

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

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Key Word Index—*Cupressus dupreziana*; *Cupressus sempervirens*; Cupressaceae; wood essential oil; wood petrol extract; sesquiterpenes; biogenesis.

Abstract—Analysis of wood essential oil of *Cupressus dupreziana* revealed 26 components: 13 monoterpenes and 13 sesquiterpenes. The main components were carvacrol methyl ether and cedrol. A petrol extract of *Cupressus sempervirens* wood was investigated and showed a mixture of sesquiterpenes very similar to those found in *Cupressus dupreziana*. The biogenesis of the identified sesquiterpenes is discussed.

INTRODUCTION

We have shown elsewhere [1–4] that about one hundred compounds, mainly terpenes and lipids, are present in the wood and leaves of *Cupressus dupreziana* A. Camus, an endemic Cypress of Ajijer's Tassili (Central Algerian Sahara). As only five monoterpenes have been identified in the petrol extract of the wood [1, 2] we now report the composition of the wood essential oil.

On the other hand, this chemotaxonomic work led us to undertake a new investigation of *Cupressus sempervirens* L., a neighbouring species of *C. dupreziana* [5]. We have previously compared the diterpenes of wood [3] and the lipids of leaves [4]. Only the secondary alcohols in the leaves are different, thus showing the relationship between the two species. We wish now to report a comparison of the sesquiterpenes of the petrol extracts of the wood and to discuss the biogenesis of these compounds.

RESULTS AND DISCUSSION

Essential oil of *Cupressus dupreziana* wood

The steam distillation of the wood of *C. dupreziana* gave 1.8% of essential oil characterized by d_{20}^{20} 0.9460, n_D^{20} 1.5018, $[\alpha]_D^{20} + 0.6^\circ$, A.I. 1.86, E.I. 14.56. This oil was analysed by GLC and GC/MS with two different capillary columns and showed four kinds of terpenic compounds: C_{10} hydrocarbons (1.5%), oxygenated monoterpenes (67%), C_{15} hydrocarbons (18.5%) and oxygenated sesquiterpenes (13%).

As expected, the major terpenes previously identified [1, 2] in the petrol extract of the wood were also present in the essential oil. The main components were carvacrol methyl ether (62%) and cedrol (12%). The presence of these two compounds together with α -cedrene seems to be characteristic feature of the Cupressaceae [1, 6–8]. The monoterpenes identified in the hydrocarbon fraction were

α -pinene, camphene, α -thujene, 3-carene, limonene, *p*-cymene, β -ocimene, β -phellandrene, γ -terpinene, α -terpinolene and *p*-cymenene (α , *p*-dimethylstyrene). All these C_{10} hydrocarbons and some C_{15} hydrocarbons, such as ϵ -cadinene, longifolene, α -copaene, β -cubebene and thujopsene, were absent from the wood petrol extract. The percentage of the different compounds was estimated by GLC of the crude essential oil (Table 1).

The physical and chemical properties of this oil were comparatively close to those of *C. sempervirens* L.: d_{20}^{20} 0.9449, n_D^{20} 1.5018, $[\alpha]_D^{20} + 10.3^\circ$, A.I. 1.40, E.I. 23.14 [18, 19]. Furthermore the percentage of carvacrol methyl ether was the same in the two species [18] showing their close relationship. However, the essential oil of *C. dupreziana* exhibited a small percentage of monoterpene hydrocarbons (1.5%) and the absence of β -pinene must be noted.

Sesquiterpenes of *Cupressus dupreziana* and *Cupressus sempervirens*

The sesquiterpenes of *C. dupreziana* have been previously isolated from wood petrol extract [1, 2]. The following groups of compounds were present. Cedrane: (+)-cedrol, (–)- α -cedrene, (+)- β -cedrene, (+)-1,7-diepi- α -cedrene, (–)-1,7-diepi- β -cedrene, (+)-1,7-diepi-cedra-8-en-15-ol and (8*BH*)-1,7-diepi-cedran-15-ol. Elemene: (+)- β -elemene. Eudesmane: α -selinene, (–)- β -selinene, (+)-selina-11-en-4 β -ol, (–)-selina-4,11-dien-13-ol (γ -costol) and (–)-selina-4(14), 11-dien-13-ol (β -costol). Alaskanene: (–)- γ -acoradiene (α -alaskanene), α -acoradiene, β -acoradiene, alaska-4,7(11)-dien-14-ol, alaska-3,11-dien-14-ol and alaska-4,11-dien-14-ol. Bisabolane: (\pm)- α -curcumene. Cuparane: (+)-cuparene. Prezizaene: prezizaene. Cadinane: calamenene.

