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WAXES OF *CUPRESSUS DUPREZIANA* AND *CUPRESSUS SEMPERVIRENS*

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Key Word Index—*Cupressus dupreziana*; *Cupressus sempervirens*; Cupressaceae; leaves; heartwood; waxes; *n*-alkanes; secondary alcohols.

INTRODUCTION

In a plant, waxes—especially epicuticular waxes—represent the "first line of defense against external attack" [1, 2]. We report now the main components of the leaf wax of *Cupressus dupreziana*. For comparative purposes the closely-related *Cupressus sempervirens* [3] was re-examined. Heartwood of these two species was also studied to complete this comparison. Previous work on this topic has been reported [4–7].

RESULTS AND DISCUSSION

The petrol extracts of the leaves (*LP*) of *Cupressus dupreziana* and *Cupressus sempervirens* were fractionated into neutral and free-acid compounds by Na_2CO_3 treatment. The neutral fraction was chromatographed (Al_2O_3 grade II) to give *n*-alkanes and secondary alcohols. Saponification of *LP* yielded fatty and ω-hydroxy acids. All compounds were identified by GLC and GC/MS. Petrol extracts of the heartwood (*HP*) were fractionated in the same way. Neutral compounds were chiefly the terpenes previously described [4–7]. Total acids, obtained by saponification of *HP*, were analysed as for the leaf extracts. Results are given below and in Tables 1 and 2.

Table 1. Composition (%) of the *n*-alkanes and secondary alcohols from *C. dupreziana* and *C. sempervirens* leaves (*LP*)

Compounds (C_n)	*C. dupreziana*	*C. sempervirens*
n-Alkanes:		
C_{23}	0.2	0.2
C_{24}	0.2	0.2
C_{25}	0.2	0.3
C_{26}	0.4	0.3
C_{27}	0.4	0.4
C_{28}	0.4	0.3
C_{29}	1.0	2.3
C_{30}	0.4	0.3
C_{31}	5.5	4.9
C_{32}	1.6	1.8
C_{33}	60.4	64.0
C_{34}	5.8	5.7
C_{35}	23.5	19.3
Secondary alcohols:		
C_{29}-10-ol	100	—*
C_{29}-5,10-diol	—	100

* GC/MS analysis of all fractions eluted after *n*-alkanes by Al_2O_3 chromatography confirmed the absence of C_{29}-10-ol.

Table 2. Composition (%) of total acids from *C. dupreziana* and *C. sempervirens* leaves (*LP*) and heartwood (*HP*)

	Total acids (%) of leaves		Total acids (%) of heartwood	
Compounds (C_n)	*C. dupreziana*	*C. sempervirens*	*C. dupreziana*	*C. sempervirens*
$C_{12:0}$	4.7	6.4	3.7	2.6
$C_{13:0}$	trace	trace	trace	trace
$C_{14:0}$	9.5	15.5	3.3	2.8
$C_{15:0}$	0.6	1.2	1.1	0.9
$C_{16:0}$	20.9	20.0	23.9	27.7
$C_{17:0}$	1.3	1.1	1.8	2.0
$C_{18:0}$	3.2	3.3	10.7	12.6
$C_{18:1}$	0.9	trace	1.5	2.0
$C_{18:2}$	trace	trace	1.0	1.5
$C_{18:3}$	trace	0.5	trace	trace
$\Sigma C > 18$	10.5	17.1	53.0	47.9
$C_{12:0}$-ω-OH	6.2	5.4	—	—
$C_{14:0}$-ω-OH	12.0	9.6	—	—
$C_{16:0}$-ω-OH	30.2	19.9	—	—

Cupressus dupreziana

Leaves: *LP*, 7% of dry leaves; neutral, 90% of *LP*; *n*-alkanes, 31% of *LP*; secondary alcohols, 27%; total acids, 30%. Heartwood: *HP*, 7–8% of dry wood; neutral, 80% of *HP*; total acids, 20%.

Cupressus sempervirens

Leaves: *LP*, 6% of dry leaves; neutral, 93% of *LP*; *n*-alkanes, 25% of *LP*; secondary alcohols, 5%; total acids, 28%. Heartwood: *HP*, 2% of dry wood; neutral, 80% of *HP*; total acids, 20%.

This comparative study shows a great similarity between the *n*-alkane compositions of the two species (Table 1). Tritriacontane and pentatriacontane were the main compounds as generally described in the Cupressaceae family [8–10]. Furthermore, the ratio ($C_{25} + C_{26}$): C_{27} was greater than one, like in all old-world *Cupressus* species [9]. The total acid compositions were also very similar (Table 2).

