Journal of Chromatography, 328 (1985) 195–206
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 17 634

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

II. THE VOLATILE CONSTITUENTS OF ALCOHOLIC EXTRACTS*

GIACOMO MAZZA
Istituto Sperimentale per l'Enologia, Sezione di Chimica Enologica, via Pietro Micca 35, 14100 Asti (Italy)
(First received December 7th, 1984; revised manuscript received February 6th, 1985)

SUMMARY

An alcoholic extract of European calamus (*Acorus calamus* L.) was preseparated by liquid–solid chromatography and analyzed by gas chromatography–mass spectrometry. Of the 243 volatile components detected, 45 are newly identified in this extract. The occurrence of β-asarone oxidation products and the formation of artifacts are also discussed.

INTRODUCTION

Dried rhizomes of *Acorus calamus* L. are used for medicinal purposes, in the formulation of vermouths, liqueurs and bitters and also in veterinary science to treat anaemia and the lack of appetite in cattle. Indian calamus is chiefly used in Indian medicine as an emetic, laxative, diuretic and also to treat nervous diseases, etc. Dried rhizomes of the European triploid variety are used exclusively for the preparation of aromatized alcoholic beverages, because they have the best organoleptic properties. Alcoholic extracts are, in general, prepared by macerating dried rhizomes (10%, w/v) in 60% ethanol.

The β-asarone content, limited in beverages to 1 mg/kg according to the directions of the Council of Europe[1], may vary widely depending on the starting material. Commercial calamus is found in three different types: dried rhizomes whole or cut in lengths, dried rhizomes cut into pieces about 4–5 mm long and also barked dried rhizomes.

In this study an alcoholic extract of *A. calamus* L. was analyzed by liquid–solid chromatography and gas chromatography–mass spectrometry (GC–MS).

EXPERIMENTAL

Dried rhizomes (20 g) were left in contact with 100 ml of ethanol (or methanol)

* Work carried out jointly with "Comitato per lo Studio delle Bevande Alcooliche Aromatizzate".