The amounts in the petrol extract as determined by GLC were: mono- and sesquiterpenes 52% (carvacrol

Table 1. Composition of the essential oil of wood of *Cupressus dupreziana*

Compounds	R_f (Carbowax 20M)	% of total oil	Evidence	References
α -Pinene	12.62	0.23	IR, MS	[9-11]*
α -Thujene	12.70	0.01	MS	[9]*
Camphene	12.98	0.03	MS	[9-11]*
3-Carene	13.83	0.05	MS	[9, 11]*
Limonene	14.23	0.11	IR, MS	[10, 11]*
β -Phellandrene	14.52	0.11	MS	[10, 11]*
β -Ocimene	14.72	0.02	MS	[11]†
γ -Terpinene	15.46	0.09	MS	[9, 11]*
<i>p</i> -Cymene	15.82	0.27	IR, MS	[10-12]*
α -Terpinolene	16.12	0.05	IR, MS	[9, 13]*
<i>p</i> -Cymenene	20.47	0.32	IR, MS	*
α -Copaene	23.62	0.27	IR, MS	[14-16]†
β -Cubebene	24.40	0.19	IR	[17]*
α -Cedrene	26.40	3.85	IR, MS	[1, 12, 16]**†
β -Elemene	27.18	0.42	IR	[1]*
β -Cedrene	27.38	1.58	{ IR, MS	[1, 16]**†
Thujopsene			{ IR	[12]*
Carvacrol methyl ether	27.85	61.78	IR, MS	[1]†
Longifolene	28.95	0.17	IR	*
Borneol	32.13	1.86	MS	[2, 10]†
β -Selinene	33.82	0.98	IR	[1]*
α -Selinene	34.08	0.69	IR	[1]*
γ -Acoradiene (α -alaskene)	34.32	1.08	IR, MS	[1, 15]†
ϵ -Cadinene	36.90	0.58	IR	*
Cuparene	40.18	0.31	IR, MS	[1]†
Cedrol	56.08	12.28	IR, MS	[1, 12, 10]†

*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.

Table 2. Sesquiterpenes of *Cupressus dupreziana* and *C. sempervirens*

Compounds	R_f (Carbowax 20 M)	% in petrol extracts of wood*	
		<i>Cupressus dupreziana</i>	<i>Cupressus sempervirens</i>
1,7-Diepi- α -cedrene	53.00	0.17	0.06
$C_{15}H_{24}$ unidentified	55.00	0.07	0.08
α -Cedrene	59.30	3.20	0.88
1,7-Diepi- β -cedrene	59.60	0.12	2.73
β -Cedrene	62.00	2.24	1.40
β -Elemene			
Carvacrol methyl ether	63.45	12.20	24.20
Preizizaene	65.60	0.02	0.02
$C_{15}H_{24}$ unidentified	66.30	0.12	—
α -Acoradiene	68.70	0.11	0.21
β -Acoradiene	69.05	0.14	0.29
β -Selinene	72.20	0.40	0.60
α -Selinene	72.60	0.20	0.50
α -Acoradine	73.05	0.32	1.30
α -Curcumene	76.60	0.20	0.23
Cuparene	79.70	0.07	0.10
Calamenene	81.80	0.07	—
Cedrol	98.90	24.00	16.00

**Cupressus dupreziana* 7-8%, *Cupressus sempervirens* 2% of dry weight.

methyl ether, borneol and sesquiterpenes cited above 46%); diterpenes 28%. In the same way, *C. sempervirens*, yielded mono- and sesquiterpenes 56% (carvacrol methyl ether, borneol and identified sesquiterpenes 50%); diterpenes 24%. The products were identified by GLC and GC/MS analysis in comparison with samples isolated from *C. dupreziana*. Most of sesquiterpenes of *C. dupreziana* were present in *C. sempervirens* (Table 2).

As previously noted for diterpenes [3], the comparison between the sesquiterpenes of both species shows their close biological relationship. In particular, the presence of 1,7-diepi- α and β -cedrenes in *Cupressus sempervirens* suggests that these compounds may be of chemotaxonomic significance in the genus *Cupressus*. Only one sesquiterpene belonging to the 1,7-diepi-cedrane group, α -funebreene, has been reported previously from *C. funebris* [20, 21]. However, some differences (Table 2) seem able to distinguish between the two species: (a) *C. sempervirens* lacks calamenene and an unidentified sesquiterpenic hydrocarbon (*R*_s 66.30 on Carbowax 20 M, C₁₅H₂₄, gem-dimethyl, allylic methyl on trisubstituted double bond, methylenic insaturation [1]). (b) *C. sempervirens* contains a significant amount of 1,7-diepi- β -cedrene (2.73%), whereas α -cedrene (0.88%) and β -cedrene (1.40%) decrease appreciably. (c) Carvacrol methyl ether (24.2%) is present in larger amount in *C. sempervirens*, whereas cedrol diminishes (16%).