On the other hand the secondary alcohols, nonacosan-10-ol from *Cupressus dupreziana* and nonacosan-5,10-diol from *Cupressus sempervirens*, distinguished these two species. Nonacosan-10-ol has been found chiefly in primitive plants such as *Chamaecyparis obtusa* [11], *Chamaecyparis lawsoniana*, *Picea sitchensis*, *Picea pungens*, *Agathis australis* [12], *Diplopteringium glaucum* [13], *Cycas revoluta* [14] and *Ginkgo biloba* [1, 12]. Long chain secondary diols, which are novel constituents of plant waxes, have been recently reported in *Pinus radiata* needle epicuticular wax [15, 16] and one of them was nonacosan-5,10-diol.

EXPERIMENTAL

Extractions. Cupressus dupreziana and *Cupressus sempervirens* leaves were collected from trees growing in the Botanical Garden of Algiers University and on the University Campus of Perpignan (France) respectively. Dry leaves (400 g) were extracted with petrol (40–60°) to give *LP* (28 g for *Cupressus dupreziana* and 24 g for *Cupressus sempervirens*). A part of this extract was fractionated into neutral- and free-acid compounds by treatment with 1N Na_2CO_3. The other part was saponified with 4N KOH–MeOH to obtain the total acid fraction.

Cupressus dupreziana heartwood was collected by the Algerian ORTF "Office de Recherches et Travaux Forestiers" from a tree growing in Ajjers Tassili. The sample of *Cupressus sempervirens* came from the same tree as the leaves. Petrol extracts (*HP*) were obtained from 2 kg of dry heartwood (145 g for *Cupressus dupreziana* and 40 g for *Cupressus sempervirens*) and fractionated by 1N Na_2CO_3 as previously described [4]. A part of this extract was also saponified by 4N KOH–MeOH.

Separations. Leaf neutral fraction was chromatographed on Al_2O_3 grade II. *n*-Alkanes were eluted by petrol. Tritriacontane ($C_{33}H_{68}$) was isolated from this fraction by preparative GLC on 20% OV-17, 6 m × 9 mm, at 290°, T.C. 310°, H_2 200 ml/min.; R_t 20 min. Elution by CH_2Cl_2–Et_2O (75:25) yielded nonacosan-10-ol from *Cupressus dupreziana*. Nonacosan-5,10-diol was obtained by CH_2Cl_2–Et_2O (1:1) from *Cupressus sempervirens*.

Leaves and heartwood total acid fractions were methylated by 20% BF_3–MeOH and analysed by GLC on DEGS and Apiezon L. In the case of the leaves the Me esters were chromatographed on Al_2O_3 grade II. Fatty Me esters were eluted by petrol and ω-hydroxy Me esters by C_6H_6. The latter compounds were characterized by a spot at R_f 0.6 (Si gel; $CHCl_3$–EtOAc, 7:3).

Analysis. GLC was carried out on 3 columns: 5% OV.1, 3 m × 3 mm, at 280°, FID 320°, N_2 25 ml/min; 15% DEGS, 3 m × 3 mm, at 175°, FID 250°, N_2 30 ml/min; 10% Apiezon L., 1.5 m × 3 mm, at 200°, FID 310°, N_2 40 ml/min. The first column was used for *n*-alkane and secondary alcohol analysis, the others for fatty and ω-hydroxy Me esters. Authentic samples and relationship log R_t = f(n) were used for identification.

GC/MS was carried out at 70 eV electron energy. A SE-30 WCOT glass capillary column, 25 m × 0.3 mm, programmed from 200 to 300° at 2°/min. He 1.4 ml/min was coupled directly to the MS ion source.

Nonacosan-10-ol: R_t (OV.1) = 8.99 min.; MS *m/e* (rel. int.): 424 (M^+, 1), 406 ($M^+ - H_2O$, 6), 297 ($C_{20}H_{40}OH$, 36), 157 ($C_{10}H_{20}OH$, 69), 139 ($C_{10}H_{19}$, 12), 125 (C_9H_{17}, 14), 111 (C_8H_{15}, 24), 97 (C_7H_{13}, 65), 83 (C_6H_{11}, 100), 69 (C_5H_9, 65).

Nonacosan-5,10-diol: R_t (OV.1) = 13.74 min.; MS, *m/e* (rel. int.): 422 ($M^+ - H_2O$, 1), 404 ($M^+ - 2H_2O$, 6), 365 ($C_{25}H_{48}OH$, 8), 347 ($C_{25}H_{47}$, 3), 297 ($C_{20}H_{40}OH$, 19), 173 ($C_{10}H_{19}(OH)_2$, 16), 155 ($C_{10}H_{18}OH$, 64), 137 ($C_{10}H_{17}$, 100), 125 (C_9H_{17}, 22), 123 (C_9H_{15}, 22), 111 (C_8H_{15}, 42), 109 (C_8H_{13}, 26), 97 (C_7H_{13}, 77), 95 (C_7H_{11}, 77), 87 ($C_5H_{10}OH$, 29), 83 (C_6H_{11}, 87), 81 (C_6H_9, 81), 69 (C_5H_9, 90), 67 (C_5H_7, 55).

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