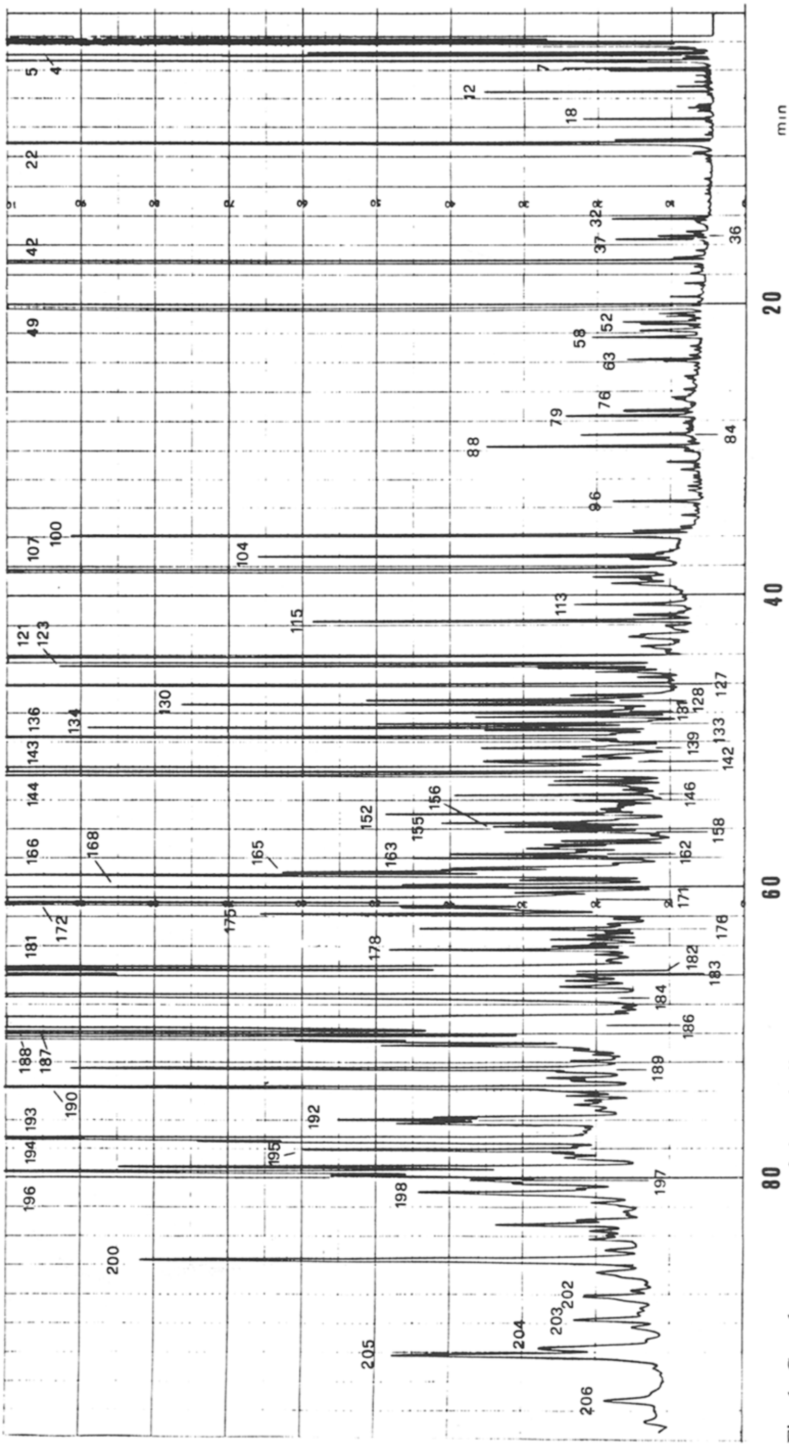

Fig. 1. Gas chromatogram of the volatile components from an ethanolic extract of European calamus rhizome.

for 48 h at room temperature. The liquid was filtered and the residue washed repeatedly with distilled water. The filtrate was diluted to 500 ml in distilled water and continuously extracted for 12 h with pentane–methylene chloride (60:40), after adding 5.506 mg of 2-heptanol as an internal standard. (After preseparation by liquid–solid chromatography, 2-heptanol, added at this stage, will be found entirely in fraction IV.) The final extract was dried over sodium sulphate and concentrated to a volume of *ca.* 2 ml by using a Vigreux column.

Liquid–solid chromatography was performed on a glass column (35 × 1.8 cm I.D.) packed with silica gel 60 (35–70 mesh). The analysis temperature was 10–11°C and four fractions were obtained. Fraction I was eluted with 200 ml of pentane, fractions II and III with 200 ml of pentane containing 10 and 20% of diethyl ether respectively and fraction IV was eluted with 200 ml of diethyl ether. To fractions I–III, 5.506 mg of 2-heptanol were added as an internal standard.

Gas chromatography and GC–MS were carried out as described for the essential oils (see Part I[2]). The concentrations of the individual components were computed assuming equal responses for each, with respect to 2-heptanol as standard.

RESULTS AND DISCUSSION

An alcoholic extract of dried rhizome from European triploid calamus, chosen from five commercial samples of the same variety and very similar quality, was analyzed by GC and GC–MS. Table I shows the analytical results, and Fig. 1 displays the gas chromatogram of the volatile fraction of the alcoholic extract. As the composition is much more complex than that of the corresponding essential oil, the organic extract was preseparated by silica gel chromatography as described above. Even a superficial examination of the results (Table I and Figs. 1–5) clearly shows considerable qualitative and quantitative differences between the volatile fraction of an alcoholic extract and that of an essential oil from the same variety (see Part I[2]).

The following classes of compound are examined: hydrocarbons, carbonyl compounds and alcohols. The formation of oxidation products of β-asarone and of artifacts is also discussed.

Hydrocarbons

Contrary to what was observed for essential oils, hydrocarbons represent only 2.6% of the volatile fraction, camphene (peak 5) being the only one present in appreciable amount (2.1%).

Carbonyl compounds

Practically all the carbonyl compounds occur in fraction II, although a very small quantity can also be found in fraction III. Acorone (peak 186), pre-isocalamendiol (peak 121), acorenone (peak 143), shyobunones (peaks 99, 100, 103 and 107), isoshyobunones (peaks 104, 105 and 108) and camphor (peak 42) make up 46.2% of the volatile fraction. Acorone, a minor component in essential oil, is the most abundant component in the alcoholic extract. Acoragermacrone is absent, while traces of calamusenone (peak 142), calamusenone isomer (peak 160) and tropone (peak 199) have been found.

