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GAS CHROMATOGRAPHIC AND MASS SPECTROMETRIC STUDIES OF THE CONSTITUENTS OF THE RHIZOME OF CALAMUS

I. THE VOLATILE CONSTITUENTS OF THE ESSENTIAL OIL

GIACOMO MAZZA
Istituto Sperimentale per l'Enologia, Sezione di Chimica Enologica, via Pietro Micca 35, 14100 Asti (Italy)
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SUMMARY

The composition of the volatile oil of European calamus (*Acorus calamus* L.) was investigated by liquid–solid chromatography and gas chromatography–mass spectrometry (GC–MS). A total of 184 volatile components were detected, including 67 hydrocarbons, 35 carbonyl compounds, 56 alcohols, 8 phenols, 2 furans and 4 oxido-compounds. The essential oil of Indian calamus was also analyzed by GC–MS and 93 volatile components were detected, β-asarone being the major component. A probable oxidation scheme for β-asarone is shown and the importance of minor components for the development of calamus aroma is also discussed. Of the 125 and 79 components identified in the European and Indian oils, respectively 72 and 39 had not been reported previously in calamus oil.

INTRODUCTION

Acorus calamus L., a member of the family of Araceae, is a perennial plant native to the East Indies. It grows wild in the temperate zones of Europe, East Asia and North America, along swamps, brooks, rivers, lakes, etc. In Italy, it grows spontaneously in the Po Valley, Tuscany, Umbria, Latium and Apulia. Calamus is widely cultivated in Hungary, Poland, Yugoslavia, Bulgaria, U.S.S.R., India, Holland, U.S.A. and Japan.

Various species of *A. calamus* L. exist: the diploid variety with $2\times = 24$ chromosomes (North America), the triploid variety with $3\times = 36$ chromosomes (Europe), the tetraploid variety with $4\times = 48$ chromosomes (East Asia, India and Japan) and the hexaploid variety with $6\times = 72$ chromosomes, whose presence in the Kashmir area was reported by Vashist and Handa[1]. The European triploid variety is sterile, and propagates by vegetative reproduction, while the diploid and tetraploid varieties are fertile and propagate by seeds. Polyploidism in *A. calamus* L. affects the qualitative and quantitative composition of its essential oil. The most

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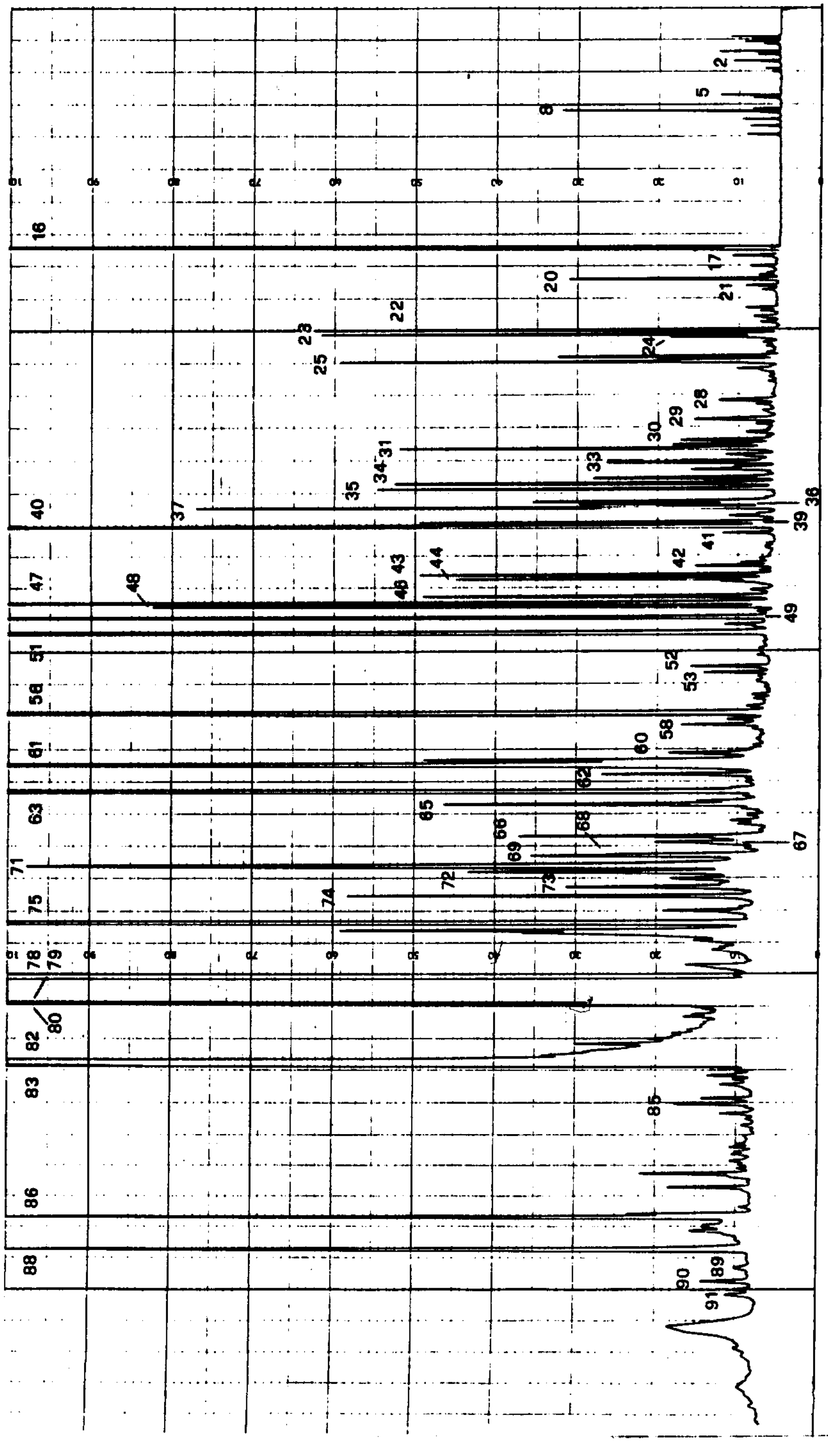

Fig. 1. Gas chromatogram of Indian calamus oil. For peak identification see Table I.

obvious example of this is the amount of β-asarone, a characteristic component of calamus, which is absent in some diploid varieties[2] but constitutes 96% of the essential oil in the tetraploid variety[3]; the European triploid variety contains an average of 5% of β-asarone[4–6].