The diversity of the different sesquiterpene skeletons present in both species and also the stereochemical features for some of them (e.g. 1,7-diepi-cedrenes, alaskadienes, selinenes) merit consideration of their biogenesis. The structure of isolated compounds permits the pathway to 1,7-diepi-cedrenes to be outlined, thus showing the relationship between the stereochemistry in the cedrane group and the chemotaxonomy of Cupressaceae, Taxodiaceae and Gramineae species [22]. On this basis and from consideration of "in vivo" [1, 2, 15, 20, 21, 23–29] and "in vitro" [23, 30–33] results, we suggest (+)JPPN as the only isoprene precursor from which all the sesquiterpenes of *C. dupreziana* and *C. sempervirens* can be elaborated.

EXPERIMENTAL

Plant material. The sample of *C. dupreziana* was collected by Algerian ORTF (Office de Recherches et Travaux Forestiers) in Ajjers' Tassili and that of *C. sempervirens* was collected in Perpignan (France). The latter was identified by Dr. L. Serve, Perpignan University, France.

Essential oil of *Cupressus dupreziana*. Powdered wood (2 kg) was steam distilled for 3 hr in a glass apparatus. The organic fraction was decanted and dried with Na₂SO₄, giving 16 g of essential oil. Cedrol (3 g) which crystallized on the cool parts of the apparatus was also recovered.

The essential oil (15 g) was prefractionated on a Si gel column eluted first with pentane and pentane–Et₂O (95:5), giving the hydrocarbon fraction (3 g). Oxygenated compounds were obtained after elutions by pentane–Et₂O (9:1), Et₂O and MeOH. The hydrocarbon fraction was separated on a AgNO₃–Si gel (1:9) column and the compounds present in the fractions obtained were isolated by preparative GLC on an 8% SE-30 column, 2 m \times 3 mm, temp. programmed from 60 to 210° at 4°/min, FID 210°, N₂ 30 ml/min. The individual compounds were identified by IR, MS and comparison with reference spectra or data published in the lit. (Table 1).

Analytical GLC was carried out on WCOT capillary columns of stainless steel coated with Carbowax 20 M, 100 m \times 0.5 mm, temp. programmed from 80 to 180° at 2°/min, FID 190°, He 2.3 ml/min and glass WCOT SE-30, 39 m \times 0.4 mm, temp. programmed from 80 to 180° at 1.5°/min, FID 190°, N₂ 1.1 ml/min. Results given in Table 1 were obtained with Carbowax.

GC/MS analysis was performed on a glass WCOT SE-30 column, 25 m \times 0.3 mm, temp. programmed from 100 to 200° at 2°/min. He 1.4 ml/min, MS operating at 70 eV electron energy.

Sesquiterpenes of *Cupressus sempervirens*. Wood (2 kg) was extracted with Et₂O. The petrol-soluble fraction (40 g) was separated by 1N Na₂CO₃ and 0.5N NaOH [1]. The neutral fraction yielded 80% of petrol extract.

C. sempervirens neutral fraction (10 g) was submitted to chromatography on alumina (grade II). A first elution with C₆H₆ gave sesquiterpene hydrocarbons and carvacrol methyl ether (5 g); by a second elution, cedrol (2 g) was obtained. Elutions with Et₂O and with MeOH led to diterpene compounds (3 g). The first fraction was submitted again to chromatography on alumina (grade I), eluted with petrol to give separate sesquiterpenes and carvacrol methyl ether. All compounds were identified by GLC, GC/MS and comparison with authentic samples previously extracted from *C. dupreziana* (Table 2).

GLC analysis was performed on 3 columns: 5% OV-1, 3 m \times 3 mm, temp. programmed from 80 to 300° at 2°/min, FID 340°, N₂ 23 ml/min; Carbowax 20 M WCOT Inox capillary column, 100 m \times 0.5 mm, at 80° during 30 min and programmed from 80 to 180° at 2°/min, FID 190°, He 2.3 ml/min; SE-30 WCOT glass capillary column, 39 m \times 0.4 mm, at 80° for 30 min and programmed from 80 to 180° at 1.5°/min, FID 190°, N₂ 1.1 ml/min. The first column was used for analysis of the neutral fraction and the other columns for the sesquiterpene fraction.

GC/MS analysis was carried out using an instrument operating at 70 eV electron energy. A Carbowax 20 M WCOT quartz capillary column, 25 m \times 0.2 mm, programmed from 80 to 180° at 2°/min, He 1.5 ml/min, was coupled directly to the MS ion source.

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