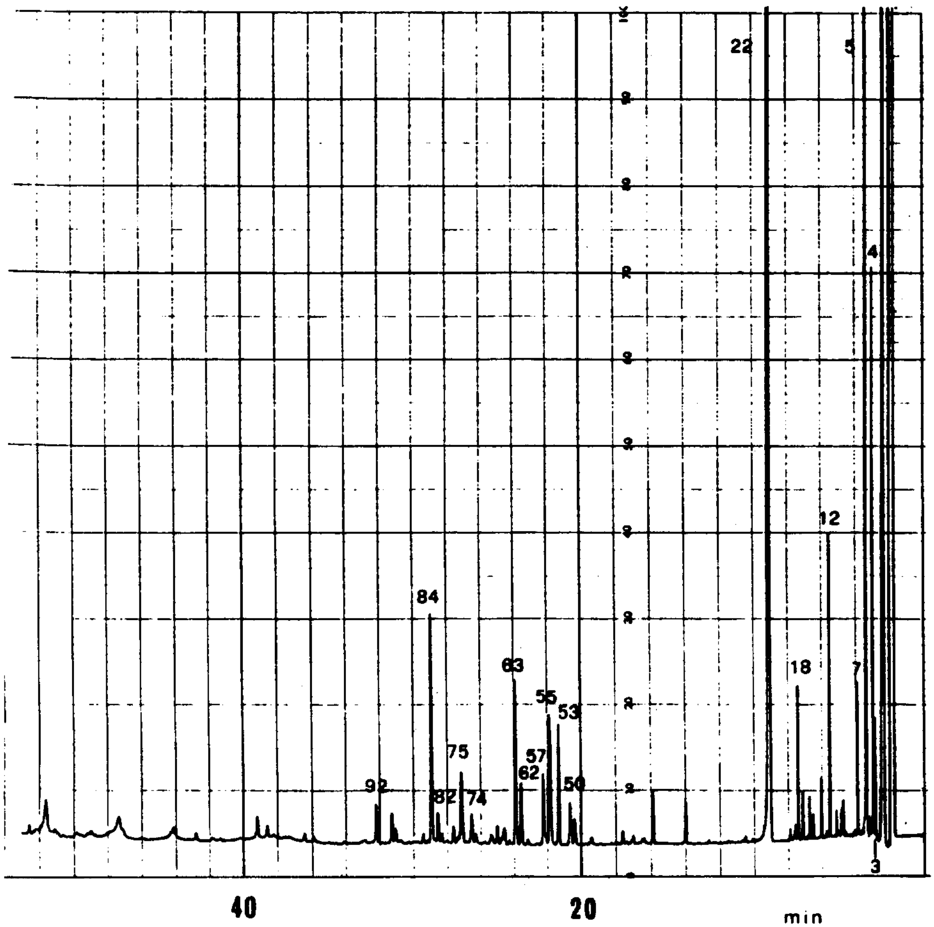

Fig. 2. Gas chromatogram of fraction I.

Alcohols

Alcohols have been found in fractions III and IV. Calamendiol (peak 183), spathulenol (peak 136) and linalool (peak 49) are the main representatives in this class. Compared to the essential oil, the additional presence of borneol (peak 77), *trans*-pinocarveol (peak 69), *cis*- and *trans*-sabinene hydrate (peak 47 and 36) and 3,7-dimethyl-1,5-octadien-3,7-diol (peak 115) was observed.

β-Asarone oxidation products

β- and *α*-asarone form 8.4 and 0.1% respectively of the volatile fraction, while *β*-asarone oxidation products constitute 1.3%. Their importance is not connected with the aroma of calamus, but with the supposed toxicity of *β*-asarone, whose content in beverages is at present limited by legislation.

Regarding the essential oils, a chemical oxidation of *β*-asarone was considered, while in the case of the rhizome an alternative enzymatic oxidation is possible. The suggested oxidation pathway for *β*-asarone also takes into account a reaction be-