The essential oil is obtained by steam distillation of fresh rhizome, or unpeeled dried rhizome. Calamus oil is generally a pale yellow to pale brown viscous liquid, having a pleasant woody and spicy odour, but which is bitter to the taste. The odour of good quality oils bears some resemblance to that of dried milk or of leather, slightly nutty. The oil is used both in perfumery and flavourings. In perfumery, the oils from fresh and dried rhizome are used for formulations of the woody oriental type, in leather-bases, ambers, etc. They blend excellently with cananga, cinnamon, costus, etc. In flavouring the oil finds some applications with cardamom, angelica, ginger, etc. in spice blends. In India the essential oil is also used as an insecticide and an insect repellent[3].

In the present study the composition of the volatile oil of *A. calamus* L. was investigated by liquid–solid chromatography and gas chromatography–mass spectrometry (GC–MS).

EXPERIMENTAL

The essential oil (200 μl) was chromatographed at 10–11°C using a glass column (30 × 1.8 cm I.D.) packed with silica gel 60 (35–70 mesh), and three fractions were obtained: I, eluted with 200 ml of pentane; II, eluted with 200 ml of 20% diethyl ether in pentane and III, eluted with 200 ml of diethyl ether.

Gas chromatography was performed on a Perkin-Elmer Sigma 3 gas chromatograph, fitted with a fused-silica capillary column (25 m × 0.2 mm I.D.) coated with Carbowax 20M. The splitting ratio was 60:1. Temperature program: 2 min at 60°C, then raised from 60 to 200°C at 2°C/min. Injector and detector temperatures: 250°C. Carrier gas (helium) flow-rate: 1 ml/min.

A Hewlett-Packard 5992 B gas chromatograph–mass spectrometer, equipped with a fused-silica capillary column (50 m × 0.3 mm I.D.) coated with Carbowax 20M was used for mass spectral identification of the volatile components under the following conditions: column temperature, held at 54°C for 5 min then increased at 4°C/min to 200°C; injector temperature, 250°C; splitless injection; carrier gas (helium) flow-rate, 3 ml/min; ionization voltage, 70 eV.

RESULTS

The essential oil of Indian tetraploid calamus and of European triploid calamus, chosen from five commercial essential oils of very similar quality, were analyzed by GC–MS. Tables I and II show the analytical results and Figs. 1 and 2 the gas chromatograms for the two samples. The European essential oil differs markedly from the Indian one, which is characterized by a higher amount of β-asarone (77.68%). The former was preseparated by liquid–solid chromatography as described and the resulting three fractions were analyzed by capillary gas chromatography and by GC–MS (Figs. 3–5). In this way many interferences were avoided and cleaner mass spectra were obtained, permitting easier identification of the individual components.

TABLE I

IDENTIFICATION OF PEAKS IN FIG. 1

Peak No.	*Compound*	*Amount* (%)*	*Mol. wt.*	*Peak*	*Compound*	*Amount* (%)*	*Mol. wt.*
1	α-Pinene	t	136	46	2,6-Diepishyobunone	0.12	220
2	Camphene	t	136	47	Isoshyobunone	0.46	220
3	β-Pinene	t	136	48	An isoshyobunone epimer**	0.20	220
4	Myrcene**	t	136	49	Shyobunone	0.38	220
5	Limonene	t	136	50	An isoshyobunone epimer**	t	220
6	1,8-Cineole	t	154	51	α-Calacorene	0.89	200
7	2-Pentylfuran**	t	138	52	A calacorene	0.03	200
8	*cis*-Ocimene**	0.05	136	53	Caryophyllene oxide**	0.02	220
9	γ-Terpinene	t	136	54	Unknown	t	94***
10	*trans*-Ocimene**	t	136	55	Cadala-1,4,9-triene	t	202
11	*p*-Cymene	t	134	56	Pre-isocalamendiol	0.91	220
12	Terpinolene**	t	136	57	Viridiflorol ?**	0.01	222
13	An *allo*-ocimene**	t	136	58	Eugenol methyl ether	0.02	178
14	4-Methylisopropenylbenzene**	t	132	59	Caprylic acid**	t	144
15	Acetic acid**	t	60	60	Unknown	0.04	200
16	2-Furaldehyde	1.02	96	61	Sesquiterpene alcohol	0.83	222
17	δ-Elemene**	t	204	62	Elemol**	0.06	220
18	α-Copaene**	t	204	63	*cis*-Isoeugenol methyl ether	1.33	178
19	2-Hydroxy-3-methylvalerianic acid methyl ester**	t	146	64	Spathulenol**	t	220
				65	Sesquiterpene alcohol	0.15	220
20	Furyl methyl ketone**	0.06	110	66	Eugenol	0.09	164
	Camphor		152	67	T-Cadinol**	0.04	222
21	Benzaldehyde**	t	106	68	10-Epi-α-cadinol**	0.05	222
22	Linalool	0.10	154	69	Unknown	0.10	210

