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GC-MS-SPECMA BANK ANALYSIS OF *JUNIPERUS COMMUNIS* NEEDLES AND BERRIES

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Key Word Index—*Juniperus communis*, Cupressaceae, juniper, needles, berries, essential oil, monoterpenes, sesquiterpenes, gas chromatography, mass spectrometry, headspace, SPECMA data bank

Abstract—Using a combination of gas chromatography-mass spectrometry (electron impact) as well as the headspace technique, more than 70 compounds were found in the juniper needle oil obtained from plants growing in southeastern France (Provence) A comparison between the headspace of fresh and bottled black berries shows a significant loss of the monoterpenoid fraction with time In the green berries (unripe) this loss is more marked. A sesquiterpenoid fraction of the *Juniperus communis* needle oil was also investigated by means of the GC (FID) and GC-MS (EI, PCI). Among more than 100 isolated constituents, 64 were sesquiterpenoids The presence of several oxygen-containing derivatives based on the calamene and calacorene isomers was established but their structures were not fully determined The various compounds were identified on the basis of our SPECMA data bank (mass spectra and Kováts indices) and our own files

INTRODUCTION

Whereas a number of papers have been devoted to the analysis of essential oils from juniper berries [1–10], with about 174 compounds recorded by Lamparsky and Klimeš [10], there is very little known about the composition of aerated juniper needles with the exception of the monoterpenes [11–17]. These essential oils are of interest because of their high content of sabinene which, however, varies depending on the geographical origin of the essential oil. Because juniper is quite widespread in southeastern France, especially at elevations above 800 m in the Sainte Baume Massif about 30 km from Marseilles, it seemed of interest to investigate the composition of the essential oil from this shrubby tree. First, we have carried out a GC-MS analysis of the essential oil obtained by steam distillation of fresh-collected juniper needles and compared its monoterpene composition with that of other essential oils of different origin.

Secondly, we have undertaken an investigation of the heavy fraction of this oil in order to identify sesquiterpenoids. On the basis of ^1H NMR and IR data, De Pascual Teresa and co-workers [7] found 20 sesquiterpenes as components of a juniper berry oil. Using GC-MS analysis, Bonaga and Galetti [9] showed the presence of 40 components in an oil originating from the Appenines near Bologna However, the mass spectrum attributed to α-cadinol is incorrect

Our interest in natural products in connection with our efforts to complete a specialized data bank in this field [18, 19] has prompted us to study this essential oil of reliable origin Former applications of this approach include our studies of several essential oils, natural aromas and model systems related to the Maillard reaction.

RESULTS AND DISCUSSION

GC-MS data bank analysis of the juniper (needles) essential oil from southeastern France (Sainte Baume, Marseilles)

The constituents identified by their mass spectra and Kováts indices, obtained from the chromatograms reconstructed on the basis of the total ionization current of the essential oil (i) and its heavy fraction (ii), are summarized in Table 1

Among the monoterpenes identified in the heavy fraction of the juniper needle oil, the main components are δ- and γ-cadinenes, (*E*)-β-elemene, (*E*)-α-cadinol, spathulenol, and α-muurolene Besides these compounds, we have also found aromatic hydrocarbons, aldehydes and ketones, alcohols (α-terpineol), esters, phenols, and even C_6 to C_{16} carboxylic acids

A number of sesquiterpenoids with M_rs of 218 and 220 have not been identified By analogy with the base peak at *m/z* 159 and 157 in calamene and calacorene conformers, respectively, one can expect that these compounds are the corresponding alcohols or oxides. However, there are not enough synthetic and/or analytical data available in the literature to make it possible to determine their structures A hydroxy derivative of calamenene was found in the essential oil of *Psiadia salvifolia* by Dennis [20]. The mass spectrum for this compound showed a

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Table 1 GC-MS data bank analysis of the juniper needle essential oil*

