Z Lebensm Unters Forsch (1983) 176:363–366

Zeitschrift für
Lebensmittel-
Untersuchung
und-Forschung

Chemotaxonomy of Wormwood (Artemisia Absinthum L.)

I. Composition of the Essential Oil of Several Chemotypes

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Chemotaxonomie von Wermut (Artemisia Absinthum L.)

I. Zusammensetzung des ätherischen Öls einiger Chemotypen

Zusammenfassung. Die ätherischen Öle des Wermuts, aus mehreren Herkünften und aus verschiedenen Höhen, wurden durch Capillargaschromatographie bestimmt und durch MS, IR, ^{1}HNMR, ^{13}CNMR identifiziert.

Dabei konnten verschiedene Chemotypen festgestellt werden. In den Westalpen ist der cis-Epoxycimen-Typ über 1 000 m der wichtigste Typ, während der β-Thujon-Typ in niedrigeren Zonen vorherrscht.

Französische Muster gehörten zu den Chrysanthenylacetat- und Sabinylacetat-Chemotypen, Muster aus Sibirien, Rumänien und dem Aostatal dagegen zu einem gemischten Typ.

Summary. A series of essential oils obtained from samples of *Artemisia absinthium* L. from various geographical origins and collected at different heights were analysed by means of glass-capillary gas chromatography. The main components were identified by means of GC, GC-MS, IR, ^{1}HNMR and ^{13}CNMR.

Several chemotypes were detected: in the western Alpine arc (Italy) the most important chemotype, above 1,000 m., was a *cis*-epoxy-ocimene type, while at lower levels a α-thujone type predominated.

Plants originating from France could be divided into a chrysanthenyl acetate and a sabinyl acetate chemotype; plants from Siberia, Rumania and some from Valle d'Aosta belonged to a mixed type.

Introduction

The essential oil of the herb of *Artemisia absinthium* L. has been the object of studies in the past, especially for its contents of compound such as β-thujone and a prochamazulene with pharmacodynamic properties; other compounds reported are hydrocarbons derived from thujane and camphane and, in smaller amounts, pinanes, monocyclic and acyclic monoterpenes, sesquiterpenes, sesquiterpenes lactones, tetrahydrofurofuran lignans, 1,8-cineole, as well as satured and unsatured fatty acids [1–17].

Recently two other compounds have been found in significant quantities in herbs of different origins: *cis*-epoxy-ocimene [18] and *trans*-sabinyl-acetate [19].

The observations carried out in recent years on essential oils and alcoholic extracts of *Artemisia absinthium* of different geographical origins and, within the same region, collected at different altitudes and exposures, have enabled us to understand that considerable qualitative and quantitative differences exist, and that these can be brought to light by means of GC analysis and by organoleptic tests.

Results and Discussion

From previous experience (unpublished data), we have found that the only differences encountered between dried whole plants and dried communited leaves and flowers were in percentage yields, rather than in the qualitative composition, of the essential oils. In addition, variations in quantitative composition between fresh and dried plants were negligible, compared to the differences encountered between different collections or different geographical areas.

A comparison between the different samples, from an analytical point of view, is thus easier than might be supposed at first sight, given their heterogenous nature.

By means of GC and GC-MS of the essential oils of *Artemisia absinthium* it was possible to detect 56 compounds, of which 50 were identified, some are reported for the first time in this oil (Table 1). In Table 2 the