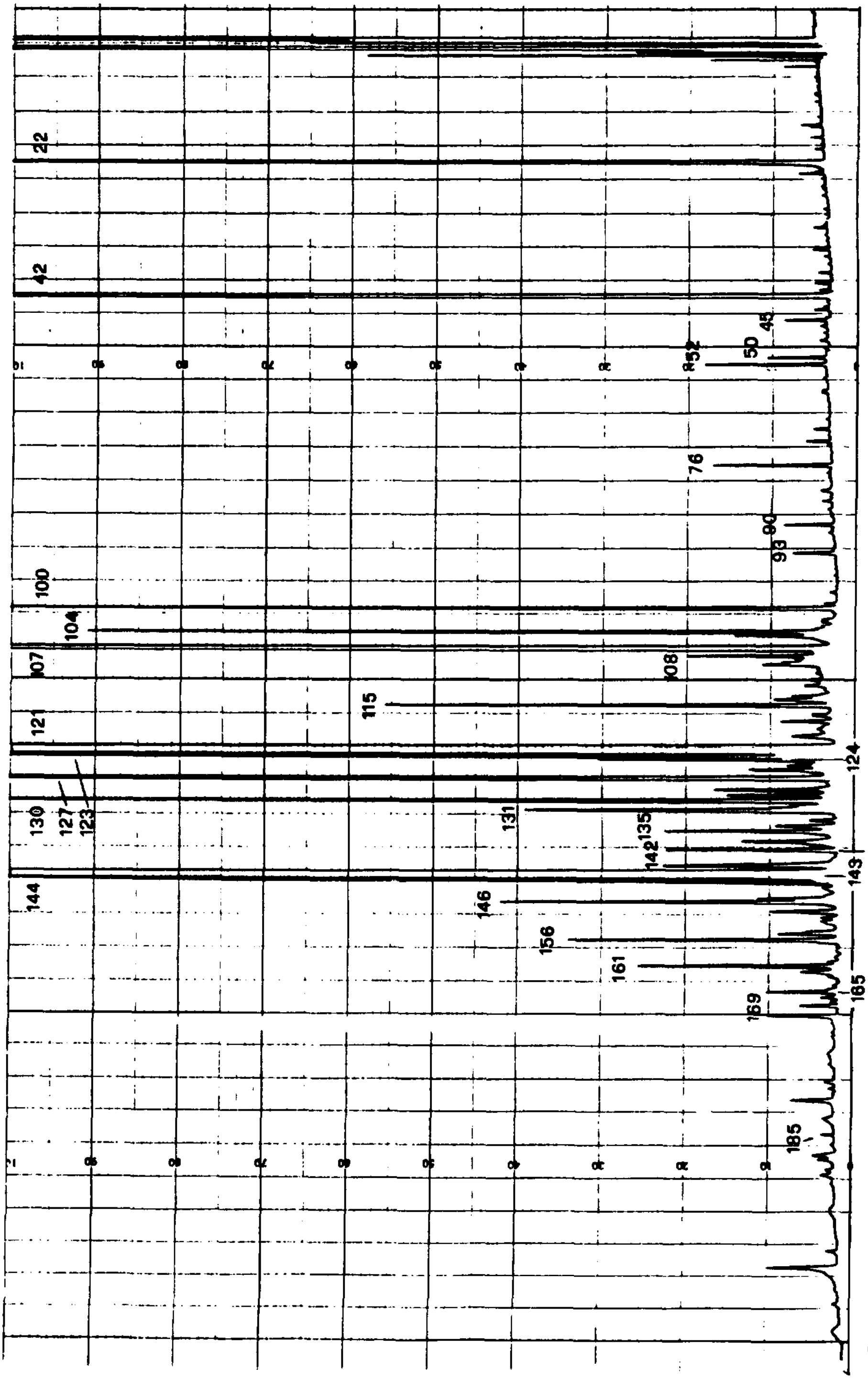

Fig. 3. Gas chromatogram of fraction II.

