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Composition of the essential oil of *Juniperus cedrus* Webb & Berth. grown on Madeira

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ABSTRACT: The essential oils isolated from twigs of *Juniperus cedrus* Webb & Berth. grown on Madeira were analysed by GC, GC–MS and ^{13}C-NMR. The oils consisted mainly of monoterpene hydrocarbons (53.1–87.8%), the main ones being α-pinene (19.6–55.3%), limonene (17.3–32.7%) and Δ-3-carene (5.5–15.7%). The sesquiterpenoid fraction (4.1–22.3%) was dominated by *E*-caryophyllene (1.6–7.4%), while sandaracopimaradiene (0.1–6.1%), isoabienol (0.5–1.3%) and *trans*-totarol (0.4–2.2%) were the main diterpenoids (2.2–11.9%). Oct-1-en-3-ol (1.0–2.2%) was the major constituent of the non-terpenic fraction (1.3–2.7%). The composition of our oil samples differed to some extent from that reported for *J. cedrus* oil grown on the Canary Islands. Copyright © 2002 John Wiley & Sons, Ltd.

KEY WORDS: *Juniperus cedrus* Webb & Berth.; Cupressaceae; essential oils; twigs; GC; GC–MS; ^{13}C-NMR

Introduction

Juniperus cedrus Webb & Berth. (= *J. oxycedrus* L. ssp. *maderensis* Menezes; *J. oxycedrus* L. var. *grandifolia* Link in Buch.; *J. webbii* Car.) is a resinous shrub or tree, endemic in Madeira and the Canary archipelago.[1,2] This species has an ecological area that is, in the main, restricted to the tops of high and steep rocks.

Once common in Madeira, where it is known as 'cedro' or 'cedro-da-madeira', *J. cedrus* is now rare in the wild, since it occurs only on the upper border of *laurisilva* and other areas of high altitude (ca. 1800 m).[1,2] Due to several reforestation programmes, the species was reintroduced and several cultivated specimens can now be seen.[3]

Apart from the paper published by Adams (1998),[4] which includes the chemical composition of the essential oil of *J. cedrus* from Tenerife, Canary Islands, there are no phytochemical reports about this taxon. In this paper we report on the composition of essential oils isolated from twigs of *J. cedrus* grown on Madeira.

Material and Methods

Plant Material

Twig samples of *J. cedrus* were randomly collected from the lower branches of eight individual plants (ca. 50 years old), growing in Carreiras, Madeira, a mountain valley (altitude 950 m) facing east, 6 km from the sea. Plant material was collected in February 1997 and voucher specimens were deposited in the Herbarium of the Botanical Institute of Coimbra (COI).

Isolation Procedure

Essential oil samples were isolated by water distillation for 3 h from fresh material, using a Clevenger-type apparatus, according the procedure described in the *European Pharmacopoeia* (1987).[5]

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Essential Oil Analysis

The oil samples were analysed by gas chromatography (GC) and by gas chromatography–mass spectroscopy (GC–MS). The oil components were identified by the matching of their retention indices and mass spectra (EI = 70 eV) with corresponding data of authentic compounds, components of reference oils or with literature data.[6,7] GC analyses were performed using a Hewlett-Packard 6890 gas chromatograph equipped with HP GC ChemStation Rev. A.05.04 data-handling system, a single injector port and two flame ionization detectors (FID). Two fused-silica capillary columns with different polarities were used: SPB-1 (polydimethylsiloxane, 30 m × 0.20 mm i.d., film thickness 0.20 μm) and Supelco Wax 10 (polyethyleneglycol 30 m × 0.20 mm i.d., film thickness 0.20 μm). Oven temperature program: 70 °C to 220 °C (at 3 °C/min), 220 °C (15 min); injector and detector temperatures, 250 °C; carrier gas, helium, adjusted to a linear velocity of 30 cm/s; splitting ratio, 1 : 40. Retention indices were determined by linear interpolation relative to retention times of a series of *n*-alkanes.

GC–MS analysis were performed with a Hewlett-Packard 6890 gas chromatograph, equipped with a HP1 fused-silica column (polydimethylsiloxane 30 m × 0.25 mm i.d., film thickness 0.25 μm) and interfaced with a Hewlett-Packard Mass Selective Detector 5973 (HP Enhanced ChemStation software, version A.03.00). Oven temperature program: 70 °C to 220 °C (at 3 °C/min), 220 °C (15 min); injector temperature, 250 °C; carrier gas, helium, adjusted to a linear velocity of 30 cm/s; splitting ratio, 1 : 40; interface temperature, 250 °C; MS source temperature, 230 °C; MS quadrupol temperature, 150 °C; ionization energy, 70 eV; ionization current, 60 μA; scan range, 35–350 u; scans/s, 4.51.

