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Note

Gas chromatographic-mass spectrometric investigation of the volatile components of myrtle berries (*Myrtus communis* L.)

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Myrtus communis L., pertaining to the family of Myrtaceae, is an evergreen shrub typical of the Mediterranean area. It grows spontaneously in Spain, France, Tunisia, Algeria and Morocco. In Italy it enriches the Mediterranean scrub from Tuscan Maremma to Sicily. The essential oil, obtained from leaves and flowers, is used in perfumery but is also employed in cooking as an ingredient of sauces. Terpenes and terpene alcohols make up nearly the whole of the volatile compounds of the essential oil¹⁻⁴.

Myrtle berries, spherical in shape and dark red-to-violet in colour, are used in the formulation of bitters and Myrtle liqueurs.

The purpose of this work was to obtain a detailed picture of the volatile components of Myrtle berries, as no information is available in the recent literature. The investigation was carried out on berries from Sardinia, on alcoholic extracts obtained by cold infusion in aqueous alcoholic solutions of Myrtle berries of the same origin and on commercial samples of liqueurs.

EXPERIMENTAL

Crushed berries (25 g) were left in contact with 100 ml of methanol for 48 h. The methanol extract obtained after centrifugation was diluted to 500 ml in distilled water, 195 μ g of 1-heptanol added as internal standard and the mixture continuously extracted with pentane-methylene chloride (60:40) for 8 h. The resulting extract was finally dried over Na₂SO₄ and concentrated. Liqueurs and alcoholic extracts were diluted to a 20% alcohol content before extracting them as above. The concentrations of the respective components were computed assuming equal responses for each with respect to 1-heptanol (standard).

Gas chromatography (GC) was performed using a Perkin-Elmer Sigma 3 gas chromatograph, with a 50 m \times 0.2 mm fused silica capillary column coated with Carbowax 20M. Splitting ratio: 60:1. Temperature program: 5 min at 50°C then from 50°C to 180°C at 2°C/min. Carrier gas (helium) flow-rate: 1 ml/min.

Gas chromatography (GC)-mass spectrometry (MS) was carried out using a Hewlett-Packard 5992 B instrument equipped with a 50 m \times 0.3 mm fused-silica capillary column, coated with Carbowax 20M. Splitless injection. Temperature program: 5 min at 50°C then from 50°C to 200°C at 3°C/min. Carrier gas (helium), flow-rate: 3 ml/min. Mass spectra were recorded at 70 eV.

RESULTS AND DISCUSSION

A gas chromatogram of the volatile components from Myrtle berries (harvest 1982) obtained by extraction with pentane-methylene chloride (see Experimental) is shown in Fig. 1 (sample A). α -Pinene, limonene and 1,8-cineole make up 72% of the volatile fraction (percentages calculated without inclusion of the methyl esters pres-

