 % d_2, 8 % d_1, 1 % d_0.

16,16-d_2-*13-Isopropylpodocarpa-8,11,13-trien-7-one (III).* 16,16-d_2-13-Isopropylpodocarpatriene (2 mg) in acetic acid (2 ml) was treated with chromium trioxide (1.5 mg) in water (0.5 ml) for 1 h at 60°. Work up and purification by preparative TLC gave 16,16-d_2-13-isopropylpodocarpa-8,11,13-trien-7-one (III). Isotopic composition: 58 % d_2, 31 % d_3, 6 % d_4, 5 % d_1.

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REFERENCES

1. Enzell, C. R. *Tetrahedron Letters* **1966** 2135.
2. Enzell, C. R. and Ryhage, R. *Arkiv Kemi* **26** (1967) 425.
3. Enzell, C. R. and Wahlberg, I. *Acta Chem. Scand.* **23** (1969) 871.
4. Enzell, C. R. and Ryhage, R. *Arkiv Kemi* **27** (1967) 213.
5. Audier, H. E., Bory, S., Defaye, G., Fetizon, M. and Moreau, G. *Bull. Soc. Chim. France* **1966** 3181.
6. Enzell, C. R. *Arkiv Kemi* **26** (1966) 87.
7. Thomas, A. F. and Willhalm, B. *J. Chem. Soc.* **B 1966** 219.
8. Budzikiewicz, H., Djerassi, C. and Williams, D. H. *Mass Spectrometry of Organic Compounds*, Holden-Day, San Francisco 1967, p. 110.

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