Compound	KIP†	Occurrence‡	Compound	KIP†	Occurrence
Monoterpenes and aromatic compounds					
α-Pinene	1030	i	Spathulenol	2037	i, ii
Camphene	1054	i	Corocalene (?)	2060	ii
β-Pinene	1090	i	T-Cadinol	2073	i, ii
Sabinene	1100	i	(*Z*)-α-Cadinol	2075	i ii
Δ-3-Carene	1125	i	δ-Cadinol	2093	
o-Cymene	1126	i	Eudesmol (?)	2110	ii
Myrcene	1140	i	Isospathulenol	2115	ii
α-Phellandrene	1143	i	(*E*)-α-Cadinol	2145	i, ii
α-Terpinene	1156	i	*t,t*-Farnesol	2160	ii
Limonene	1177	i	Calamenol	2166	ii
β-Phellandrene	1185	i	Calacorenol	2193	ii
γ-Terpinene	1227	i	Calamenol	2196	ii
p-Cymene	1255	i, ii	Carbonyl compounds		
α,*p*-Dimethylstyrene		ii	α-Thujone	1410	i
Sesquiterpenes and Derivatives			β-Thujone	1430	i
α-Cubebene	1460	i, ii	Piperitone	1720	i
α-Ylangene	1475	ii	2,4-Nonadienal		ii
α-Copaene	1488	ii	2,4-Undecadienal		ii
β-Bourbonene	1525	i, ii	*p*-Methadienal	-	
β-Copaene	1535	-	Cuminaldehyde	1770	i, ii
(*Z*)-β-Elemene	1578	i, ii	6,10-Dimethyl-5,9-undecadien-2-one		ii
(*E*)-β-Elemene	1593	ii			
β-Caryophyllene	1605	i, ii	Methyl undecyl ketone		ii
Santalene	1608	ii	6,10,14-Trimethylpentadecan-~~2-one~~	-	~~ii~~
α-Elemene	1628	ii			
Allo-Aromadendrene	1638	ii	~~Umbellunone~~	-	~~ii~~
Calarene	1658	—	Cryptone		ii
α-Humulene	1664	i, ii	*Alcohols*		
ε-Cadinene	1668	ii	Linalool		ii
β-Maaliene	1671	ii	*t*-Sabinene hydrate	1450	i
γ-Muurolene	1684	i, ii	4-Terpinenol	1602	i, ii
γ-Elemene	1694	ii	Borneol		ii
Germacrene D	1713	i, ii	Myrtenol	1625	i
Guaiene	1720	ii	*t*-Pinocarveol	1650	i
α-Muurolene	1725	ii	α-Terpineol	1690	i, ii
Germacrene B	1730	ii	*cis*-Sabinol (?)	1694	i
α-Farnesene	1736	ii	*cis*-Piperitol		i
δ-Cadinene	1750	i, ii	~~Citronellol~~	-	~~ii~~
γ-Cadinene	1755	i, ii	*t*-Carveol		ii
Ar-Curcumene	1760	i, ii	Geraniol	1755	ii
α-Ferulene	1778	ii	Dodecanol		ii
(*Z*)-Calamenene	1812	ii	*p*-Methadienol	1780	i
(*E*)-Calamene	1850	ii	1,5-*p*-Menthadien-7-ol	1790	i
(*Z*)-Calacorene	1880	–	*p*-Cymen-8-ol	1827	i
(*E*)-Calacorene	1914	—	Cuminyl alcohol	2038	ii
Caryophyllene oxide	1934	i, ii	*p*-Menthadienetriol (?)	2270	i
Humulene oxide (I)	1940	i, ii			
Humulene oxide (II)	1944	ii			
4(14)-Salvial-1-en-6-one	1950	ii	Phenols		
Humulol (?)	1960	ii	Carvacrol	2130	i, ii
			Esters		
Cubenol	1994	ii	Isoamyl butyrate	1245	i
Cadinenol	1997	ii	Isoamyl isovalerate	1275	i
Oplopenone	2000	ii	2-Methylbutyl isovalerate	1358	i