TABLE I
IDENTIFICATION OF PEAKS IN FIG. 1

Peak No.	Compound	Amount (mg/kg)	Mol.wt.	Peak No.	Compound	Amount (mg/kg)	Mol.wt.
	<i>Hydrocarbons</i>				<i>Esters</i>		
5	α -Pinene	77.1	136	2	Methyl butyrate	0.1	102
7	β -Pinene	1.2	136	6	Butyl butyrate	0.1	144
8	<i>p</i> -Xylene	*	106	27	Dimethyl malonate	0.1	132
9	<i>m</i> -Xylene	*	106	32	Methyl benzoate	0.1	136
10	Δ^3 -Carene	3.9	136	58	Methyl hexadecanoate	9.1	270
11	α -Phellandrene	0.6	136	62	Methyl octadecanoate	1.0	298
12	Myrcene	0.5	136		<i>Terpene esters</i>		
13	<i>o</i> -Xylene	*	106	29	Linalyl acetate	1.9	196
14	Limonene	18.4	136	38	α -Terpinyl acetate	2.7	196
16	<i>cis</i> -Ocimene	0.1	136	43	Neryl acetate	7.8	196
17	γ -Terpinene	3.6	136	47	Geranyl acetate	0.1	196
18	<i>trans</i> -Ocimene	0.3	136		<i>Phenols and phenol ethers</i>		
19	<i>p</i> -Cymene	5.7	134	36	Estragole	0.4	148
20	Terpinolene	1.3	136	46	Anethole	0.1	148
26	Unknown	*	57***	55	Phenol	1.1	94
30	β -Caryophyllene	1.0	204	56	Methyleugenol	2.3	178
35	α -Humulene	1.5	204		<i>Oxides</i>		
40	Unknown	0.7	204	15	1,8-Cineole	69.3	154
49	Unknown	0.1	204	23	Linalool oxide A	0.1	170
	<i>Alcohols</i>			25	Linalool oxide B	0.1	170
21	1-Hexanol	0.1	102		<i>Others</i>		
22	<i>cis</i> -3-Hexen-1-ol	0.1	100	1	Diethyl ketone	*	86
24	1-Heptanol	**		3	Ethyl isopropyl ketone	*	100
28	Linalool	6.0	154	4	Diisopropyl ketone	*	114
31	Terpinen-4-ol	2.2	154	41	Unknown	0.5	212
33	Unknown	0.1	154	57	Unknown	2.7	163***
34	<i>trans</i> -Pinocarveol	0.6	152	59	Unknown	0.7	152***
37	Unknown	1.8	152	60	Unknown	1.1	92***
39	α -Terpineol	8.3	154	61	Unknown	0.8	163***
42	<i>cis</i> -Piperitol	0.1	154	63	Unknown	0.9	43***
44	Myrtenol	0.1	152	64	Unknown	1.2	43***
45	Nerol	0.1	154	65	Unknown	7.9	43***
48	<i>trans</i> -Carveol	0.1	152	67	Dibutyl phthalate	*	278
50	<i>p</i> -Cymen-8-ol	0.3	150				
51	Geraniol	0.6	154				
52	Benzyl alcohol	0.3	108				
53	2-Phenylethanol	0.9	122				
54	3,7-Dimethyl-1,5-octadiene-3,7-diol	0.7	170				
66	Sesquiterpene alcohol	3.4	220				

* Compound extraneous to the berries.

