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Note

Determination by gas chromatography of terpenes in the berries of the species *Juniperus oxycedrus* L., *J. thurifera* L. and *J. sabina* L.

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Extensive studies concerning the chemical composition of leaves and berries of species of the *Juniper* genus have been carried out in the past 30 years. Analyses of the *Juniperus sabina* L. berry show that sabinene, sabinyl acetate, α-pinene, myrcene, limonene, terpinen-4-ol, γ-terpinene, cadinene, germacrene-B and γ-selinene are the major constituents of the terpenic fraction[1–5].

The most detailed studies of the essential oil of *J. oxycedrus* L. are those of Motl *et al.*[6] and De Pascual Teresa *et al.*[7,8]. These authors agree that the principal components include α-pinene, β-myrcene, limonene, cadinene and γ-muurolene. Motl *et al.* also cited ylangene and viridiflorol, whereas De Pascual Teresa *et al.* detected β- and γ-bulgarenes for the first time.

The chemical composition of the *J. thurifera* L. berry has been the subject of fewer investigations. De Pascual Teresa *et al.*[9] found two new sesquiterpenes (8-α-acetoxielemol and β-element-7-α-ol) in the neutral fraction of an hexane extract of *J. thurifera* L. berries. The same authors[10] indicate that the major component of the essential oil is limonene, followed in importance by α-pinene and terpinen-4-ol.

The present paper reports a more thorough analysis of the essential oil in berries of the species *J. oxycedrus* L., *J. thurifera* L. and *J. sabina* L.

EXPERIMENTAL

Reagents

The chromatographic standards were camphene (BDH, U.K.), thymol (Panreac, Barcelona, Spain), α-pinene, sabinene, δ-3-carene, β-myrcene, limonene, α-terpinene, 1,8-cineole, *p*-cymene, α-terpinolene, camphor, linalool, linalyl acetate, bornyl acetate, isobornyl acetate, *cis*-caryophyllene, terpinen-4-ol, myrtenyl acetate, α-terpineol, geranyl acetate, *p*-cymen-8-ol, geraniol, borneol and isoborneol (Distillery Garcia de la Fuente, Granada, Spain).

A internal standard solution was prepared by dissolving 10.325 g methyl caprylate and 11.403 g thymol in 100 ml of diethyl ether.

Apparatus

Perkin-Elmer Model Sigma 3B gas chromatograph equipped with a flame ion-

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TABLE I

TERPENIC COMPONENTS FOUND IN RIPE BERRIES OF *J. THURIFERA* L., *J. OXYCEDRUS* L. AND *J. SABINA* L. EXPRESSED IN RELATIVE % ± S.D. (n = 4)