Table 1 GC-MS data bank analysis of the juniper needle essential oil*

Compound	KIP†	Occurrence‡	Compound	KIP†	Occurrence‡
Linalyl acetate	—	ii	Miscellaneous		
Sabinyl acetate	1530	i	3-(4-Methyl-3-pentenyl)-furan	1415	i
4-Terpenyl acetate	1557	i, ii	α-Pinene oxide	1486	i
Bornyl acetate	1580	i	Even acids, C_6 to C_{16}		i
8-*p*-Cymenyl acetate	—	ii			
Isoamyl benzoate	—	i, ii			

*Compounds whose identification has not been confirmed by comparison with a reference sample are followed by a question mark

†Kováts indices were calculated from the regression line. $KIP = 0.689 \times (scans) + 870.4$, with a correlation coefficient $r = 0.996$ Reference files were No 1834–10/84, 1549A–22/11/84, and 1549C–22/11/84

‡(i) denotes compounds identified in the essential oil and (ii) the compounds identified in the heavy fraction of this oil

§Solvents, phthalates, and more than 30 compounds that have not been identified (mainly sesquiterpenoid derivatives) are not listed in the Table.

molecular ion at m/z 220 with the next peak at m/z 202 corresponding to the loss of a water molecule. The base peak at m/z 159 indicates the basic structure of calamenenes However, it is likely that the hydroxyl is not bonded to the position with the isopropyl group because of the absence of the fragment at m/z 59 characteristic of a tertiary alcohol Consequently, structures such as **1** and **2** for calamenols and calacorenols, respectively, appear more adequate Mass spectra for spathulenol and 4(14)-salvial-1-en-6-one have been recorded by Maurer and Hauser [21].

Table 2 represents a comparison of the terpenoid and aromatic hydrocarbons found in juniper essential oils (needles) from various countries The relative amount of sabinene in our oil (48.4%) is higher than in essential oils of different geographical origin and approaches that found in Norwegian essential oils from elevations between 500 and 1100 m [16]. The high percentage of α-pinene seems characteristic of juniper oils from lower elevations (5–200 m) [6].

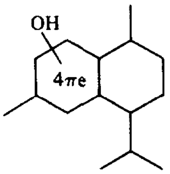
In a study of the essential oil of *Juniperus pseudosabina*, Satar [17] has found increased amounts of α-pinene and sabinene (35.5 and 38.1%, respectively) These numbers demonstrate that our oil is rich in monoterpenes (80%) and especially in sabinene. The important variations in the composition reflect the geographical origin of the oil, the elevation and the species

Headspace analysis of ripe and unripe juniper berries

Using the headspace technique we have investigated the monoterpenoid and aromatic hydrocarbons present in ripe and unripe juniper berries. Volatiles of gin obtained by maceration of black berries in an alcoholic solution (45°) followed by steam-distillation were also studied

Two samples of berries (black and green) were collected in October, 1984, and stored in bottles for two years before analysis. Fresh ripe berries were collected two years later (October, 1986) and immediately analysed by GC. The Tenax-trapped volatiles of the sample were thermally desorbed at 260°/3 sec by column chromatography Relative amounts of monoterpenoids and *p*-cymene in the headspace of juniper berries are given in Table 3 as well as those observed in gin.

The present work provides information about the computerization of data obtained after GC-MS analysis of juniper essential oil (needles) from southeastern France. The composition of monoterpenoid hydrocarbons and *p*-cymene both in the essential oil and in the



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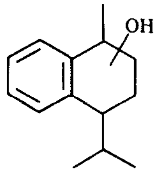

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Table 2. Relative amounts of monoterpene and aromatic hydrocarbons in the juniper needle essential oil from various countries

	Origin of the essential oil					
	I*	II†	III‡	IV§		V‖
				1	2	
Compound	Relative amount (%)					
α-Pinene	16.5	80	34	57	20	40
Camphene	0.12	—	—	—	—	—
β-Pinene	0.9	—	—	—	—	—
Sabinene	48.4	0.2	22	3	39	18
β-Terpinene	0.07	—	—	—	—	—
Δ-3-Carene	0.07	—	—	—	—	—
Myrcene	3.5	—	—	—	—	—
α-Phellandrene	1.6	—	—	—	—	—
α-Terpinene	0.3	—	—	—	—	—
Limonene	2.0	1	5	12	5	—
β-Phellandrene	0.9	—	—	—	—	—
γ-Terpinene	1.2	—	—	—	—	—
p-Cymene	3.2	—	—	—	—	—
α-Terpinolene	1.6	—	—	—	—	—

*The present study. Determined from an analytical chromatogram (50 m × 0.22 mm i d. Carbowax 20M capillary column).