** Internal standard.

*** Base mass peak.

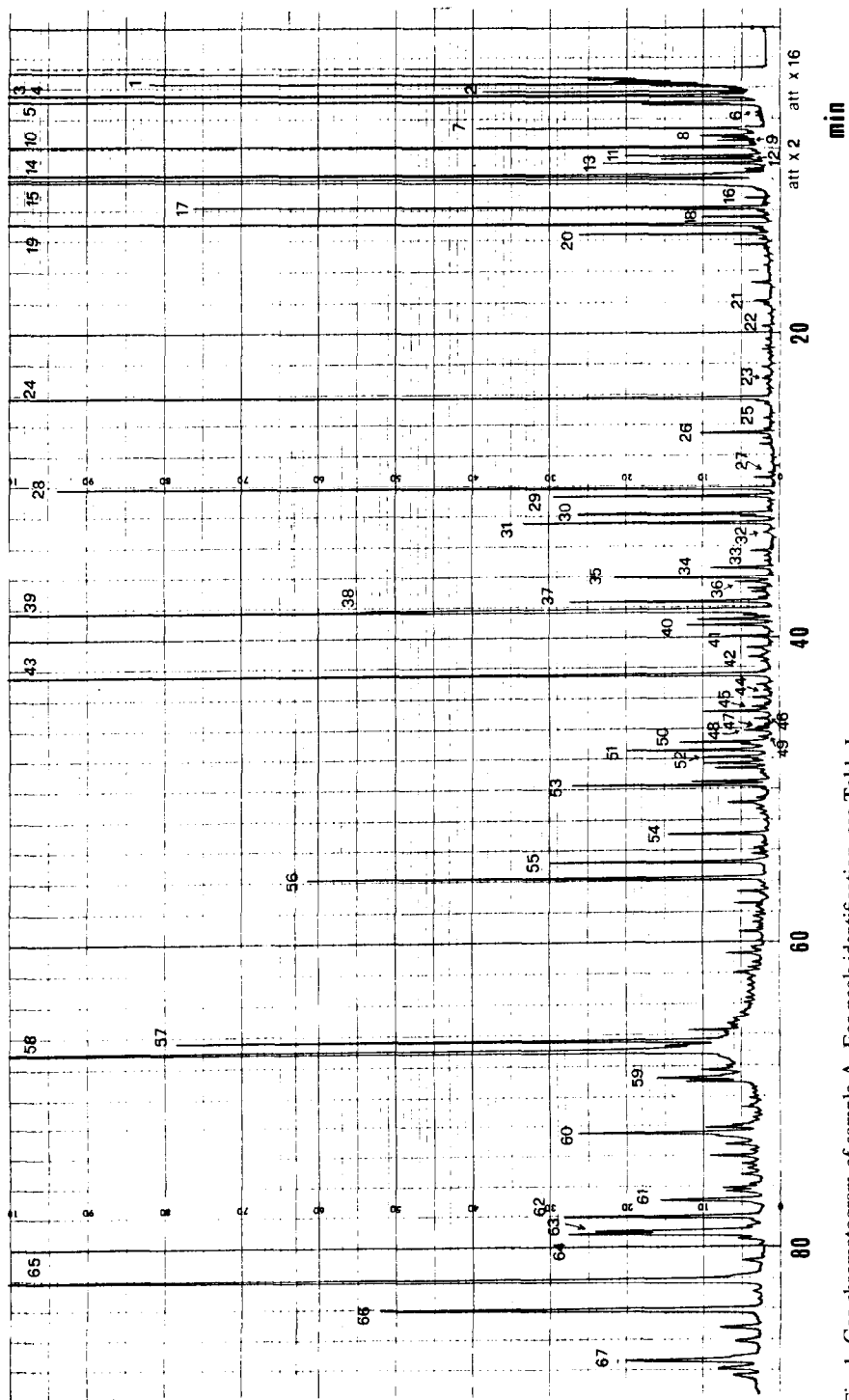


Fig. 1. Gas chromatogram of sample A. For peak identification, see Table I.

ent). Hydrocarbons form 51% of the volatile fraction. Eleven terpene hydrocarbons have been identified: the major ones are α -pinene, Δ^3 -carene, limonene, γ -terpinene, *p*-cymene and terpinolene. Alcohols represent 11.1% of the volatile fraction, linalool and α -terpineol being the most abundant (57% of alcohol fraction), together with smaller amounts of terpinen-4-ol, *trans*-pinocarveol, *cis*-piperitol, *trans*-carveol and *p*-cymen-8-ol, which are not found in the essential oil from leaves and flowers¹⁻⁴. The aromatic content of Myrtle berries differs from that of the latter essential oil also in