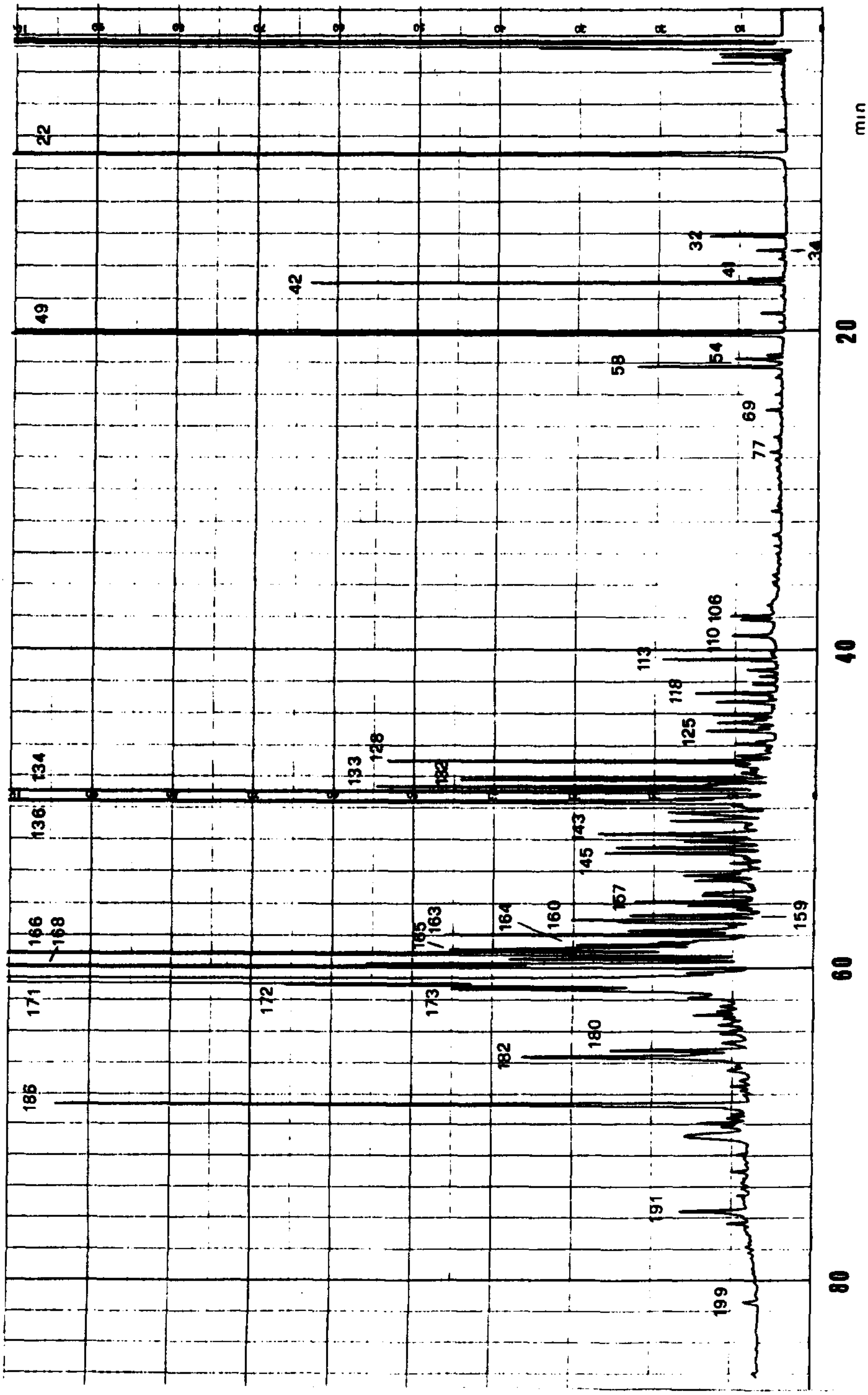

Fig. 4. Gas chromatogram of fraction III.

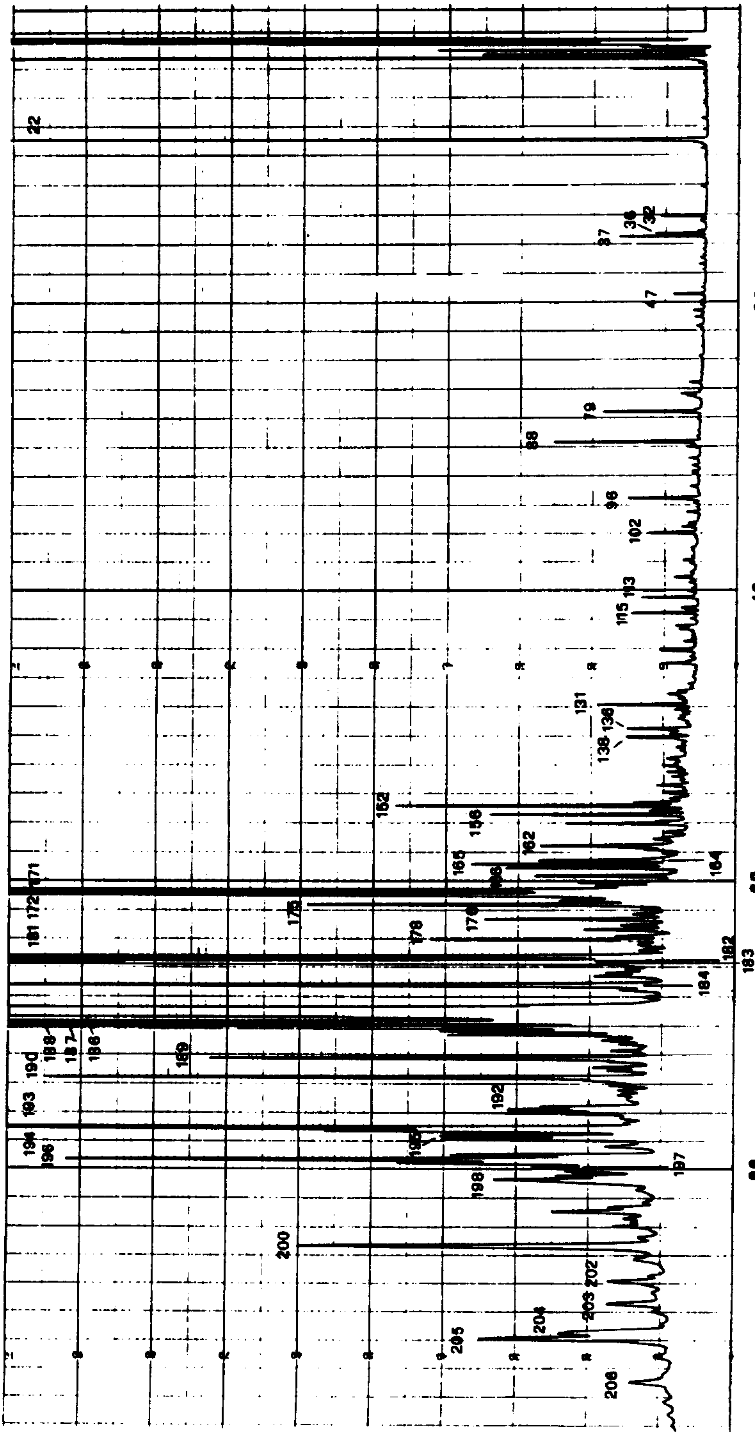

Fig. 5. Gas chromatogram of fraction IV.

TABLE I

IDENTIFICATION OF PEAKS IN FIGS. 1–5

I.S. = Internal standard; t = less than 10.0 ppm.