†From Canadian lowlands [13]

‡Norwegian oil (Central Norway) [12, 14]

§Norwegian oils, 1, lowlands and 2, high mountains [16]

‖West German oil (northwest) [15]

Table 3 Relative amounts of terpene hydrocarbons and *p*-cymene in the headspace of juniper berries and gin*

	Berries†		
Compound	Ripe (black)	Unripe (green)	Gin‡
α-Pinene	30 5	49 4	10 0
α-Thujene	traces	traces	traces
β-Pinene	1 7	2 08	0 58
Sabinene	28 0	21 8	9 8
Myrcene	27 5	9 3	6 4
α-Phellandrene	0 67	0 6	0 44
Limonene	3 1	1 21	2 8
β-Phellandrene (+ 1, 8-cineole)	0 78	1 11	0 32
cis-β-Ocimene	0 025	0 017	traces
γ-Terpinene	1 37	1 02	1 16
trans-β-Ocimene	0 05		traces
p-Cymene	0 4	0 42	0 56
α-Terpinolene	0 9	0 47	0 12
Total	95	87 42	32 18

*Analyses were carried out on a DELSI DI 700 instrument equipped with a bubbler and a trap filled with Tenax GC Nitrogen was bubbled through the flask for 15 min Volatiles trapped on Tenax GC and thermally desorbed at 250 were separated using an FFAP capillary column (50 m × 0 28 mm i d)

†Fresh berries (black and green) were collected in October 1986, and immediately analysed Prolonged storage (2 years) led to a significant loss of α-pinene (3 5%), β-pinene (0 25%) and sabinene (10%) (berries collected in 1984)

headspace of berries (ripe, unripe, fresh or bottled) and gin shows some differences in the relative amounts of components However, these differences cannot be used to explain the characteristic aroma of juniper berries and gin Also, the results provide information about the sesquiterpenoids identified in the heavy fraction of this oil

EXPERIMENTAL

Plant material Juniper trunks were harvested in October, 1984 in the Sainte Baume Massif (Marseilles) The needles (~1 kg) were separated from the trunks and berries and were steam-distilled in a stainless-steel apparatus equipped with a condenser The obtained essential oil was yellow with a metallic appearance and was separated by decantation (yield ~4 5 g)

Fractionation of the essential oil The essential oil was distilled under reduced pressure at 50°/4 mm Hg using a Vigreux column (height 10 cm) The volatile fraction was recovered in a receiver kept at −5° The residue representing the heavy fraction (approximately 20% of the essential oil) was analysed by the usual chromatographic techniques and by coupled GC-MS using electron impact (EI) and positive chemical ionization (PCI) techniques

GC analysis using the DELSI headspace technique Volatiles from 30 berries were flushed out by an inert gas (N_2) for 10 min and trapped on Tenax GC Then they were thermally desorbed at 250° and separated on an FFAP column (50 m × 0 28 mm i d) The DELSI 700 apparatus was combined with a Shimadzu C-R3A Chromatopack integrator The chromatograph was especially equipped with a DCI unit for the analysis of the headspace of aroma compounds (both in solid or liquid state)

Chromatographic and GC-MS analyses The analyses were performed under the same conditions as those already described in ref [22]

Calculation of the Kováts indices After having recognized about 10 products in the listing whose Kovats indices on the respective column are known, a linear regression allowed us to calculate the Kováts indices from the scans for all mass spectra recorded [23]

Identification of unknown compounds On the one hand, the unknowns were identified on the basis of the EPA/NIH data bank coupled to the VG 70-70F mass spectrometer, and on the other hand, on the basis of our own SPECMA data bank and files The concept of the data bank and its implementation have been developed elsewhere [18, 19]

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