TABLE II
IDENTIFICATION OF PEAKS IN FIG. 2

Peak No.	Compound	Mol.wt.	Peak No.	Compound	Mol.wt.
1	Ethyl isobutyrate	116	37	Diethyl malonate	160
2	1,1-Diethoxy-2-methylpropane	146	38	Terpinen-4-ol	154
3	Diisopropyl ketone	114	39	Unknown terpene alcohol	154
4	α -Pinene	136	40	<i>trans</i> -Pinocarveol	152
5	Butyl acetate	116	41	Ethyl decanoate/ α -Humulene	200-204
6	1,1-Diethoxy-2-methylbutane	160	42	Ethyl benzoate/Estragole	150-148
7	1,1-Diethoxy-3-methylbutane	160	43	Diethyl succinate	174
8	Isobutyl butyrate	144	44	α -Terpinyl acetate	196
9	β -Pinene	136	45	α -Terpineol	154
10	Δ^3 -Carene	136	46	Unknown	85*
11	α -Phellandrene	136	47	Unknown	212
12	Myrcene	136	48	<i>cis</i> -Piperitol	154
13	Limonene	136	49	Neryl acetate	196
14	1,8-Cineole	154	50	Myrtenol	152
15	<i>cis</i> -Ocimene	136	51	Nerol	154
16	γ -Terpinene	136	52	Geranyl acetate	196
17	<i>trans</i> -Ocimene	136	53	Anethol	148
18	<i>p</i> -Cymene	134	54	<i>trans</i> -Carveol	152
19	Terpinolene	136	55	<i>p</i> -Cymen-8-ol	150
20	1,1,3-Triethoxypropane	176	56	Geraniol	154
21	3,7-Dimethyl-3-ethoxy-1,6-octadiene	182	57	Benzyl alcohol	108
22	Ethyl lactate	118	58	2-Phenylethanol	122
23	1-Hexanol	102	59	Unknown	76*
24	Unknown ethyl ether	182	60	3,7-Dimethyl-1,5-octadiene-3,7-diol	170
25	<i>trans</i> -3-Ethoxy-2(10)-pinene?	180	61	Methyleugenol	178
26	<i>cis</i> -3-Hexen-1-ol	100	62	Diethyl malate	190
27	4-Ethoxy-1- <i>p</i> -menthene	182	63	Unknown	236
28	<i>cis</i> -3-Ethoxy-1- <i>p</i> -menthene	182	64	Unknown	163*
29	Linalool oxide A	170	65	Ethyl hexadecanoate	284
30	Ethyl octanoate	172	66	Unknown	43*
31	8-Ethoxy-1- <i>p</i> -menthene	182	67	Unknown	163*
32	1-Heptanol	**	68	Unknown	43*
33	Linalool oxide B	170	68a	Ethyl octadecanoate	312
34	8-Ethoxy- <i>p</i> -cymene	178	69	Sesquiterpene alcohol	220
35	Linalool	154	70	Unknown	43*
36	Linalyl acetate	196	71	Dibutyl phthalate	278

* Base mass peak.