23	5-Methyl-2-furaldehyde**	0.15	110	70	Unknown	t	164
24	Sesquiterpene hydrocarbon	0.04	204	71	Elemicine	0.58	208
25	β-Gurjunene	0.17	204	72	*trans*-Isoeugenol methyl ether	0.16	178
26	β-Elemene	t	204	73	10-α-Cadinol	0.11	222
27	β-Caryophyllene	0.01	204	74	Guaiazulene isomer**	0.17	198
28	γ-Elemene**	0.03	204	75	*cis*-Isoelemicine	1.29	208
	allo-Aromadendrene**		204	76	γ-Asarone	t	208
29	α-Humulene	0.04	204	77	Guaiazulene**	t	198
30	Viridiflorene**	0.04	204	78	β-Asarone	77.68	208
31	Sesquiterpene hydrocarbon	0.14	204	79	Isocalamendiol	0.09	238
32	δ-Selinene ?**	t	204	80	*trans*-β-Sesquiphellandrol**	0.45	222
33	Germacrene D**	0.07	204	81	Unknown	t	232
34	β-Curcumene**	0.14	204	82	α-Asarone	6.80	208
35	β-Bisabolene	0.14	204	83	Calamendiol	0.42	238
36	α-Bisabolene?**	0.07	204	84	Unknown	t	190
37	δ-Cadinene	0.21	204	85	Unknown	t	194
38	Unknown	t	126	86	2,4,5-Trimethoxyphenylacetone	0.33	224
39	β-Sesquiphellandrene	0.13	204	87	1-(2,4,5-Trimethoxyphenyl)-1-		
40	α-Curcumene**	0.42	202		methoxypropan-2-ol**	t	256
41	Sesquiterpene hydrocarbon	t	204	88	Asaronaldehyde	0.37	196
42	A calamenene	t	202	89	Unknown	t	256
43	A shyobunone epimer**	0.12	220	90	2,4,5-Trimethoxypropiophenone**	0.03	224
44	6-Epishyobunone	0.11	220	91	1-(2,4,5-Trimethoxyphenyl)-1-		
45	Capronic acid**	t	116		methoxypropan-2-ol**	t	256

* Percentage of total peak area; t = less than 0.01%.
** Identified for the first time in calamus oil.
*** Base peak.

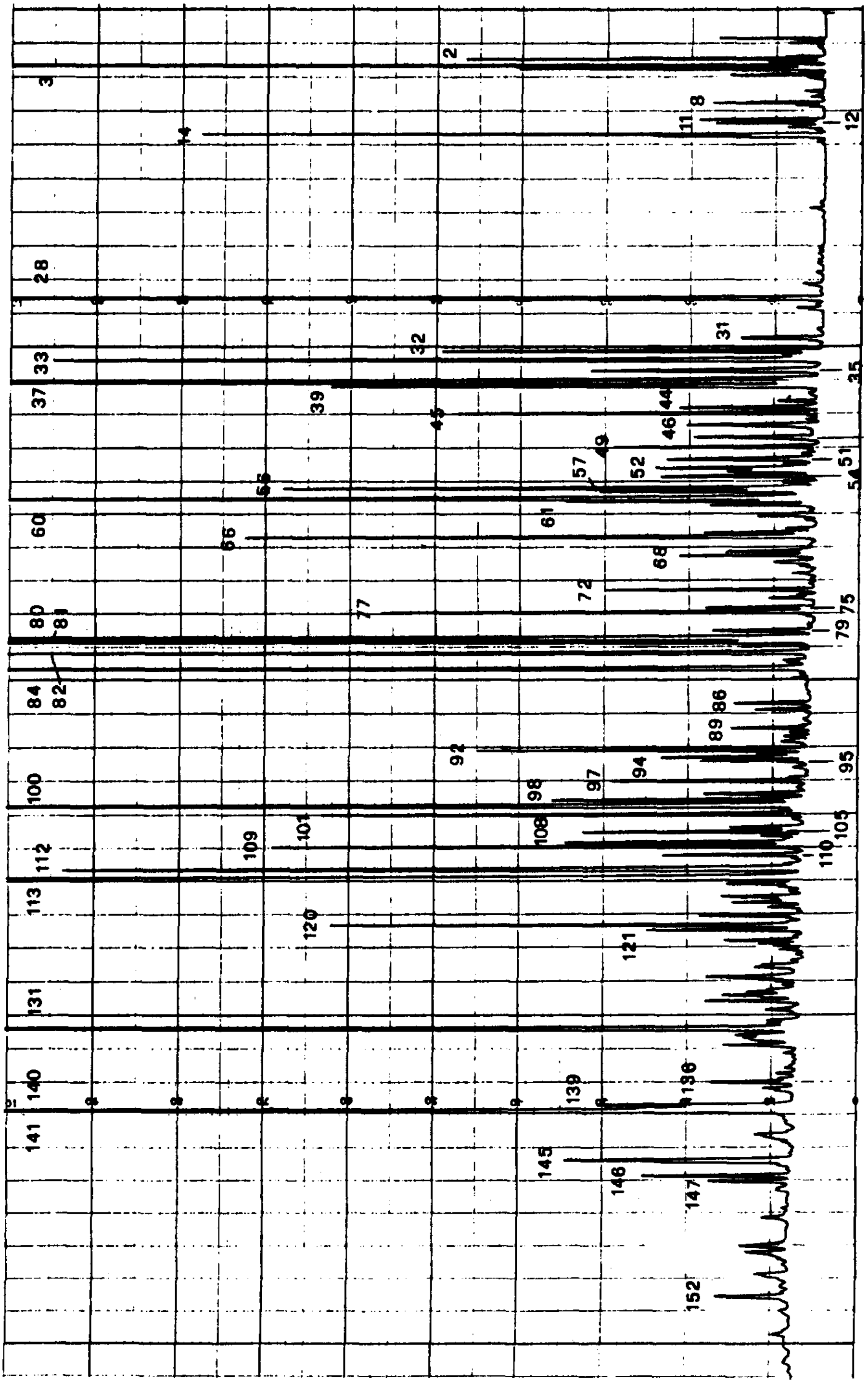

Fig. 2. Gas chromatogram of European calamus oil. For peak identification see Table II.