Table 1. Components found in analysed samples

Components	Identification	Components	Identification
Thujene	GC-MS[a]	18 Sabinyl acetate	GC-MS[b] GC[c,d] Hydrolysis
1 α-Pinene	GC-MS[b] GC[c,d]	Humulene	GC-MS[b]
Camphene	GC-MS[b] GC[c,d]	Lavandulol	GC-MS[b]
β-Pinene	GC-MS[b] GC[c,d]	19 β-Curcumene (°)	GC-MS[a]
2 Sabinene	GC-MS[b] GC[c,d]	20 Germacrene-d (°)	GC-MS[a]
3 Myrcene	GC-MS[b] GC[c,d]	21 Sabinol	GC-MS[b] GC[c,d]
α-Terpinene	GC-MS[b]	22 Neryl acetate	GC-MS[b] GC[c,d]
4 Limonene	GC-MS[b] GC[c,d]	ar-Curcumene (°)	GC-MS[a]
5 1,8-Cineole	GC-MS[b] GC[c,d]	23 Chrysanthen-diol? (°)	GC-MS GC[c,d]
6 β-Ocimene	GC-MS[b] GC[c,d]	24 Geranyl acetate	GC-MS[b]
7 γ-Terpinene	GC-MS[b] GC[c,d]	25 Nerol	GC-MS[b]
8 p-Cymene	GC-MS[b] GC[c,d]	26 Neryl iso butyrate (°)	GC-MS[b] GC[c,d]
9 Terpinolene	GC-Ms[b] GC[c,d]	27 Geranyl iso butyrate (°)	GC-MS[b] GC[c,d]
n-Hexanol (°)	GC-MS[b]	Geraniol	GC-MS[b]
Nonanal (°)	GC-MS[b]	28 Neryl n-butyrate (°)	GC-MS[b] GC[c,d]
10 α-Thujone	GC-MS[b] GC[c,d]	29 Neryl iso valerate (°)	GC-MS[b]
11 β-Thujone	GC-MS[b] GC[c,d]	30 M. W. 186	GC-MS
12 *cis*-Epoxy ocimene	GC-MS[b(18)] GC[c,d], IR, ^{1}H-NMR, ^{13}C-NMR	31 Geranyl n-butyrate (°)	GC-MS[b] GC[c,d]
		32 Geranyl iso valerate (°)	GC-MS[b] GC[c,d]
1-Octen-3-ol (°)	GC-MS[b]	M. W. 186	GC-MS
α-Copaene	GC-MS[a]	M. W. 186	GC-MS
13 *trans*-Epoxy ocimene	GC-MS[b(18)] GC[c,d], IR, ^{1}H-NMR, ^{13}C-NMR	Caryophyllene oxide (°)	GC-MS[b]
		Nerolidol (°)	GC-MS[b]
14 Camphor (°)	GC-MS[b] GC[c,d]	33 Spathulenol (°)	GC-MS[a]
15 Linalool	GC-MS[b] GC[c,d]	34 α-Bisabolol (°)	GC-MS[b]
16 Chrysanthenyl acetate (°)	GC-MS[b] GC[c,d], IR, Hydrolysis	119–132	GC-MS
17 β-Caryophyllene	GC-MS[b]	119–132	GC-MS
Terpinen-4-ol	GC-MS[b]	119–132	GC-MS
β-Farnesene (°)	GC-MS[a]	Chamazulene	GC-MS[a]

?=tentative, [a]=Literature spectra [21, 22, 23], [b]=Original sample spectra, [c]=Carbowax 20 M, [d]=OV 1, (°)=Found for the first time in wormwood oil

data concerning the 32 components identified are summarized.

Four constituents predominate in some samples. In some cases they constitute more than 40% of the total essential oil. We consider as chemotypes [20] these plants whose oils present such a characteristic.

Hence, there occur "pure" chemotypes of *cis-epoxy-ocimene* (*e.g.* sample n. 9 in which the *cis*-epoxy-ocimene represents 54% of the oil), of *sabinyl acetate* (e.g. sample n. 18 with 41%), and of β-thujone (*e.g.* sample n. 19 with 40%). Similarly there exist "mixed" chemotypes, plants of which the oils contain two or more of the compounds mentioned in large amounts, for example β-thujone + *cis*-epoxy-ocimene (sample n. 5 and 6), β-thujone + sabinyl acetate (sample n. 4), *cis*-epoxy-ocimene + chrysanthenyl acetate + sabinyl acetate (sample n. 3), etc.

However, as to the constituents that provide the matrix of the "mixed" chemotypes, it should be pointed out that there is not a clear demarcation between the various "mixed" types, even though a certain chemical relationship exists.

Particularly in the Western Alpine arc we observed only one "pure" chemotype (*cis*-epoxy-ocimene), whereas of the "mixed" chemotypes the most frequently occurring one was the *cis*-epoxy-ocimene + β-thujone type.

Depending on the altitude at which the plant material was collected, as well as the degree of development of the plants, the ratio *cis*-epoxy-ocimene/β-thujone was found into increase.

Experimental

Material

All the plant material investigated was uncultivated. Of the 19 samples in question, 14 considered of dried and communited leaves and flowers, two of entire dried plants, and the remaining three of fresh entire plants.

Eight of the samples were obtained from commercial sources, the rest were collected by ourselves in different geographical areas (Table 3).

Essential Oil

The essential oil was obtained by steam distillation of the herb and the quantitiy was determined using the apparatus described in *Pharmacopoea Helvetica* (Vol. I, p. 175).