The identity of the major compounds was also confirmed by ^{13}C-NMR without previous separation of the components, following the pioneering work done by Formàcek and Kubeczka (1982) and according to an experimental procedure and computerized method.[8,9] This technique has proved to be useful in the investigation of the composition of essential oils, particularly in the identification of sesquiterpenoids or diterpenoids poorly separated by GC or having similar MS spectra. ^{13}C-NMR spectra were recorded on the whole sample or on pentane and diethyl ether fractions on a Bruker AC 200 Fourier Transform spectrometer, operating at 50.323 MHz, equipped with a 10 mm probe in deuterated chloroform (around 200 mg oil in 2 ml of $CDCl_3$), with all shifts (δ) referred to internal tetramethylsilane (TMS). Parameters: pulse width (PW), 5.0 μs (flip angle, 45°); acquisition time, 1.3 s; relaxation delay D_1, 2 s (total recycling time, 3.3 s) for 32 K data table with a spectral width (SW) of 12500 Hz (250 ppm); composite phase decoupling (CPD) of the proton band; digital resolution, 0.763 Hz/pt; 5000 scans were accumulated for each sample. An exponential multiplication of the free induction decay with the line broadening of 1.0 Hz was applied before Fourier transformation. The components were identified by comparison of the values of the carbon chemical shifts in the mixture spectrum with those of reference spectra compiled in a computerized databank. Each compound is identified by taking into account three parameters directly available from the computer program: (a) the number of observed signals with respect to that expected; (b) the difference between the chemical shift of each signal in the mixture and in the reference ($\Delta\delta$); (c) the number of overlapped signals of carbons belonging to two components which by chance possess the same chemical shift.

The relative amounts of individual components were calculated based on GC peak areas without using correction factors.

Results and Discussion

The essential oils from twigs of *J. cedrus* showed a yellowish colour and a strong odour and gave a yield of 0.14–0.34% (v/w).

The identified components, representing over 95% of the total oil, are listed in Table 1 in order of their elution on the SPB-1 column. The oils consisted

Table 1. Percentage composition of the essential oils isolated from twigs of *Juniperus cedrus* Webb & Berth. grown on Madeira

RI[a]	Compound	Present (%) in samples:							
		1	2	3	4	5	6	7	8
921	Tricyclene			0.1	0.1	t	t	0.1	t
922	α-Thujene	t	0.1	0.1	0.1				t
930	α-Pinene[b]	22.2	28.3	55.3	31.5	19.6	25.8	25.2	33.2
941	α-Fenchene[c]	0.3	0.4	t	0.4	1.5	1.4	t	0.3
942	Camphene[c]	0.1	0.1	1.1	0.2	0.8	0.5	0.3	0.2
959	Oct-1-en-3-ol	2.2	1.0	1.6	1.8	1.8	1.8	1.3	1.7
964	Sabinene	0.3	t	t	t	0.2	0.5	0.3	t
970	β-Pinene	0.5	0.4	0.7	0.5	0.3	0.5	1.1	0.5

Table 1. (*Continued*)