** Internal standard.

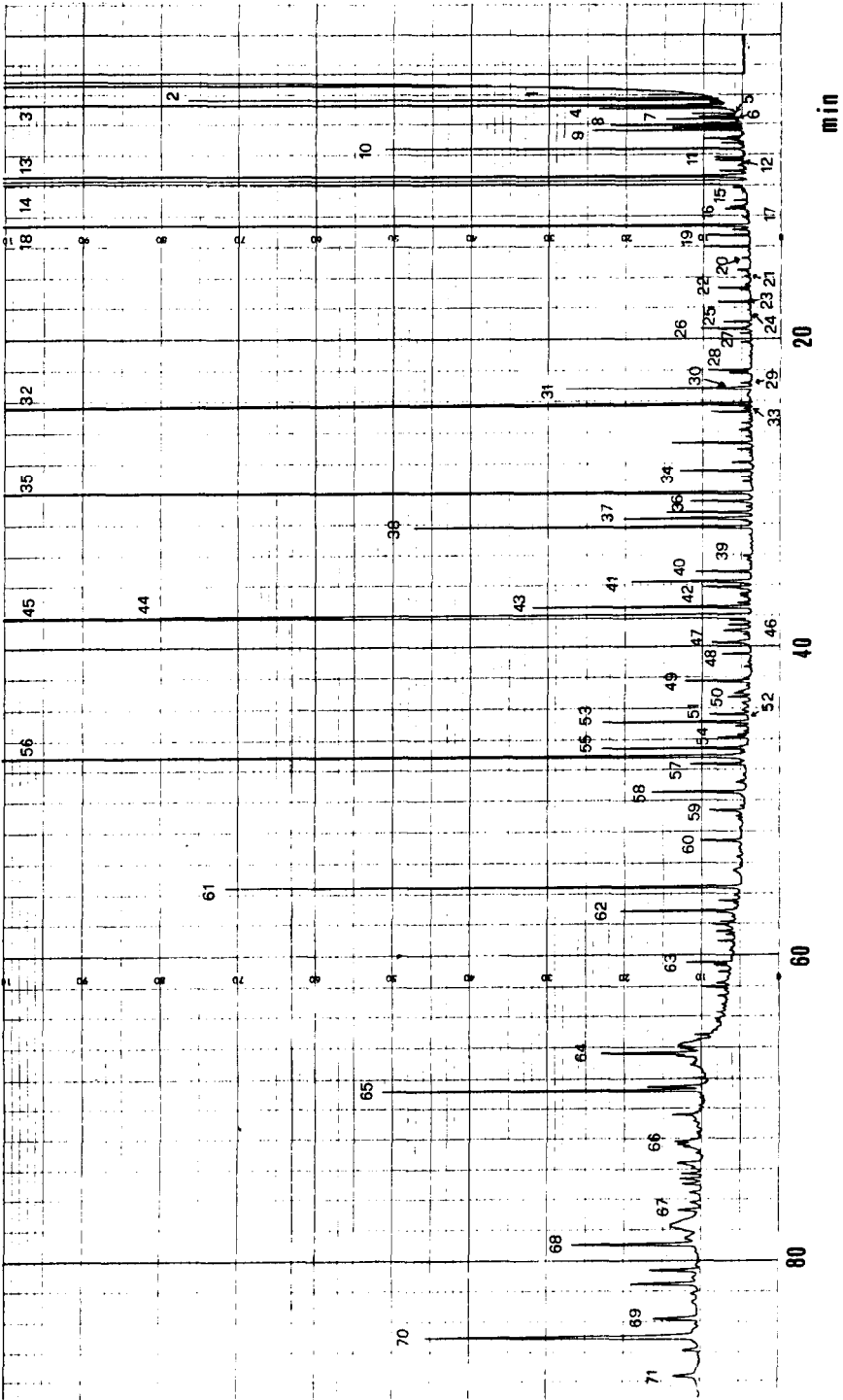


Fig. 2. Gas chromatogram of sample B. For peak identification, see Table II.