Table 2. The most important compounds (%) found in all the samples

No.	Components	Sample No.																		
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
1	α-Pinene	–	0,54	0,26	0,27	0,18	0,34	0,86	0,45	1,51	0,13	0,45	2,04	1,11	0,37	0,10	0,24	0,10	1,46	0,57
2	Sabinene	1,35	0,26	0,29	1,40	0,33	1,48	1,38	2,20	4,01	0,53	1,10	6,70	2,69	1,07	0,37	6,14	0,83	0,81	6,34
3	Myrcene	0,54	0,23	0,40	3,62	0,26	0,64	1,98	2,11	3,24	0,25	0,89	5,73	0,78	–	2,09	9,06	0,47	2,90	1,39
4	Limonene	–	–	–	–	–	0,12	0,19	1,18	0,27	–	–	–	–	–	–	0,29	0,10	–	0,33
5	1.8-Cineole	0,68	–	0,15	0,17	–	0,10	1,14	0,13	0,21	–	–	–	–	–	–	0,29	0,22	0,15	0,32
6	β-Ocimene	0,13	0,73	0,36	0,15	0,24	0,43	1,46	0,79	3,70	0,31	2,08	0,47	0,72	0,14	–	0,22	0,10	–	0,51
8	p-Cymene	0,52	–	–	0,54	–	0,10	0,45	0,11	0,21	–	–	0,11	–	–	0,20	1,80	0,13	0,22	0,17
10	α-Thujone	0,90	–	0,20	9,37	0,70	0,74	–	0,29	0,24	–	0,30	0,32	0,17	0,10	0,12	0,63	0,20	1,32	1,68
11	β-Thujone	8,75	0,81	0,33	7,57	21,68	20,90	–	5,22	–	0,11	7,78	6,85	0,38	2,43	0,58	14,76	0,71	18,72	40,60
12	cis-Epoxy ocimene	3,83	42,20	25,73	1,68	24,19	28,90	38,80	31,46	54,38	30,84	45,14	28,73	46,98	40,29	–	3,80	0,12	0,34	22,39
13	trans-Epoxy ocimene	–	1,61	0,81	0,11	1,03	1,42	3,51	2,29	2,32	1,48	1,40	1,09	1,91	1,06	–	0,24	–	–	0,76
14	Camphor	–	0,74	0,35	0,24	–	–	9,30	4,86	2,17	2,39	1,77	0,33	0,29	0,19	0,31	0,27	–	0,18	–
15	Linalool	9,35	0,36	1,29	3,37	2,35	2,15	1,54	0,83	0,33	0,39	0,24	2,54	1,65	0,68	2,53	5,57	0,60	0,41	0,53
16	Chrysanthenyl acetate	–	9,85	15,59	0,92	3,17	4,32	–	0,59	1,32	–	2,90	–	–	10,96	5,80	–	–	41,54	1,49
17	β-Caryophyllene	2,95	1,67	2,19	1,28	0,64	0,66	2,05	1,75	0,60	1,99	1,48	1,29	1,06	1,46	0,82	0,95	0,11	–	0,22
18	Sabinyl acetate	–	0,27	7,41	7,88	0,27	0,43	10,76	11,52	–	–	1,06	–	–	3,54	31,47	1,57	84,48	0,73	0,92
19	β-Curcumene	2,2	0,89	0,56	0,99	–	0,69	0,55	0,31	0,43	0,44	0,80	–	–	0,44	1,65	1,40	0,31	0,29	–
20	Germacrene-D	–	2,64	2,03	–	0,78	1,43	1,16	0,60	0,85	1,34	1,87	1,16	0,97	1,61	–	0,48	0,26	–	0,40
21	Sabinol	2,09	0,38	0,42	1,51	–	–	1,67	1,73	–	2,53	0,19	3,66	2,53	0,26	0,81	1,57	4,18	0,49	0,19
22	Neryl acetate	–	0,18	0,12	0,62	2,12	0,43	–	0,57	–	0,89	0,11	0,11	–	–	0,79	0,18	0,43	0,62	–
23	Chrysanthen-diol?	–	1,02	1,84	0,96	5,30	6,57	0,69	2,60	8,30	0,55	0,94	13,95	17,25	5,37	0,47	0,12	–	2,40	1,78
24	Geranyl acetate	2,41	0,64	1,04	2,10	1,64	1,42	0,75	0,64	0,39	1,69	0,60	0,61	0,55	0,54	4,17	1,68	0,14	0,22	0,45
25	Nerol	1,30	0,49	1,04	1,29	0,43	0,35	2,17	0,78	0,81	0,91	0,95	0,73	0,88	1,11	3,16	0,71	0,19	1,15	0,97
26	Neryl iso butyrate	1,65	1,14	0,46	2,25	–	0,16	1,17	0,48	0,27	3,35	0,89	0,68	0,56	0,49	2,59	1,38	0,45	0,25	0,21
27	Geranyl iso butyrate	–	0,26	0,13	0,21	–	0,18	–	–	–	0,92	0,19	–	–	–	0,75	0,40	0,19	0,12	0,33
28	Neryl n-butyrate	5,33	3,07	1,70	6,98	1,30	1,22	0,49	0,85	0,28	8,06	1,86	0,98	1,03	0,86	7,89	1,83	0,24	1,10	0,97
29	Neryl iso valerate	3,87	2,15	1,58	6,56	1,14	0,98	0,52	0,70	0,29	3,89	1,47	1,06	0,96	0,62	9,05	1,65	0,22	0,67	1,19
30	M. W. 186	–	1,83	0,41	0,45	2,26	1,27	0,23	1,01	0,21	1,73	1,30	1,33	1,27	–	–	5,02	–	0,15	0,26
31	Geranyl n-butyrate	1,15	0,43	0,19	0,94	–	0,14	–	–	–	1,21	0,21	0,12	0,12	0,10	0,85	0,47	0,10	–	–
32	Geranyl iso valerate	0,80	0,20	0,16	0,69	–	0,13	–	–	–	0,38	0,14	0,12	0,15	–	0,77	0,21	–	–	–
33	Spathulenol	2,41	0,41	0,64	1,34	0,69	0,45	0,91	1,02	–	0,62	0,36	0,32	0,55	0,22	1,55	–	0,14	0,12	–
34	α-Bisabolol	1,07	1,47	2,54	2,34	2,27	1,71	1,70	1,94	1,47	3,38	1,55	1,28	1,28	2,32	–	–	–	0,44	0,56