Peak	*Terpenic components*	*J. thurifera L.*	*J. oxycedrus L.*	*J. sabina L.*
2	α-Pinene	3.48 ± 0.76	60.60 ± 3.33	4.95 ± 0.82
3	Camphene	–	0.22 ± (<0.01)	0.05 ± 0.01
4	β-Pinene	0.24 ± 0.05	1.08 ± 0.07	0.16 ± (<0.01)
5	Sabinene	2.20 ± 0.14	0.30 ± 0.07	82.89 ± 0.20
6	β-Myrcene	3.82 ± 0.18	24.97 ± 1.76	5.80 ± 0.22
7	α-Terpinene	0.16 ± 0.04	0.05 ± (<0.01)	0.37 ± 0.03
8	Limonene	84.32 ± 1.07	1.77 ± 0.15	1.85 ± 0.08
9	1,8-Cineole	0.27 ± 0.05	0.22 ± 0.02	0.04 ± (<0.01)
10	γ-Terpinene	0.21 ± 0.04	0.10 ± 0.01	0.56 ± 0.01
11	*p*-Cymene	0.15 ± 0.02	0.07 ± 0.01	0.06 ± 0.02
12	α-Terpinolene	1.39 ± 1.39	0.51 ± 0.07	0.86 ± 0.08
14	α-Copaene	–	0.64 ± 0.06	–
	Germacrene-B	–	–	0.37 ± 0.03
15	Camphor	–	0.17 ± 0.03	–
	Monoterpene I	–	–	0.08 ± (<0.01)
	Monoterpene alcohol I	–	–	0.21 ± 0.03
16	Linalool	020 ± 0.06	0.15 ± 0.04	0.12 ± 0.01
	Monoterpene alcohol II	–	–	0.07 ± (<0.01)
	Linalyl acetate	0.25 ± 0.03	–	–
	Bornyl acetate	–	–	0.02 ± (<0.01)
	Isobornyl acetate	0.10 ± 0.02	–	–
17	*cis*-Caryophyllene	0.33 ± 0.03	0.51 ± 0.07	0.02 ± (<0.01)
18	Terpinen-4-ol	0.44 ± 0.06	0.14 ± 0.03	1.07 ± 0.12
	Sabinol	–	–	0.04 ± (<0.01)
19	Cadinere, type sesquiterpene	–	0.19 ± 0.04	–
20	α-Muurolene	–	0.29 ± 0.04	–
	Sabinyl acetate	–	–	0.03 ± (<0.01)
21	β-Farnesene	0.24 ± 0.007	0.42 ± 0.08	–
	β-Selinene	–	–	0.01 ± (<0.01
22	α-Humulene	0.05 ± 0.01	0.37 ± 0.07	–
23	Mirtenyl acetate	–	0.23 ± 0.03	–
24	α-Terpineol	0.47 ± 0.06	0.10 ± 0.01	0.03 ± 0.01
25	γ-Muurolene	0.37 ± 0.10	5.19 ± 0.76	0.04 ± 0.01
26	γ-Elemene	–	0.33 ± 0.09	–
27	γ-Cadinene	0.25 ± 0.08	0.93 ± 0.19	0.06 ± 0.02
	Elemene, type sesquiterpene	0.52 ± 0.10	–	–
	p-Cymen-8-ol	–	–	0.01 ± (<0.01)
	Cadinol	0.20 ± 0.09	–	0.07 ± 0.03
28	Sesquiterpene alcohol I	–	0.16 ± 0.03	–
	Sesquiterpene alcohol II	0.23 ± 0.06	–	–

ization detector, Perkin-Elmer Model Sigma 15 data station and a fused-silica Carbowax 20M column (50 m × 0.25 mm I.D.) (Perkin-Elmer, U.K.) was employed. Operating conditions: injector, 250°C; detector, 250°C; initial column temperature, 60°C, raised at 1°C/min to 180°C; carrier gas (nitrogen) flow-rate, 0.70 ml/min; splitting 1:100; injection volume, 0.5 μl.

An Hewlett-Packard Model 5992 B gas chromatograph–mass spectrometer

was equipped with a fused-silica SE-30 column (25 m × 0.32 mm O.D. × 0.22 mm I.D.) (Supelco; Teknokroma, S. Coop. Ltd., Barcelona, Spain). Chromatographic operating conditions: injector, 250°C; detector, 250°C; initial column temperature, 60°C, held for 2 min, then raised at 3°C/min to 220°C and held for 5 min; helium carrier gas flow-rate, 0.7 ml/min; splitting 1:50; injection volumes 0.2 μl. Mass spectrometry operating conditions: ionization voltage, 70 eV; acceleration voltage, 2000 V.

Samples

Ripe berries of *J. thurifera* L., *J. oxycedrus* L. and *J. sabina* L. were collected in Spain during the autumn season, the first two species at an altitude of 850–900 m and the third at an altitude of approximately 2600 m.

RESULTS AND DISCUSSION

Terpenes in the essential oil

A 50–100 g amount of freshly picked ripe berries was steam distilled to obtain the essential oil in a volatile-oil trap (Clevenger type[10]). The essential oil was dissolved in 1 ml of diethyl ether and 0.1 ml of the internal standard solution, and 0.5-μl samples were injected in triplicate into the chromatograph equipped with fused-silica Carbowax 20M column (50 m × 0.25 mm I.D.).

The identies of the peaks were confirmed by means of the addition of pure components, by this retention times relative to methyl caprylate and thymol and by the agreement of their mass spectra obtained by gas chromatography–mass spectrometry (GC–MS) using a fused-silica SE-30 column (25 m × 0.32 mm O.D. × 0.22 mm I.D.) with those in the literature[14].

The relative percentages of the individual components were obtained by summation of peak areas using digital electronic integrators (Perkin-Elmer, Sigma 15); triplicate experiments for four different samples of each species, were performed.