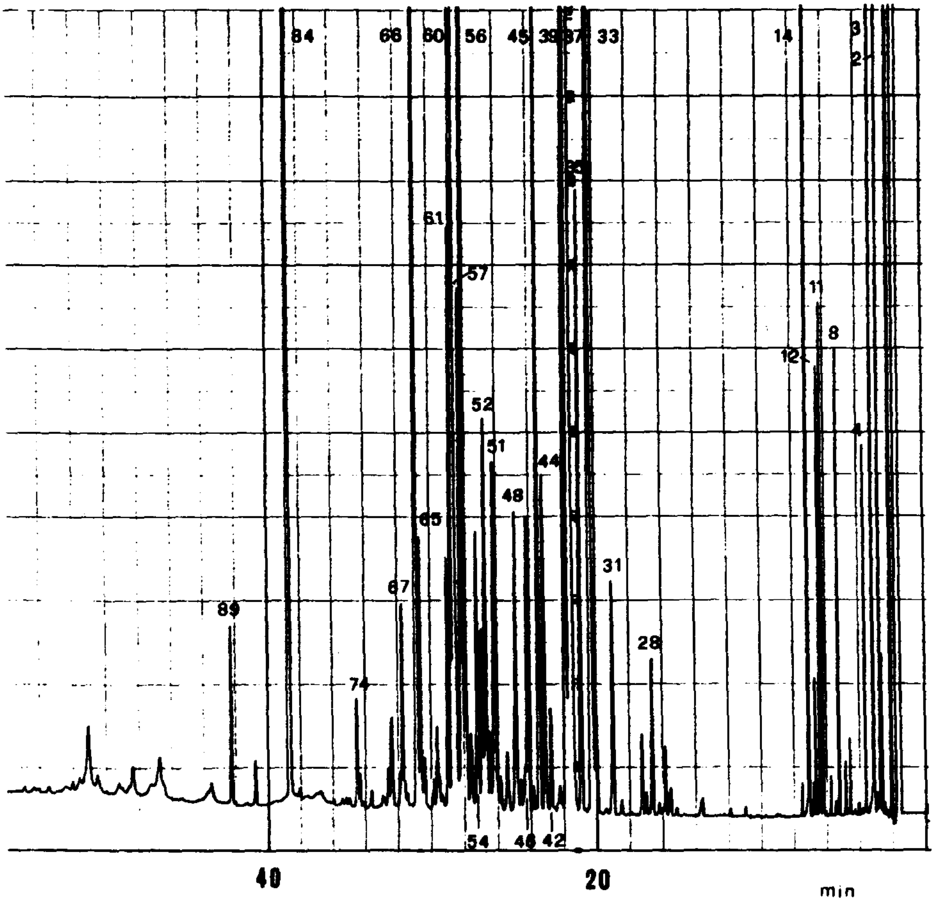

Fig. 3. Gas chromatogram of fraction I of European calamus oil. For peak identification see Table II.

In the European oil 184 volatile components have been found, and 93 in the Indian one. Of the 125 and 79 volatile components found in the European and Indian oil, respectively 72 and 39 have been identified for the first time in calamus oil. To ensure a clear presentation of the analytical results, the identified substances have been divided into six main classes: hydrocarbons, carbonyl compounds, alcohols, phenols, furans and oxido-compounds. Furthermore, as some oxidation products of β-asarone have been identified, a probable scheme for their formation is also provided.

Hydrocarbons

In European calamus, hydrocarbons represent about 34% of the essential oil, the terpene and sesquiterpene hydrocarbons forming 4 and 30% of the oil, respectively. The major representatives are camphene (peak 3), *p*-cymene (peak 14), β-gurjunene (peak 37), α-selinene (peak 60), δ-cadinene (peak 66) and α-calacorene (peak 84) (Table II). In the Indian calamus there is the additional presence of *allo*-aromadendrene (peak 28), γ-elemene (peak 28), viridiflorene (peak 30), germacrene