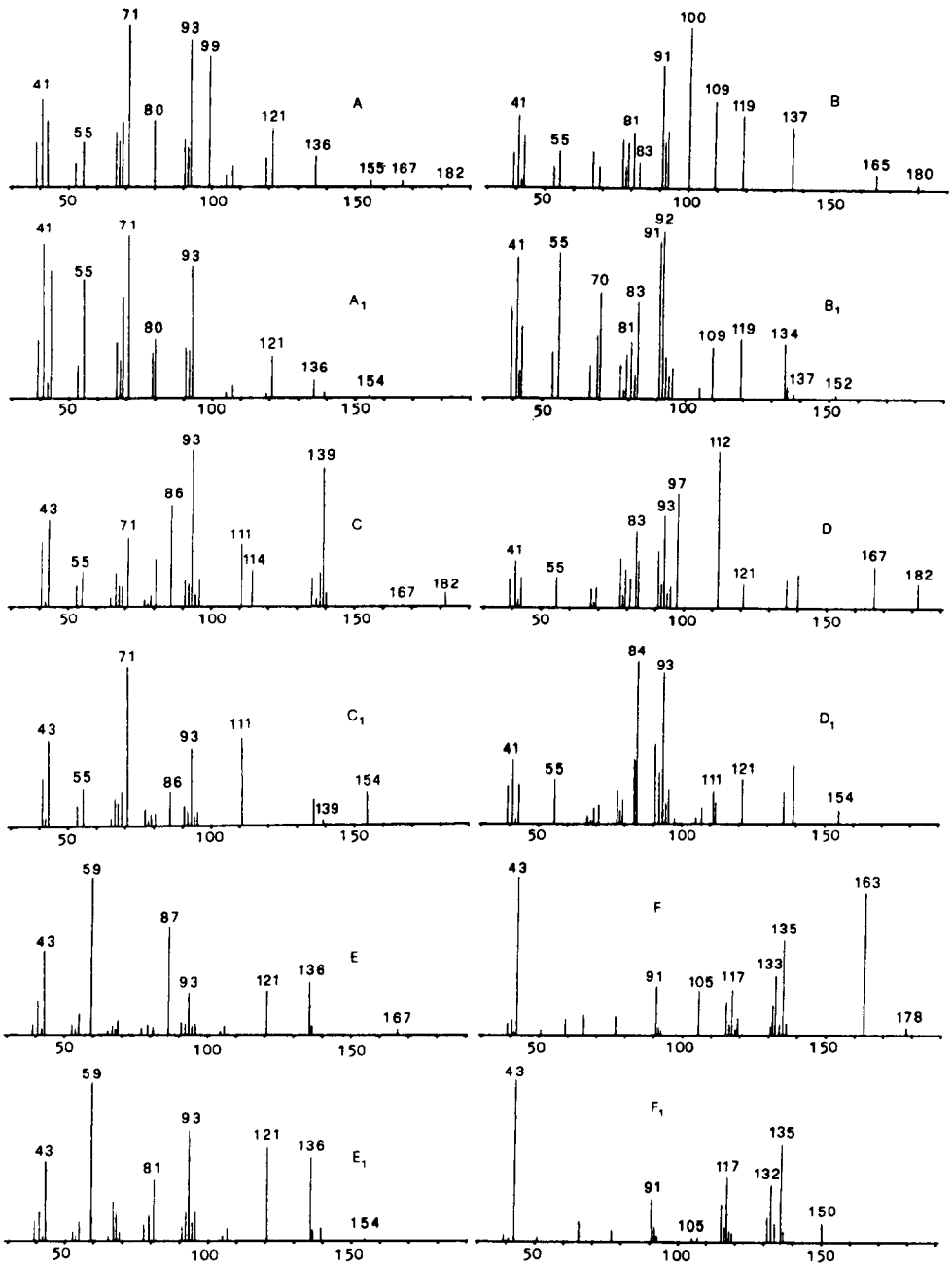


Fig. 3. Mass spectra of 3,7-dimethyl-3-ethoxy-1,6-octadiene (A), linalool (A₁), *trans*-3-ethoxy-2(10)-pinene? (B), *trans*-pinocarveol (B₁), 4-ethoxy-1-*p*-menthene (C), terpinen-4-ol (C₁), *cis*-3-ethoxy-1-*p*-menthene (D), *cis*-piperitol (D₁), 8-ethoxy-1-*p*-menthene (E), α -terpineol (E₁), 8-ethoxy-*p*-cymene (F) and *p*-cymen-8-ol (F₁).

respect to the small amount of myrtenol present and the lack of myrtenyl acetate, which are important and typical components of the essential oil. Terpene esters constitute 5.5% of the volatile fraction. Linalyl-, α -terpinyl-, neryl- and geranyl acetates have been identified. Also, significant amounts of phenol and methyleugenol have been found.

Fig. 2 shows a gas chromatogram of an extract (60% ethanol) obtained by cold infusion of Myrtle berries harvested in January 1981 and subjected to analysis 2 months after preparation (sample B). In this case, the profile is even more complex owing to the presence of acetals and ethers formed through equilibrium reactions dependent on the alcohol concentration and pH.

