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VOLATILE CONSTITUENTS OF THE LEAVES OF *CUPRESSUS DUPREZIANA* AND *CUPRESSUS SEMPERVIRENS*

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Key Word Index—*Cupressus dupreziana*; *Cupressus sempervirens*; Cupressaceae; leaf essential oil; monoterpenes; sesquiterpenes.

Abstract—The essential oils of the leaves of *Cupressus dupreziana* and *Cupressus sempervirens* were compared. The composition of the hydrocarbon fraction showed a great similarity between the two species.

INTRODUCTION

Studies of mono-, sesqui- and diterpenes of the wood of *Cupressus dupreziana* and *C. sempervirens*, two related species [1], and of the leaf lipids have been reported previously [2–7]. Only the secondary alcohols in the leaves are different and small differences in the sesquiterpene composition of the wood have also been noted. We now report a comparison of the essential oil of the leaves of these two species.

RESULTS AND DISCUSSION

Steam distillation of the leaves of *C. dupreziana* gave 0.6% of essential oil characterized by d_{20}^{20} 0.9160, n_D^{19} 1.5070, $[\alpha]_D^{20}$ +16.46°, A.I. 1.8, E.I. 16.5. This oil was analysed by GC and GC/MS with two capillary columns and showed five groups of terpenes: C_{10} hydrocarbons, oxygenated monoterpenes, C_{15} hydrocarbons, oxygenated sesquiterpenes and diterpenes.

In order to simplify identification, the hydrocarbons were separated from the oxygen fraction by CC on Si gel. This fraction was separated on a silver nitrate–Si gel column and the compounds were isolated by micropreparative GC. The individual compounds were identified by IR, MS and comparison with reference spectra or data published in the literature (Table 1). Many of them, such as α-fenchene, β-pinene, β-myrcene, α-terpinene, limonene, α-cubebene, β-caryophyllene, α-humulene, γ-muurolene, α-muurolene, germacrene D, δ-cadinene and γ-cadinene were absent from the essential oil of the wood [7]. On the other hand, some major constituents of the wood, i.e. α-cedrene, β-cedrene, α-selinene, β-selinene and γ-acoradiene [2, 7], were not identified in the leaves.

In the same way, *C. sempervirens* afforded an essential oil (0.7% of the leaves), characterized by d_{20}^{20} 0.8670, n_D^{20} 1.4740, $[\alpha]_D^{20}$ +27.28°, A.I. 1.3, E.I. 19.7. This oil was also analysed by GC, GC/MS and the products were identified by comparison with samples isolated from *C. dupreziana*. Composition of the hydrocarbon fraction is shown in Table 1.

As previously reported for sesquiterpenes and diterpenes of wood [4, 7], the comparison of leaf essential oils of both species shows their close biological relationship. In particular, all monoterpene hydrocarbons identified in the oil of *C. dupreziana* were also present in *C. sempervirens*. α-Pinene and 3-carene were the major compounds in both cases, as expected for essential oils of most *Cupressus* [16–18]. Our results differ from the report that *C. sempervirens* growing in Azerbaijan lacks 3-carene in the leaf oil [19]. In this case the absence of 3-carene could be due to a genetic variation, as has been shown for *Pinus monticola* [20] and *P. pinaster* [21].

The main components of both sesquiterpene fractions were a mixture of cadinene–muurolene isomers, probably produced by thermal rearrangement of germacrene D [22]. The presence of small amounts of germacrene D in both oils is in good agreement with this hypothesis. However, some differences may be noted (Table 1): (a) the paucity of calamenene in *C. sempervirens* oil; and (b) the larger amounts of α-humulene, γ-muurolene and δ-cadinene in *C. dupreziana*.

In addition to the compounds listed in Table 1, we identified also trace amounts of some oxygenated mono- and sesquiterpenes by IR and MS: α-terpenyl acetate, linalyl acetate, bornyl acetate, α-terpineol, 1-terpinen-4-ol, carvacrol methyl ether, cedrol and α-cadinol [2, 9, 23]. It must be noted that cedrol and carvacrol methyl ether were the major constituents of wood extracts and essential oils of *C. dupreziana* and *C. sempervirens*. These compounds are present only in trace amount in the leaves. The last component identified was manoyl oxide, an oxygenated diterpene related to manool, this compound being a constituent of the wood [4].