Table 3. Key of samples

No. Locality	Height a.s.l. meters	Harvest year	% Ess. oil.	Type
1 Aosta Valley		1979	0.26	1a
2 Aosta Valley		1980	1.20	1a
3 Macra Valley		1981	0.70	1a
4 Siberia II		(1980)	1.20	1a
5 Vermenagna Valley	1300	1981	0.60	1a
6 Stura Valley	1400	1981	0.50	1a
7 Cret Tzavanis (Aosta Valley)	2100	1981	0.50	1b
8 Crest (Aosta Valley)	1900	1981	0.50	1b
9 Gimillan Le Selve (Aosta V.)	1950	1981	0.80	1b
10 Champ Mort (Aosta Valley)		1980	0.96	1b
11 Champillon (Aosta Valley)		1980	1.24	1b
12 Roya Valley (France)	1100	1980	1.54	1b
13 Roya Valley (France)	1400	1980	1.60	1b
14 Chianale (Varaita Valley)	1800	1980	0.75	1b
15 Siberia I		(1980)	0.30	2a
16 Rumania		(1980)	0.25	2a
17 Maine & Loire (France)		1980	n.d.	3b
18 Isère Valley (France)		1980	n.d.	3b
19 Chivasso	250	1981	0.50	3b

1=dried browsed leaves and flowers, 2=whole dried herb, 3=whole fresh herb, a=commercial sample, b=collected plant material

Physico-chemical Apparatus

GC-MS: Hewlett-Packard 5992 A with Carbowax 20 M packed fused-silica columns (25 m × 0.3 mm) operated at E.I., 70 eV. IR: Perkin-Elmer 257. H NMR: Jeol C 60 HL. C NMR: Jeol PFT 100 at 25.15 MHz. GC. Carlo Erba model 4160 using glass capillary columns (25 m × 0.2 mm) with Stationary phase a) Carbowax 20 M, film tickness 0.1–0.5 nm and b) OV1 film tickness 0.2–0.25 nm, carrier gas Hydrogen with flow ratio a) 2.5 ml min, b) 2.2 ml min. Split ratio a) 1:30 b) 1:40, injector 250 °C. Programmed column a) 3 min at 60 °C, 60–180 °C 3°/min b) 3 min 25 °C, 25–180 °C 3°/min. Integration: Perkin-Elmer Sigma 10 CDS.

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Received November 11, 1982