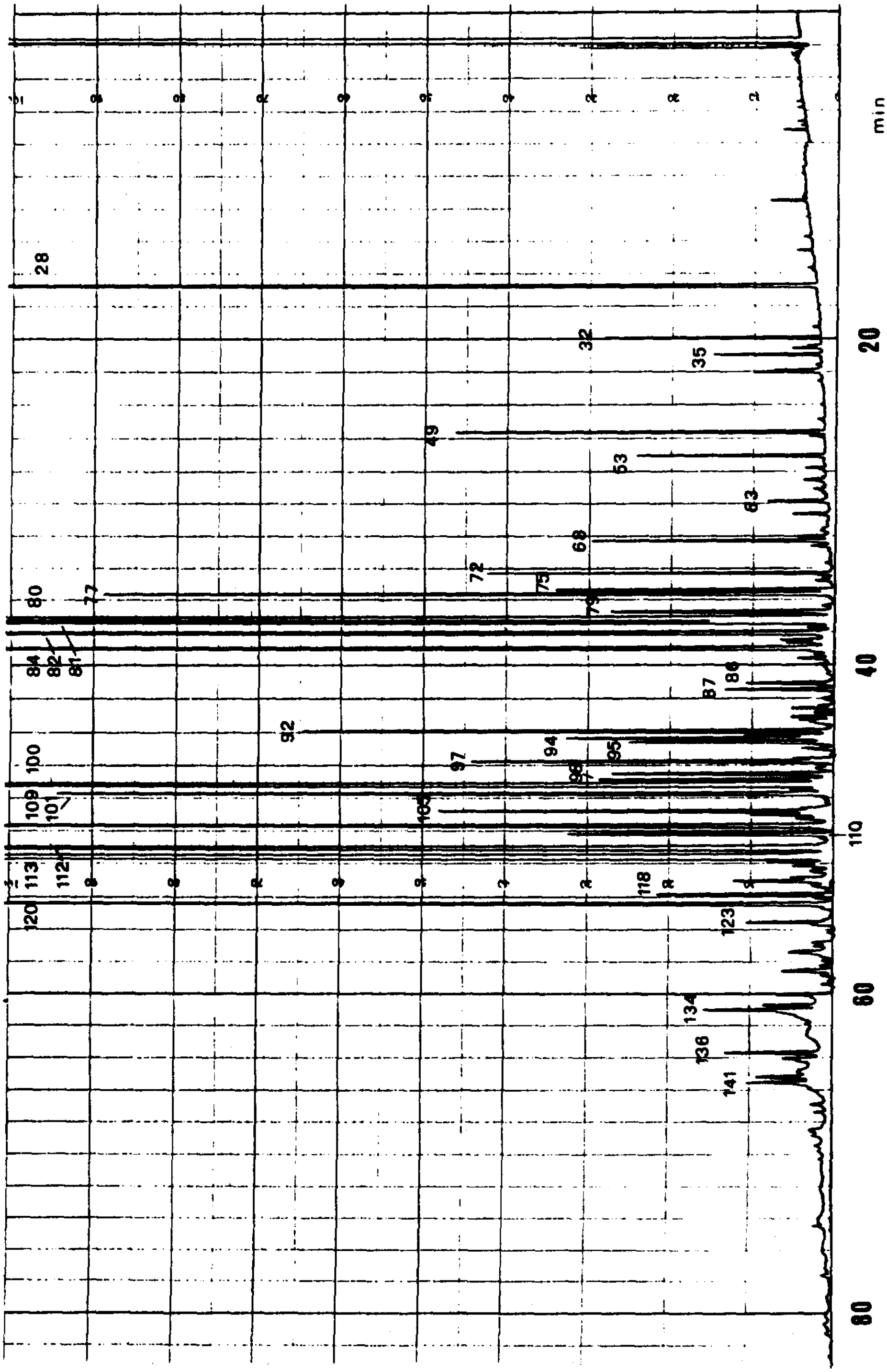

Fig. 4. Gas chromatogram of fraction II of European calamus oil. For peak identification see Table II.

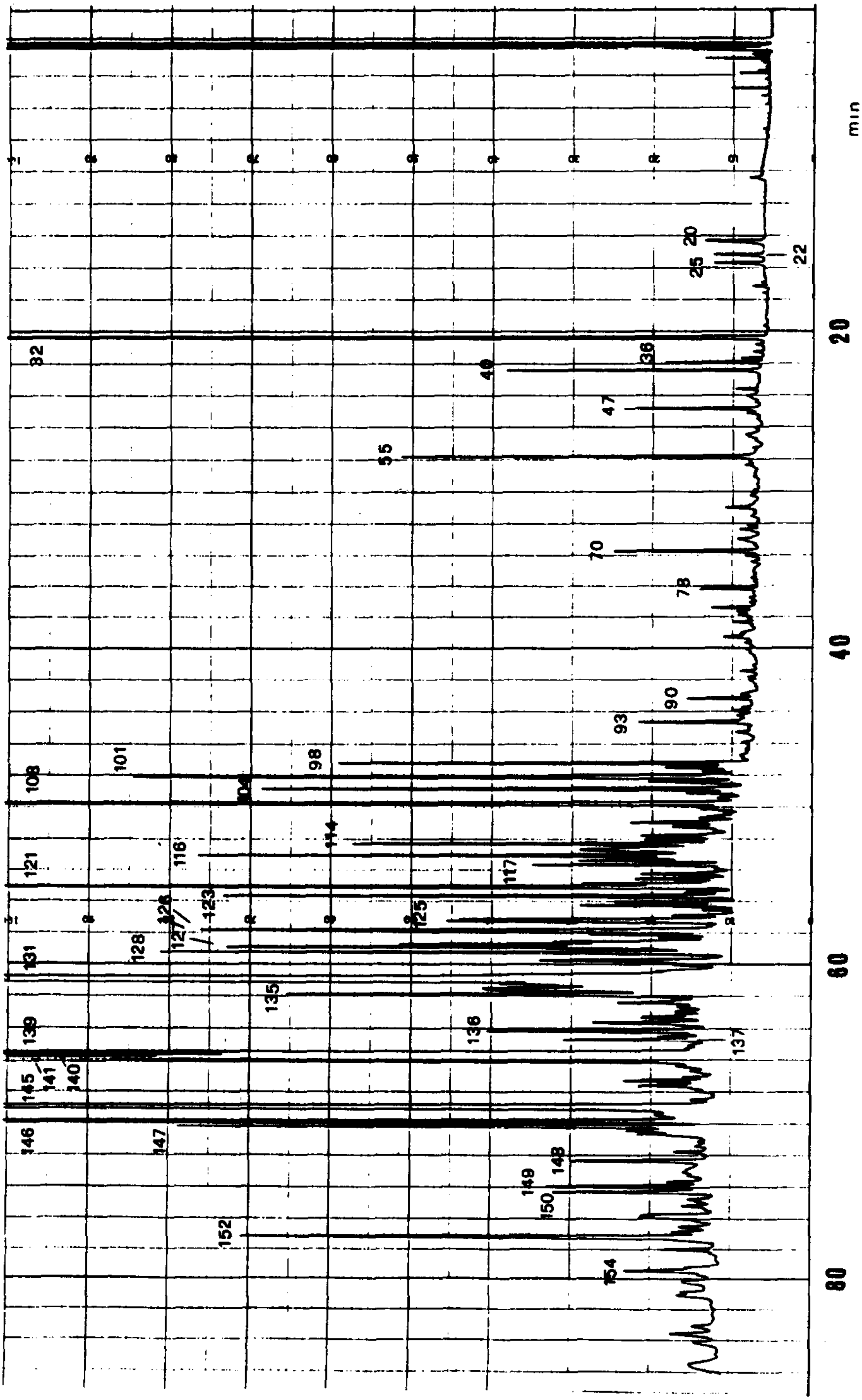

Fig. 5. Gas chromatogram of fraction III of European calamus oil. For peak identification see Table II.