RI[a]	Compound	Present (%) in samples:							
		1	2	3	4	5	6	7	8
980	β-Myrcene[b]	2.3	1.8	2.0	2.5	1.4	1.9	2.4	2.1
998	Δ-2-Carene	0.7	0.9	0.4	1.4	0.3	0.2	1.1	1.0
998	α-Phellandrene		0.2				0.1		
1005	Δ-3-Carene[b]	10.0	5.5	9.0	6.1	7.3	15.7	8.5	10.2
1010	α-Terpinene	0.2		0.1	0.1	0.1		0.2	0.1
1011	*p*-Cymene	0.3	0.3	0.1	0.2	0.3		0.3	0.3
1020	β-Phellandrene[c]	0.7	0.8	0.4	1.3	0.6	0.5	1.0	0.6
1020	Limonene[b,c]	27.2	17.3	18.0	32.7	19.8	18.9	28.5	24.1
1025	*cis*-β-Ocimene				0.2		t		t
1035	*trans*-β-Ocimene	t	0.1	t	0.3	0.1	t	t	0.1
1046	γ-Terpinene	0.1	0.1	t	0.1	0.1		0.1	t
1052	Octanol			0.1	0.2	0.2		0.2	0.1
1077	Terpinolene[b]	0.8	0.7	0.5	1.2	0.7	1.5	0.7	0.5
1082	Linalool							0.3	
1094	Oct-1-en-3-yl acetate	0.4	0.3		0.4	0.7	0.7	0.5	0.6
1106	*cis*-*p*-Menth-2-en-lol	0.3	0.3		0.2	0.4		0.4	0.2
1120	*trans*-*p*-Menth-2-en-lol		0.2		0.2				
1125	*trans*-Verbenol							0.7	
1135	Pinocarvone							0.3	
1158	Terpineol-4[c]	0.3	0.3	0.1	0.2	0.5	0.5	0.3	0.5
1158	*p*-Cymene-8-ol[c]	0.1	0.2	t	0.1	t	t	0.1	0.1
1169	α-Terpineol[b]	0.4	0.5	0.1	0.5	0.4	0.6	0.6	0.6
1177	*cis*-Piperitol		0.1		0.2	0.1		0.6	
1187	*trans*-Piperitol		0.1						
1222	Piperitone	0.4	0.4	t	0.2	0.3	t	0.3	0.2
1223	Methyl carvacrol	0.1	0.2	0.1	0.2		0.3		0.1
1267	Bornyl acetate[b]	0.4	0.4	0.2	0.5	0.7	0.9	0.6	0.6
1275	Carvacrol	1.5	0.9	0.2	0.4	2.1	0.3	0.2	0.9
1328	α-Terpinyl acetate[b]	2.7	4.2	0.8	2.6	2.4	3.5	3.7	3.1
1342	α-Cubebene	0.2	0.4	0.1	0.2	0.3	0.3	0.2	0.1
1369	α-Copaene	0.1	0.2	t	0.2	0.2	t	0.1	t
1380	β-Cubebene		0.1						t
1382	β-Elemene		0.1						t
1408	*E*-Caryophyllene[b]	5.3	7.0	1.6	3.0	6.8	7.4	3.8	3.3
1442	α-Humulene[b]	1.5	1.8	0.4	0.8	1.9	1.7	0.9	1.0
1467	Germacrene D	1.5	1.3	0.4	0.9	1.6	1.6	0.6	1.3
1479	Valencene		1.0	0.2	0.4	1.2		0.4	t
1486	α-Muurolene	0.1	0.2	t	0.1	0.1			0.1
1498	γ-Cadinene	0.4	1.4	0.2	0.4	0.8	0.8	0.7	0.3
1508	δ-Cadinene	1.0	1.5	t	0.6	1.1	1.3	0.8	0.6
1540	Germacrene B		0.1		0.1	0.2			t
1545	*E*-Nerolidol	0.2	0.3	t	0.3	0.2	t	t	t
1557	Caryophyllene oxide[b]	3.6	4.9	0.8	1.4	5.0	2.0	3.7	1.5
1582	Humulene oxide	0.9	1.2	0.2	0.3	1.2	0.3	0.8	0.2
1605	1-*epi*-Cubenol						0.8	0.9	
1616	T-Cadinol		0.4	0.1		0.4	0.3		t
1627	α-Cadinol[c]			0.1	0.1	0.2			
1627	T-Muurorol[c]		0.4						
1941	Sandaracopimaradiene[b]	5.4	4.2	0.7	0.1	6.1	2.4	1.2	3.1
1969	Isopimaradiene[b]	0.9	0.9	0.6	0.1	1.5	0.5	0.5	0.7
2019	Abietatriene	0.8	0.5	0.1	0.2	0.6	0.3	0.3	0.3
2070	Isoabienol[b]	1.3	0.9	0.5	1.1	1.1	0.8	1.2	0.9
2090	8-β-Hidroxisandaracopimaradiene	0.2	0.1	t		0.3			t
2230	*cis*-Totarol		t			0.1			
2253	*trans*-Totarol[b]	1.1	0.7	0.4	0.7	2.2	0.7	1.7	0.9
2268	*trans*-Ferruginol			0.1		t			t
	Total identified	99.0	95.7	97.5	97.6	95.8	97.3	97.7	96.2
	Monoterpene hydrocarbons	65.7	57.0	87.8	78.9	53.1	67.5	69.8	73.2
	Oxygen-containing monoterpenes	6.2	7.8	1.5	5.3	6.9	6.1	8.1	6.3
	Sesquiterpene hydrocarbons	10.1	15.1	2.9	6.7	14.2	13.1	7.5	6.7
	Oxygen-containing sesquiterpenes	4.7	7.2	1.2	2.1	7.0	3.4	5.4	1.7
	Diterpenoids	9.7	7.3	2.4	2.2	11.9	4.7	4.9	5.9
	Others	2.6	1.3	1.7	2.4	2.7	2.5	2.0	2.4