Peak No.	*Compound*	*Silica gel fraction*	*Amount (ppm)*	*Mol. wt.*	*Peak No.*	*Compound*	*Silica gel fraction*	*Amount (ppm)*	*Mol. wt.*
1	Propanal diethyl acetal*	II**	t	132	31	Acetic acid	IV	t	60
2	Ethyl isobutyrate*	II**	t	116	32	*trans*-Linalool oxide (furanoid)	III, IV	11.14	170
3	α-Thujene	I	t	136	33	Unknown	II	t	69***
4	α-Pinene	I	37.84	136	34	1-Octen-3-ol	III	t	128
5	Camphene	I	421.49	136	35	2-Furaldehyde	II	t	96
6	Hexanal*	II	t	100		1-Heptanol*	IV	t	116
7	β-Pinene	I	14.70	136	36	*trans*-Sabinene hydrate*	IV	t	154
8	Sabinene	I	t	136	37	*cis*-Epoxyocimene*	II	t	152
9	Pentanal diethyl acetal*	II**	t	160		*cis*-Linalool oxide (furanoid)	III, IV	11.05	170
10	Myrcene	I	t	136	38	8-Ethoxy-*p*-cymene*	II**	t	178
11	α-Terpinene	I	t	136	39	α-Copaene	I	t	204
12	Limonene	I	18.94	136	40	*trans*-Epoxyocimene*	II	t	152
13	*cis*-Ocimene	I	t	136	41	2-Hydroxy-3-methylvalerianic			
14	γ-Terpinene	I	t	136		acid methyl ester	III, IV	t	146
15	Hexanal diethyl acetal*	II**	t	174	42	1,7-Diepi-α-cedrene	I	t	204
16	Octan-3-one*	II	t	128		Camphor	II, III	619.44	152
17	*trans*-Ocimene	I	t	136	43	2-Hydroxy-3-methylvalerianic	III**	t	160
18	*p*-Cymene	I	12.81	134		acid ethyl ester*	IV**		
19	Terpinolene	I	t	136	44	α-Gurjunene	I	t	204
20	Unknown	II	t	140		Unknown	II	t	152
21	Terpene hydrocarbon	IV**	t	136	45	Unknown	II	t	152
22	2-Heptanol	–	I.S.	116	46	Menthyl acetate	II, IV**	t	198
23	6-Methyl-5-hepten-2-one*	II	t	126	47	*cis*-Sabinene hydrate*	IV	t	154
24	Unknown	II	t	140	48	Unknown	II	t	154
25	An *allo*-ocimene	I	t	136	49	Linalool	II III	171.52	154
26	Unknown hydrocarbon	IV**	t	134	50	α-Cedrene	I	t	204
27	4-Methylisopropenylbenzene	IV**	t	132		Linalyl acetate	II	t	196
28	Unknown	II	t	137***	51	Isomenthyl acetate	II, IV**	t	198
29	*trans*-3-Ethoxy-2(10)pinene ?*	II**	t	180	52	Bornyl acetate	II	11.02	196
30	4-Ethoxy-1-*p*-menthene*	II**	t	182	53	Sesquiterpene hydrocarbon	I	t	204

54	α-*trans*-Bergamotene	I	t	204
	Camphene hydrate	III, IV	t	154
55	β-Gurjunene	I	t	204
56	β-Elemene	I	t	204
57	Sesquiterpene hydrocarbon	I	t	204
58	Terpinen-4-ol	III	t	154
59	β-Caryophyllene	I	t	204
60	Myrtenal*	II	t	150
61	Hotrienol	IV	t	154
62	Sesquiterpene hydrocarbon	I	t	204
63	Sesquiterpene hydrocarbon	I	t	204
	endo-Isocamphanone	III	t	152
64	γ-Butyrolactone*	IV	t	86
65	Butyric acid*	IV	t	88
66	Acetophenone*	IV**	t	120
67	Menthol	IV	t	156
68	An acoradiene	I	t	204
	Sesquiterpene hydrocarbon	IV**	t	202
69	*trans*-Pinocarveol*	III	t	152
	2-Vinyl-2-methyltetra-hydrofuran-5-one*	IV	t	126
70	Unknown	II	t	150
71	α-Humulene	II	t	204
72	α-Acoradiene	I	t	204
73	Unknown	III	t	206
74	An acoradiene*	I	t	204
75	β-Acoradiene	I	t	204
76	*trans*-Pinocarvyl acetate	II	10.11	194
77	An acetate	II	t	194
	Borneol*	III	t	154
78	γ-Gurjunene ?	I	t	204
79	α-Terpineol	IV	20.71	154
80	Carvenone*	IV**	t	152
81	Naphthalene*	II, III	t	128
82	β-Selinene	I	t	204
83	Carvone	IV	t	150
84	α-Selinene	I	t	204
	Piperitone	IV**	t	152
85	*p*-Mentha-1(7)-2-dien-8-ol ?*	IV	t	152
	Valerianic acid*	IV	t	102
86	γ-Acoradiene ?	I	t	204
87	*trans*-Linalool oxide (pyranoid)	IV	t	170
88	Unknown	IV	35.82	164
89	*cis*-Linalool oxide (pyranoid)	IV	t	170
90	Unknown	II	t	210
91	δ-Cadinene	I	t	204
	Unknown	IV	t	206
92	β-Sesquiphellandrene	I	t	204
93	α-Curcumene	II	t	202
94	Terpene alcohol	IV	t	152
95	4,10-Dimethyl-7-isopropyl-bicyclo(4.4.0]-1,4-decadiene*	I	t	204
96	Terpene alcohol	IV	15.94	152
97	An acetate ?	II	t	194
98	A calamenene	I	t	202
99	A shyobunone epimer	II	t	220
	trans-Carveol*	III, IV	t	152
100	6-Epishyobunone	II	109.94	220
101	A calamenene	I	t	202
102	*p*-Cymen-8-ol	III, IV	t	150
	Capronic acid*	IV	t	116
103	2,6-Diepishyobunone	II	t	220
104	Isoshyobunone	II	74.57	220
	Benzyl alcohol*	IV	t	108
105	An isoshyobunone epimer	II	t	220
106	Sesquiterpene alcohol	IV	t	222
107	Shyobunone	II	490.86	220
	Unknown sesquiterpene	IV	t	220
108	An isoshyobunone epimer	II	13.83	220
109	2-Phenylethanol*	IV	t	122
110	α-Calacorene	III**, IV**	12.32	200