TABLE II

IDENTIFICATION OF PEAKS IN FIGS. 2–5

Peak No.	Compound	Silica gel fraction	Amount* (%)	Mol. wt.	Peak No.	Compound	Silica gel fraction	Amount* (%)	Mol. wt.
1	α-Thujene**	I	t	136	31	Calarene ?**	I	0.37	204
2	α-Pinene	I	0.25	136		Sesquiterpene hydrocarbon	I		204
3	Camphene	I	1.50	136	32	Linalool	II, III	1.11	154
4	β-Pinene	I	0.16	136	33	α-Cedrene	I	t	204
5	Sabinene**	I	t	136		Sesquiterpene hydrocarbon	I	2.90	204
6	Myrcene	I	t	136		Linalyl acetate**	II	t	196
7	α-Terpinene**	I	t	136	34	Isomenthyl acetate**	II	t	198
8	Limonene	I	0.24	136	35	Sesquiterpene hydrocarbon	I	0.58	204
9	1,8-Cineole	II	t	154		Bornyl acetate	II	t	196
10	2-Pentylfuran**	II	t	138	36	α-*trans*-Bergamotene**	I	t	204
11	*cis*-Ocimene**	I	0.25	136		Camphene hydrate**	III	t	154
12	γ-Terpinene	I	0.24	136	37	β-Gurjunene	I	6.74	204
13	*trans*-Ocimene**	I	t	136	38	β-Elemene	I	t	204
14	*p*-Cymene	I	1.38	134	39	Sesquiterpene hydrocarbon	I	1.78	204
15	Terpinolene**	I	t	136	40	Terpinen-4-ol	I, III	t	154
16	An *allo*-ocimene**	I	t	136	41	β-Caryophyllene	I	t	204
17	Fenchone**	II	t	152	42	Sesquiterpene hydrocarbon	I	t	204
18	4-Methylisopropenylbenzene**	III***	t	132	43	Hotrienol**	III	t	154
19	Acetic acid**	III	t	60	44	Sesquiterpene hydrocarbon	I	0.43	204
20	*trans*-Linalool oxide (furanoid)**	III	t	170	45	Sesquiterpene hydrocarbon	I	1.10	204
21	Menthone**	II	t	154	46	Sesquiterpene hydrocarbon	I	0.47	202
22	1-Octen-3-ol**	III	t	128	47	Menthol**	III	t	156
23	2-Furaldehyde	II	t	96	48	An acoradiene**	I	0.44	204
24	δ-Elemene**	I	t	204	49	α-Humulene	I, II	0.54	204
25	*cis*-Linalool oxide (furanoid)**	III	t	170	50	Sesquiterpene hydrocarbon	III***	t	204
26	α-Copaene**	I	t	204	51	α-Acoradiene**	I	0.49	204
27	2-Hydroxy-3-methylvalerianic acid methyl esters**	III	t	146	52	β-Acoradiene**	I	0.51	204
					53	*trans*-Pinocarvyl acetate**	II	0.24	194
28	1,7-Diepi-α-cedrene**	I	t	204		Viridiflorene**	III***	t	204
	Camphor	II	3.17	152	54	γ-Gurjunene ?**	I	0.49	204
29	α-Gurjunene**	I	t	204	55	Unknown	II	t	218
30	Menthyl acetate**	II	t	198		α-Terpineol	III	t	154

56	δ-Selinene**	I	1.94	204	89	Cadala-,4,9-triene	I, III***	0.20	202
57	β-Selinene	I	0.55	204		Unknown	III	t	232
58	Carvone**	II	t	150	90	γ-Eudesmol ?**	III	t	222
59	Piperitone**	III***	t	152	91	Humulene epoxide I**	II	t	220
60	α-Selinene**	I, III***	3.84	204	92	Pre-isocalamendiol	II	1.02	220
61	γ-Acoradiene ?**	I	0.69	204		Unknown	III	t	43§
	Sesquiterpene hydrocarbon	II	t	202	93	Viridiflorol ?**	III	t	222
62	*trans*-Linalool oxide (pyranoid)**	III	t	170	94	Humulene epoxide II**	II	0.48	220
63	Sesquiterpene hydrocarbon	II	0.13	204	95	Unknown sesquiterpene	II	0.32	222
64	*cis*-Linalool oxide (pyranoid)**	III	t	170	96	Sesquiterpene alcohol	III	t	220
65	Sesquiterpene hydrocarbon	I	0.35	204	97	Unknown sesquiterpene	II	0.57	222
66	δ-Cadinene	I, III***	1.74	204	98	Unknown sesquiterpene	II	0.75	236
67	β-Sesquiphellandrene	I, III***	0.19	204		*trans*-Nerolidol**	III		222
68	α-Curcumene**	II, III***	0.48	202	99	Sesquiterpene alcohol	III	t	220
69	Sesquiterpene hydrocarbon	I	t	204	100	Sesquiterpene ketone	II	4.24	220
	Terpene alcohol	III	t	152	101	Unknown sesquiterpene	II	1.28	222
70	Sesquiterpene hydrocarbon	I	t	202		Elemol**	III	0.31	222
	Terpene alcohol	III	t	152	102	Sesquiterpene alcohol	III	t	220
71	Sesquiterpene hydrocarbon	I	t	202	103	A guiaenol**	III	t	222
72	*trans*-Anethole**	II	0.56	148	104	Cedrol**	III	0.19	222
73	Sesquiterpene hydrocarbon	I	t	202	105	*cis*-Isoeugenol methyl ether	II, III	0.57	178
74	A calamenene	I, III***	0.11	202		Sesquiterpene alcohol	II		222
75	A shyobunone epimer**	II	0.35	220	106	Acoragermacrone	II	t	220
76	A calamenene	I, III***	t	202	107	Unknown sesquiterpene	II	t	220
77	6-Epishyobunone	II	1.24	220	108	Spathulenol**	III	0.71	220
78	*p*-Cymen-8-ol**	III	t	150	109	Sesquiterpene ketone	II	1.76	220
79	2,6-Diepihyobunone	II	0.36	220	110	Sesquiterpene ketone	II	0.47	220
80	Isoshyobunone	II	6.25	220	111	An eudesmol ?**	III	t	222
81	An isoshyobunone epimer**	II	2.26	220	112	Calamusenone	II, III	3.24	218
82	Shyobunone	II	2.59	220	113	Acorenone	II	8.12	220
83	An isoshyobunone epimer**	II	t	220	114	T-Cadinol**	III	0.21	222
84	α-Calacorene	I, III***	3.55	200	115	Sesquiterpene alcohol	III	t	222
85	Sesquiterpene ketone	II	t	220	116	10-Epi-α-cadinol**	III	0.25	222
86	A calacorene	I, III***	0.19	200	117	Torreyol**	III	t	222
87	Caryophyllene oxide**	II	0.16	220	118	*trans*-Isoeugenol methyl ether	II	0.33	178
88	Unknown	III	t	232		An acorenol ?	III	t	222

(*Continued on p. 190*)

TABLE II (*continued*)