[a] Relative to C_9–C_{23} *n*-alkanes on the SPB-1 column.
[b] Identity confirmed by ^{13}C-NMR.
[c] Quantitative determination on Supelco Wax column.
t, trace (<0.05%).

mainly of monoterpene hydrocarbons (53.1–87.8%) with α-pinene (19.6–55.3%), limonene (17.3–32.7%), Δ-3-carene (5.5–15.7%) and β-myrcene (1.4–2.5%) being the major components. The oxygen-containing monoterpene fraction represented 1.5–8.1% of the total oil, being dominated by α-terpinyl acetate (0.8–4.2%). Among the sesquiterpenoids (4.1–22.3%), *E*-caryophyllene (1.6–7.4%) was the main compound, whereas sandaracopimaradiene (0.1–6.1%), isoabienol (0.5–1.3%) and *trans*-totarol (0.4–2.2%) were the main diterpenoids (2.2–11.9%). The non-terpenic fraction (1.3–2.7%) was dominated by oct-1-en-3-ol (1.0–2.2%).

This composition differs in some extent from those reported for the oils of formerly related species, particularly *J. oxycedrus, J. brevifolia* and *J. navicularis*. The monoterpene hydrocarbons are also dominant in their compositions (over 65%), but the major compounds and their relative amounts are rather different. Generally, the oil of *J. oxycedrus* is reported as consisting mainly of α-pinene (25–85%), β-phellandrene and often Δ-3-carene (both up to 10%).[10–12] Limonene, one of the major components of the oil of *J. cedrus*, is usually described in the oil of *J. oxycedrus* as a minor compound, in spite of some exceptions, such as the report for an oil from Greece, in which it attains 27%.[11] Conversely, limonene is the major component of the oil of *J. brevifolia*, ranging from 66% to 79%,[13] more than twice the percentage we recorded in the oil of *J. cedrus*. In *J. brevifolia* oil sandaracopimaradiene is, as in *J. cedrus* oil, the major diterpene, representing up to 5% of the total oil. The most similar oil of *J. cedrus*, in terms of the main components, is that from *J. navicularis*.[4,14] In this taxon, limonene (7–34%) and α-pinene (6–38%) are the major components and their relative amounts are similar to those of the oil of *J. cedrus*. Nevertheless, the oil of *J. navicularis* is clearly distinct, particularly in the relatively high amounts of β-myrcene (up to 8%) and α-phellandrene (up to 13%) and low amount of Δ-3-carene (up to 0.8%).

The composition of the essential oils isolated from twigs of *J. cedrus* grown on Madeira is also different from that previously reported by Adams for a sample collected at Tenerife, Canary Islands.[4] Indeed, several monoterpenes, such as α-pinene (70.7% vs. 19.6–55.3%), limonene (4.5% vs. 17.3–32.7%), β-pinene (4.1% vs. 0.3–1.1%) or β-phellandrene (4.6% vs. 0.4–1.3%), exhibit different relative amounts in the oils from Canary and in those from Madeira. Δ-3-Carene, not found in the oil from Canary, is one of the major constituents in the oils from Madeira (5.5–15.7%). Apart from the monoterpenes, only *E*-caryophyllene (0.4% vs. 1.6–7.4%), α-humulene (traces vs. 0.4–1.9%) and abietatriene (0.1 vs. 0.1–0.8%) were reported in the oil from Canary.

Although some of the quantitative differences could be explained by the different techniques used for quantification (the ITD total ion current used by Adams[4] vs. the FID response used in this work), the most striking quantitative and the qualitative differences, as for instance the absence of several sesqui- and diterpenes, cannot be explained purely by the different analytical methodologies. In view of this, our results suggest that there is some chemical variability in the essential oil from *J. cedrus*, a species whose geographical distribution is restricted to the Canary archipelago and Madeira.

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