The results of this phytochemical comparison of *C. dupreziana* and *C. sempervirens* complete our chemotaxonomic study on *C. dupreziana* and shows the close relationship between these two species. They are probably two subspecies derived from the same ancestral taxon, *C. sempervirens* being a Mediterranean subspecies and *C. dupreziana* a Saharan subspecies.

EXPERIMENTAL

Plant material—essential oils. *C. dupreziana* and *C. sempervirens* leaves were collected from trees growing in the Botanical

Table 1. Composition of the hydrocarbon fraction of the essential oils of the leaves of *Cupressus dupreziana* (*cd*) and *Cupressus sempervirens* (*cs*), determined by GC analysis (PPGS)

Compounds	R_t	% in hydrocarbon fraction *cd*	*cs*	Evidence	References
α-Pinene	13.80	72.38	74.66	IR, MS	[8–10]*
α-Fenchene	14.90	0.33	0.50	MS, R_t	[8, 10]*
Camphene	15.50	0.21	0.22	MS, R_t	[8–10]*
β-Pinene	16.90	1.79	1.61	IR, MS	[8–10]*
β-Myrcene	17.82	1.97	2.15	IR, MS	[8–10]*
3-Carene	18.26	11.24	12.87	IR, MS	[8–10]*
α-Terpinene	19.44	0.02	0.32	MS, R_t	[8–10]*
Limonene	20.18	1.02	2.78	IR, MS	[9, 10]*
β-Phellandrene	20.82	0.30	0.22	MS, R_t	[9, 10]*
γ-Terpinene	21.70	0.09	0.79	IR, MS	[8, 10]*
Terpinolene	22.98	1.52	2.05	IR	*
p-Cymene	27.38	0.03	< 0.02	MS, R_t	[9, 10]*
α-Cubebene	33.76	0.15	0.03	MS, R_t	[11]*
α-Copaene	34.62	0.06	< 0.02	MS, R_t	[11–13]†
β-Caryophyllene	37.18	0.84	0.09	IR, MS	[11, 14]*
α-Humulene γ-Muurolene	39.22	3.14	0.47	IR, MS IR, MS	[11, 14]* *; †
α-Muurolene Germacrene D	40.04	0.78	0.09	MS, R_t MS, R_t	*; † *
δ-Cadinene	41.16	2.33	0.50	IR, MS	[11, 14]†
γ-Cadinene	41.98	0.66	0.13	IR, MS	[11, 15]*
ε-Cadinene	42.98	0.27	0.06	R_t	—
Calamenene	44.00	< 0.02	—	MS, R_t	†

*Also identified by comparison with reference spectra of the collection of the Laboratoire de Physiologie Cellulaire Végétale, Université de Bordeaux I, 33405 Talence, France.

†Also identified by comparison with reference spectra of the Roure Bertrand Dupont collection, 06332 Grasse, France.

Garden of Algier University. Ground fresh leaves (4 kg) of each cypress were steam distilled for 3 hr in a glass apparatus. The organic fraction was decanted and dried, giving 24 g essential oil for *C. dupreziana* and 28 g for *C. sempervirens*. Both oils were fractionated and analysed in the same way.

Separations. The essential oil of *C. dupreziana* (12 g) was prefractionated on a Si gel column eluted first with pentane and pentane–Et_2O (19:1), giving the hydrocarbon fraction (10.5 g). Oxygenated compounds were obtained after elutions with pentane–Et_2O (9:1), Et_2O and MeOH. The hydrocarbon fraction was separated on a $AgNO_3$–Si gel (1:9) column and the compounds present in the fractions obtained were isolated by prep. GC on two columns: (a) 8% SE-30, 2 m × 6 mm, temp. programmed from 60 to 210° at 4°/min, FID 250°, N_2 60 ml/min; (b) PPGS 10%, 1.70 m × 6 mm, temp. programmed from 70 to 220° at 4°/min, FID 250°, N_2 60 ml/min. The individual compounds were identified by IR, MS, R_t and comparison with reference spectra or data published in the lit. (Table 1). Oxygenated compounds identified were also isolated by prep. GC on the same columns.

The essential oil of *C. sempervirens* was separated and analysed in the same way as that of *C. dupreziana.*

Analysis. Analytical GC was carried out on a 10% PPGS column, 2.5 m × 3 mm, temp. programmed from 70 to 220° at 4°/min, FID 250°, N_2 25 ml/min (results in Table 1). GC/MS analysis was performed on a Carbowax 20M quartz capillary column, 25 m × 0.2 mm, temp. programmed from 80 to 180° at 2°/min, He 1.5 ml/min, MS operating at 70 eV electron energy.

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