Peak No.	*Compound*	*Silica gel fraction*	*Amount* (%)*	*Mol. wt.*	*Peak No.*	*Compound*	*Silica gel fraction*	*Amount* (%)*	*Mol. wt.*
119	An eudesmol ?	III	t	222	135	Isocalamendiol	III	0.24	238
120	Carvacrol**	II	1.38	150	136	Sesquiterpene ketone	II	0.27	218
121	10-α-Cadinol	III	0.49	222		Sesquiterpene alcohol	III		222
122	Isospathulenol**	III	t	220	137	Sesquiterpene alcohol	III	t	220
123	Guaiazulene isomer**	II		198	138	Unknown	II	t	232
	Unknown	II	0.30	218	139	Unknown	III	0.63	218
	Sesquiterpene alcohol	III		222	140	α-Asarone	III	0.49	208
124	Calamusenone isomer	II	t	218	141	Unknown	II	t	218
	cis-Isoelemicine	III	t	208		Calamendiol	III	5.21	238
125	Sesquiterpene alcohol	III	t	222	142	Sesquiterpene alcohol	III	t	218
126	Humulenol II**	III	0.34	220	143	Sesquiterpene alcohol	III	t	218
	Sesquiterpene alcohol	III		222	144	Oplopanone ?**	III	t	238
127	γ-Asarone	II, III	0.26	208	145	Acorone	III	0.71	236
	Sesquiterpene alcohol	III		220	146	Isoacorone	III	0.48	236
128	Sesquiterpene alcohol	III	0.28	222	147	An acorone	III	0.28	236
129	Guaiazulene**	II	t	198	148	An acoronene**	III	t	234
130	Calamusenone isomer	II	t	218	149	An acoronene**	III	0.19	234
	4-Isopropyl-6-methyl-1,2,3,4-					Unknown	III		232
	tetrahydronaphthalen-1-one**	II	t	202	150	Unknown	III	0.16	232
131	β-Asarone	III	5.24	208	151	2,4,5-Trimethoxyphenylacetone	III	t	224
132	Sesquiterpene alcohol	III	t	220	152	Unknown	III	0.26	252
133	Sesquiterpene ketone ?	II	t	236	153	Asaronaldehyde	III	t	196
	A hydroxycalamenene**	III	t	218	154	Unknown	III	t	252
134	Sesquiterpene ketone	II	t	236	155	2,4,5-Trimethoxypropiophenone**	III	t	224
	Sesquiterpene alcohol	III	t	220	156	Unknown	III	t	252

* Percentage of total peak area; t = less than 0.10%.
** Identified for the first time is calamus oil.
*** Artifact.
§ Base peak.

D (peak 33), β-curcumene (peak 34) and β-bisabolene (peak 35) (Table I). Guaiazulene and one of its isomers displaying the same mass spectrum have been found in both essential oils.

Carbonyl compounds

In European calamus the major components present are ketones which represent about 35% of the essential oil. The most abundant representatives are acorenone (8.12%), the shyobunones (4.55%), the isoshyobunones (8.51%), calamusenone (3.24%), camphor (3.17%) and pre-isocalamendiol (1.02%). Acorenone (peak 113), a spirosesquiterpene isolated by Sŏrm and co-workers[7] from sweet flag oil (*A. calamus* L.) and synthesized by several authors[8–11], is the major component of triploid European calamus oil (Table II), and also of diploid American calamus oil[2].

The monocyclic ketones shyobunone, epishyobunone and isoshyobunone were first isolated from Japanese calamus by Iguchi and Nishiyama[12]. The structure of epishyobunone was later revised by Fráter[13], who synthesized 6-epishyobunone, 2-epishyobunone, shyobunone and 2,6-diepishyobunone. The synthesis of isoshyobunone and one of its epimers has been described by Alexandre and Rouessac[14], while Williams and Callahan[15] synthesized (−)-shyobunone and its epimers from methylcyclobutane and (−)-piperitone. In triploid calamus, in addition to shyobunone (peak 82), 6-epishyobunone (peak 77), 2,6-diepishyobunone (peak 79) and isoshyobunone (peak 80), a new shyobunone epimer (peak 75) and two new isoshyobunone epimers (peaks 81 and 83) have been found (Table II). Unfortunately, the structures of these isomers cannot be established by mass spectrometry alone.

Calamusenone and a calamusenone isomer were isolated by Rohr *et al.*[16] from Eastern European sweet flag oil. These two sesquiterpene ketones occurred in fraction II (peaks 112 and 124), together with a small quantity of a new calamusenone isomer (peak 130), whose mass spectrum is very similar to that of calamusenone itself. Also present in fraction II was pre-isocalamendiol (peak 92), a germacrane-type sesquiterpene isolated by Iguchi *et al.*[17] from Japanese calamus.

Another monocyclic sesquiterpene, acoragermacrone, was isolated by Niwa and co-workers[18] from Japanese calamus. From a biogenetic point of view, this ketone is an important precursor of shyobunones, isoshyobunones, pre-isocalamendiol, calamendiols and acolamones[19,20]. Recently Ueda *et al.*[21] isolated epoxyisoacoragermacrone, a derivative of acoragermacrone. Small amounts of the latter ketone (peak 106) have been found in the European essential oil.

Acorone (peak 145) and isoacorone (peak 146), two spirosesquiterpenes isolated by Sŏrm and Herout[22], were also present in fraction III.

Only pre-isocalamendiol, four shyobunones and two isoshyobunones were found in Indian calamus.

Alcohols

In *A. calamus* L. only three terpenic alcohols, namely linalool, terpinen-4-ol and α-terpineol[23], a sesquiterpene alcohol, α-cadinol and two diols[2], calamendiol and isocalamendiol[24], have hitherto been reported. In European and Indian calamus I found 56 and 14 alcohols, respectively, in the course of the present work.

Calamendiol and isocalamendiol, two bicyclic diols derived from pre-isocalamendiol, occur in both essential oils. Sesquiterpene alcohols represent about 10% of the European calamus essential oil, spathulenol (peak 108), cedrol (peak 104) and elemol (peak 101) being the main representatives; also, small quantities of 1-octen-3-ol (peak 22) and of terpene alcohols are present. The alcohols represent 1.7% of the Indian oil including an unknown sesquiterpene alcohol (peak 61) and *trans-β*-sesquiphellandrol (peak 80), as major component.