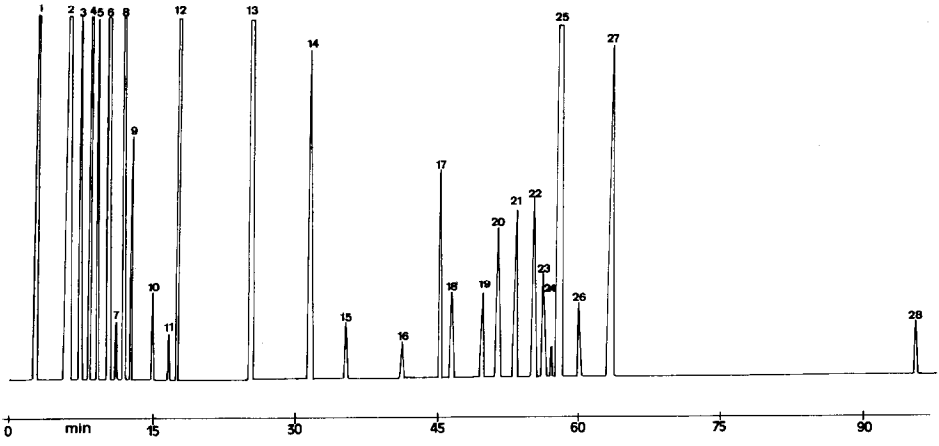

Fig. 1. Chromatogram of *J. oxycedrus* L. oil. Column: fused-silica Carbowax 20M. Initial temperature: 60°C, raised at 1°C/min to 180°C. See Table I for peak numbers [peak 1 is diethyl ether (dissolvent)].

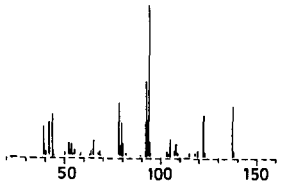

Fig. 2. Mass spectrum of γ-terpinene.

Table I shows the mean percentage of each terpenic compound in the essential oil of the berries of *J. thurifera* L., *J. oxycedrus* L. and *J. sabina* L. Fig. 1 is a chromatogram corresponding to the berries of *J. oxycedrus* L. An example of the mass spectrum of one terpene is shown in Fig. 2.

The essential oil content differed considerably from one species to another: 0.13% (v/w) was found for *J. thurifera* L., 0.50% (v/w) for *J. oxycedrus* L. and 2.25% (v/w) for *J. sabina* L.

In the essence of *J. thurifera* L., the principal components were limonene (84.32%), β-myrcene (3.82%), α-pinene (3.48%) and α-terpinolene (1.39%). α-Pinene was predominant (60.5%) in the essence of *J. oxycedrus* L., followed by β-myrcene 24.97%) and γ-muurolene (5.19%). In the essential oil of *J. sabina* L., sabinene was predominant (83%) and β-myrcene (5.80%), α-pinene (4.95%), limonene (1.85%) and terpin-4-ol (1.07%) were also found in substantial quantities.

The results obtained for *J. thurifera* L. essence by De Pascual Teresa *et al.*[11] differ from ours in that they found greater proportions of essential oil (1.7%) and terpinen-4-ol, and lower percentages of β-myrcene and α-terpinolene.

It should be noted that we confirmed the presence of linalool, linalyl acetate, α-terpineol, α-humulene and γ-cadinene by mass spectrometry. These components have not been cited previously.

Motl *et al.*[6] found 70% monoterpenes and 30% sesquiterprenes in the species *J. oxycedrus* L. and De Pascual Teresa *et al.*[8] found 43.7% sesquiterpenes. We found only 9% sesquiterpenes. In addition to detecting α-copaene (as did the above authors), we detected camphene, sabinene, α-terpineol, 1,8-cineole, γ-terpinene, α-terpinolene, terpinen-4-ol, β-farnesene, camphor and linalool. These components, which were not detected by the above authors, were confirmed by mass spectrometry.

Our findings for *J. sabina* L. do not agree with those of Booth[1], Koedam and Looman[12] and Satar[13], who found high proportions of sabinyl acetate (30–40%) and sabinol (1.4–6%). These two components appeared only in very small proportions in the berries we analyzed.

Finally, we should stress that the monoterpenic compositions of the berries of the three species is qualitatively very similar. The only notable differences occur in the quantities of some of the terpenes of the analyzed species.

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