(Continued on p. 204)

TABLE I (*continued*)

Peak No.	Compound	Silica gel fraction	Amount (ppm)	Mol. wt.	Peak No.	Compound	Silica gel fraction	Amount (ppm)	Mol. wt.
111	Sesquiterpene ketone	II	t	220	134	*cis*-Isoeugenol methyl ether	III	115.85	178
112	A selinadienol*	III	t	222	135	Acoragermacrone	II	38.41	220
113	A selinadienol*	III, IV	19.42	222		Sesquiterpene ketone	II		236
114	Sesquiterpene oxide	II	12.01	220	136	Spathulenol	III, IV	194.30	220
	A calacorene	III**		200	137	Unknown	II	t	220
		IV**			138	Unknown	IV	t	222
115	Carophyllene oxide	II	55.08	220	139	Sesquiterpene ketone	II	30.27	220
	3,7-Dimethyl-1,5-octadien-3,7-diol*	IV	14.35	170		A selinadienol*	III, IV		222
116	Unknown	III, IV	t	232	140	An eudesmol ?	III, IV	t	222
117	Unknown	II	t	165***	141	Unknown sesquiterpene	III	t	220
118	Cadala-1,4,9-triene	I, IV**	12.31	202	142	Calamusenone	II	30.02	218
	Unknown	III	t	232	143	Acorenone	II, III	1431.16	220
119	γ-Eudesmol ?	III	t	222	144	Unknown	II	164.72	220
120	Humulene epoxide I	II	t	220		T-Cadinol	III	t	222
121	Pre-isocalamendiol	II	1557.98	220	145	10-Epi-α-cadinol	III, IV	23.21	222
	Sesquiterpene alcohol	IV	t	220	146	Unknown sesquiterpene	II	32.25	220
122	Viridiflorol ?	III	t	222	147	Elemicine	III	t	208
123	Humulene epoxide II	II	119.62	220	148	Torreyol	IV	t	222
124	Sesquiterpene ketone	II	20.06	220	149	An acorenol ?	IV	t	222
125	Unknown	II	15.62	220		*trans*-Isoeugenol methyl ether	III	t	178
	Sesquiterpene alcohol	III, IV		222	150	An eudesmol	IV	t	222
126	Unknown sesquiterpene	II	t	222	151	Unknown sesquiterpene	II	t	238
127	Unknown sesquiterpene	II	182.65	222		Carvacrol	III	t	150
128	*trans*-Nerolidol	III, IV	45.42	222	152	10-α-Cadinol	IV	42.82	222
129	A selinadienol*	III	t	222	153	Sesquiterpene ketone	II	t	220
130	Sesquiterpene ketone	II	82.85	220		A spiroketone	III	t	124***
	Caprylic acid	IV	t	144	154	Unknown sesquiterpene	II	t	218
131	Unknown sesquiterpene	II	39.69	220	155	Sesquiterpene alcohol	IV	36.94	222
	Elemol	III, IV	t	222	156	Unknown sesquiterpene	II	29.74	218
132	A guaienol	IV	27.86	222	157	*cis*-Spiro[4.5]decenone*	III	t	206
	Sesquiterpene alcohol	III		222	158	Unknown	IV	28.58	218
133	Cedrol	III	43.20	222	159	Isospathulenol	III	t	220