Phenols

The phenols comprise about 9% of the European oil, as compared to *ca.* 87% of the Indian oil, β-asarone being the most abundant in both oils (5.24 and 77.68%, respectively). In Indian oil, α-asarone (peak 82), *cis-* and *trans*-isoeugenol methyl ether (peaks 63 and 72), elemicine (peak 71) and *cis*-isoelemicine (peak 75) are major components, while in European oil carvacrol (peak 120) and *trans*-anethole (peak 72) were also found.

Furans

These compounds are practically absent in triploid calamus, while they represent 1.2% of the Indian oil. 2-Furaldehyde (peak 16) and 5-methyl-2-furaldehyde (peak 23) are the main components, but furyl methyl ketone (peak 20) and 2-pentylfuran (peak 7) are also found.

Oxido-compounds

Caryophyllene oxide (peak 87), humulene epoxide I (peak 91) and humulene epoxide II (peak 94) have been identified for the first time in European calamus, while only 1,8-cineole (peak 6) is present in Indian oil.

β-Asarone oxidation products

2,4,5-Trimethoxybenzaldehyde, or asaronaldehyde (peak 153, Table II), 2,4,5-trimethoxyphenylacetone (peak 151) and 2,4,5-trimethoxypropiophenone (peak 155), found in both essential oils, had already been identified, in previous work on *A. calamus*[25], as oxidation products of β-asarone. Moreover, two diastereoisomers of 1-(2,4,5-trimethoxyphenyl)-1-methoxypropan-2-ol (peaks 87 and 91, Table I) have been found for the first time in a natural source; their occurrence may be explained by the initial presence of small amounts of methanol in Indian calamus oil. In Fig. 6 a probable oxidation pathway of β-asarone to form these products is outlined. The presence of these compounds in essential oils may be due to a chemical oxidation promoted by certain environmental factors (light, air, etc.), but further studies need be made in this respect.

Although asaronaldehyde, the main oxidation product of β-asarone, is absent in essential oils obtained from fresh rhizome[26], its presence and that of other oxidation products cannot always be taken as an index of the extent of ageing of the oil. In this connection, it is necessary to know whether the oil has been obtained from fresh or dried rhizome, because the (often used) air-dried rhizome already contains the mentioned oxidation products, probably due to other enzymatic oxidation reactions. Therefore, these oxidation products may have different origins, but while the chemical oxidation is rather slow the probable enzymatic oxidation is much more rapid.

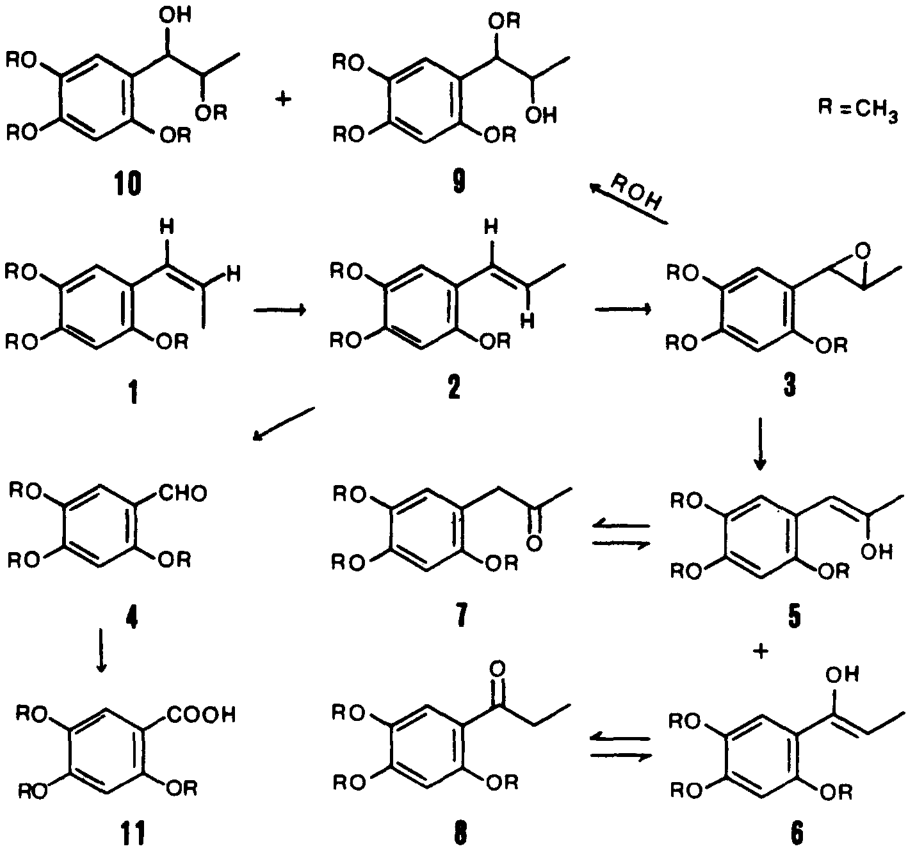

Fig. 6. Oxidation pathway of β-asarone (1) and α-asarone (2) to asaronaldehyde (4), 2,4,5-trimethoxyphenylacetone (7), 2,4,5-trimethoxypropiophenone (8), 1-(2,4,5-trimethoxyphenyl)-1-methoxypropan-2-ol (9) and asaronic acid (11).

DISCUSSION

The identified volatile compounds represent 80 and 98%, respectively, of the European and Indian essential oils. Many of them are minor components. Often, in investigating the volatile components of essential oils, only the main components are considered, not always correctly, to be responsible for the aroma. In fact, from an organoleptic point of view certain minor components exhibiting low odour thresholds are much more important than many major components. For example, in Indian calamus, only eight constituents account for 91% of the total oil, but a mixture of these will not reproduce the odour pattern of calamus oil. Since β-asarone and α-asarone are odourless, the accompanying minor components are decisive in the calamus fragrances.

Finally, when analysing essential oils, special attention should be paid to artifacts whose presence is pointed out in Table II. This will be dealt with in Part II, devoted to alcoholic extracts, in which this phenomenon is obviously more important.

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