In sample B and C (liqueurs in 35% ethanol), six ethyl ethers have been identified, derived respectively from linalool (peak 21), *trans*-pinocarveol? (peak 25), terpinen-4-ol (peak 27), *cis*-piperitol (peak 28), α -terpineol (peak 31) and *p*-cymen-8-ol (peak 34). The mass spectra of these ethyl ethers (some of which are published for the first time) are compared with those of the parent terpene alcohols in Fig. 3. Possible fragmentation modes are shown in Fig. 4⁵.

The earliest information about ethyl ethers of monoterpene alcohols is provided by the work of Taskinen and Nykänen (1974–1975)^{6,7}, who investigated the

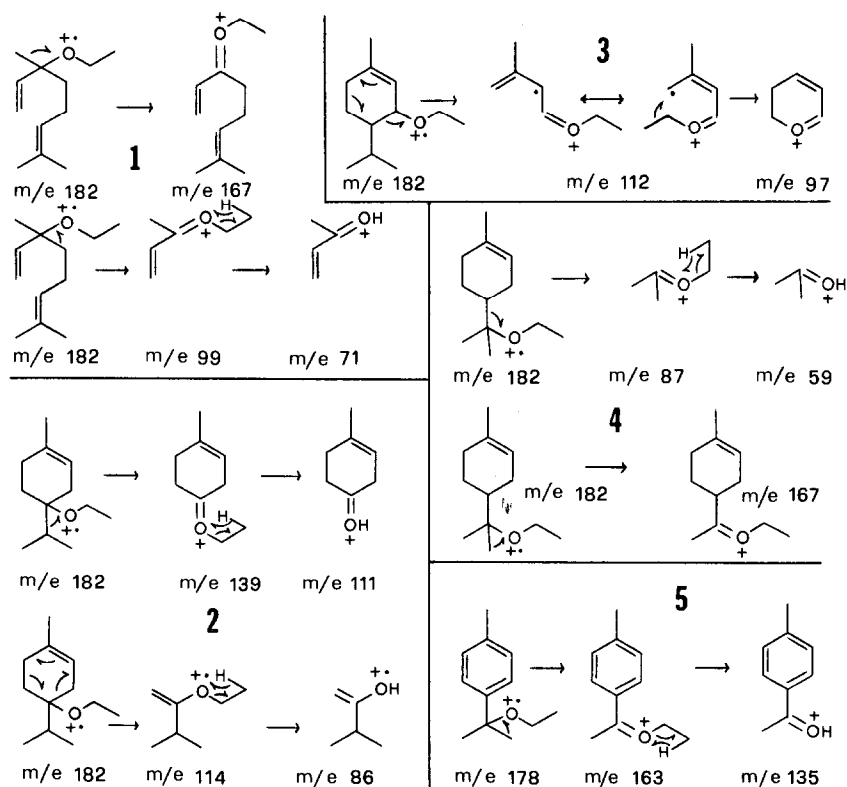


Fig. 4. Fragmentation pathways of 3,7-dimethyl-3-ethoxy-1,6-octadiene (scheme 1), 4-ethoxy-1-*p*-menthene (scheme 2), *cis*-3-ethoxy-1-*p*-menthene (scheme 3), 8-ethoxy-1-*p*-menthene (scheme 4) and 8-ethoxy-*p*-cymene (scheme 5).

composition of the essential oils of *Origanum majorana* L. and *Angelica archangelica* L., and that of distillates from the corresponding aqueous alcoholic infusions. These authors found various ethyl ethers, namely those of *cis*-piperitol and terpinen-4-ol, and described their most significant masses and respective fragmentation pathways. Subsequently, De Smedt and Liddle (1976)⁸ identified linalyl ethyl ether (the mass spectrum of which was reported) in alcoholic extracts of Coriander and wild mint, and pointed out the possibility that analogous ethyl ethers can be formed from citronellol, nerol, α - and β -terpineol and geraniol.

After 2 months of ageing, the overall flavour profile of alcoholic extracts of Myrtle can undergo noticeable changes brought about by the formation of terpenyl ethyl ethers and other "artifacts" (acetals, esters).

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