160	Calamusenone isomer	II	t	218
	cis-Isoelemicine	III	28.33	208
161	Sesquiterpene ketone	II	22.44	236
162	Sesquiterpene alcohol	III, IV	38.50	222
163	A spiroketone ?	III	40.37	206
164	Sesquiterpene alcohol	III, IV	t	222
165	Sesquiterpene alcohol	III	37.21	222
	Sesquiterpene alcohol	IV	42.31	238
166	Sesquiterpene alcohol	III	122.18	222
	Sesquiterpene alcohol	IV	33.18	220
167	Unknown sesquiterpene	II	t	218
168	Sesquiterpene ketone	III	118.23	236
169	4-Isopropyl-6-methyl-1,2,3,4-tetrahydronaphthalen-1-one	II	t	202
170	A hydroxycalamenene*	III, IV	19,21	218
171	*β*-Asarone	III, IV	1723.13	208
172	Sesquiterpene alcohol	III, IV	307.98	220
173	A hydroxycalamenene	III, IV	43.07	218
174	Unknown	III	t	236
175	Isocalamendiol	IV	76.82	238
176	Unknown sesquiterpene	IV	39.02	236
177	Unknown	IV	t	218
178	Unknown	IV	76.18	238
179	*trans*-*β*-Sesquiphellandrol	IV	t	220
180	Unknown	III	22.35	238
181	Unknown	IV	307.06	218
182	*α*-Asarone	III, IV	21.72	208
183	Calamendiol	IV	1709.07	238
184	Oplopanone ?	III, IV	522.14	238
185	Unknown	II	t	218
186	Acorone	III, IV	5245.77	236
187	Isoacorone	III, IV	329.26	236
188	An acorone	III, IV	414.78	236
	An acoronene ?	IV	t	234
189	An acoronene	IV	159.60	234
190	An acoronene*	IV	192.92	234
	Unknown	IV		232
191	An acoronene*	III IV	t	234
192	2,4,5-Trimethoxyphenylacetone	IV	57.45	224
193	1-(2,4,5-Trimethoxyphenyl)-1-ethoxypropan-2-ol**	IV**	92.32	270
194	Unknown	IV	301.24	236
195	Asaronaldehyde	IV	64.62	196
196	Unknown	IV	234.53	252
197	2,4,5-Trimethoxypropiophenone	IV	t	224
198	1-(2,4,5-Trimethoxyphenyl)-1-ethoxypropan-2-ol*	IV**	50.23	270
199	A hydroxycalamenene ?*	III	t	218
	Tropone	III	t	174
200	Unknown	IV	155.25	252
201	Unknown	IV	14.02	252
202	Unknown	IV	19.01	252
203	Unknown	IV	20.42	252
204	Unknown	IV	25.21	252
205	Unknown	IV	61.10	252
206	Unknown	IV	17.57	252

* Identified for the first time in *A. calamus* L.
** Artifact.
*** Base peak.

tween ethanol and a probable epoxidic intermediate giving two diastereoisomers of 1-(2,4,5-trimethoxyphenyl)-1-ethoxypropan-2-ol (peak 193 and 198). These two compounds, which represent 53% of the oxidation products in the alcoholic extract, are absent from European calamus oil but present as traces in Indian calamus oil, as diastereomers of 1-(2,4,5-trimethoxyphenyl)-1-methoxy-propen-2-ol (peak 87 and 91, see Part I, Table I[2]).
their methanolic derivatives.

Mass spectra of these compounds have been published recently[3].

Artifacts

Three hydrocarbons (peaks 21, 26 and 27) have been found in fraction IV, instead of fraction I as expected. The same compounds, on the other hand, are presented in the organic extract before preseparation by liquid–solid chromatography. As the occurrence of these hydrocarbons seems to be linked to the presence of well determined alcohols, a thermal dehydration taking place in the gas chromatograph injector may be responsible for the formation of such hydrocarbons from the corresponding alcohols, namely 4-methylisopropenylbenzene (peak 27) from *p*-cymen-8-ol (peak 102), compounds 21 and 26 from two unidentified alcohols. Similar conclusions can be reached regarding the two isomers of calacorene.

The presence of several sesquiterpene hydrocarbons in fractions III and IV (fraction III for essential oil, see Part I, Table II) is probably an artifact. Cadala-1,4,9-triene, for instance, is present in fractions I and IV as well as in the organic extract before the preseparation. The amount in fraction I must be taken as naturally, while that found in fraction IV might come from a kind of dehydration as pointed out previously.

Menthyl and isomenthyl acetates already found in fraction II are unexpectedly present in fraction IV. The simultaneous occurrence of menthol and acetic acid in fraction IV might be responsible for the formation of these compounds.

Other artifacts are found in the alcoholic extract as ethers, acetals, esters, etc.; β-asarone oxidation products themselves must also be regarded as artifacts. Mass spectra of the identified ethyl ethers have already been published[4].

In order to elucidate the occurrence of artifacts both in essential oils and alcoholic extracts from natural sources, further research is being carried out.

REFERENCES

1 *Flavouring Substances and Natural Sources of Flavourings*, Council of Europe, Maisonneuve, Moulins-Lès-Metz, 3rd ed., 1981, Appendix A, p. 34.
2 G. Mazza, *J. Chromatogr.*, 328 (1985) 179.
3 G. Mazza, *Sci. Aliments*, 4 (1984) 473.
4 G. Mazza, *J. Chromatogr.*, 264 (1983) 304.