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ISOLATION OF A NEW PHENOLIC COMPOUND FROM LEAVES OF *POPULUS DELTOIDES*

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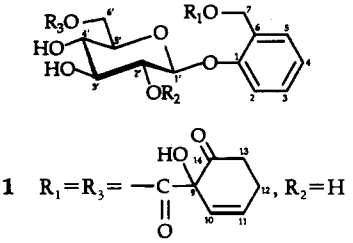
ABSTRACT.—A new phenolic glycoside was isolated from *Populus deltoides* leaves and was identified by spectral methods as salicortin-6′-*O*-(1-hydroxy-6-oxo-2-cyclohexen-1-carboxylate).

Phenolic glycosides are the main secondary compounds of *Populus* species and are important markers for poplar chemotaxonomy. Their biological activity in humans and their importance in plant-herbivore relations have been demonstrated (1–5). Previous studies on *Populus deltoides* leaves using a hot-H_2O extraction technique led to the identification of the following components: salicin, salicyl alcohol, pyrocatechol, 1-*O*-*p*-coumaroyl-β-D-glucoside, populoside, salicortin, chrysin-7-glucoside, ω-salicyloylsalicin, and deltoidin (identified as 2′-*O*-salicyloylsalicin) (6,7).

The relative instability of phenolic glycosides explains the different glycoside patterns obtained with different extraction methods, particularly in hot solvents. The methodology we used to determine the phenolic glycosides of Leuce poplar clones appeared suitable, as our compositional profiles and concentration data are comparable to those of Lindroth *et al.* (8,9).

The major crystalline components isolated by flash chromatography monitored by tlc and hplc, according to the method of Clausen *et al.* (10), from the leaves of *P. deltoides*, were salicortin, tremulacin, populoside, and a previously unknown phenolic glycoside, salicortin-6′-*O*-(1-hydroxy-6-oxo-2-cyclohexen-1-carboxylate) [**1**].

The extraction method, employing a



1 $R_1=R_3=$, $R_2=H$

90% aqueous Me_2CO system and the freeze-drying of the fresh leaves in liquid nitrogen presented no detectable changes in the relative amount of phenolic glycosides, when compared to a 90% aqueous MeOH extraction of fresh leaves. Accordingly, it is improbable that the new product **1** is an artifact of extraction or of a recombination of enzymatically hydrolyzed components.

The structure of the novel isolate was determined by spectral methods (^{1}H-nmr, ^{13}C-nmr, mass spectroscopy). The ^{1}H-nmr spectrum of **1** showed a set of signals (δ 5.20–6.10 ppm) which indicated the presence of two -CH=CH-CH_2- moieties (H-10, H-10′, H-11, H-11′) that can also be observed in the ^{1}H-nmr spectra (H-10, H-11) of salicortin and tremulacin. Chemical shifts of two H-6′ glucoside protons (δ 4.05–4.42 ppm) suggested the 6′-*O*-substitution of salicortin. The ^{13}C-nmr spectrum of **1** presents analogies with that of salicortin and particularly a duplication of the (1-hydroxy-6-oxo-2-cyclohexen-1-carboxy-

late) unit (C-8′ to C-14′). An HMBC ^{1}H-^{13}C-nmr (MeOH-d_4) correlation confirmed both a 6′-*O*-substitution (H-6′a, H-6′b/C-8′; δ 4.05–4.5 ppm/172.2 ppm) and a 7-*O*-substitution (H-7a, H-7b/C-8; δ 5.2 ppm/172.3 ppm) of salicin by the (1-hydroxy-6-oxo-2-cyclohexen-1-carboxylate) group. The cims of **1** using NH_3 exhibited a peak at *m/z* 580, corresponding to [M+H$^+$+17] that confirmed the molecular formula of $C_{27}H_{30}O_{13}$. The principal observed fragment peaks were consistent with successive losses of 1-hydroxy-6-oxo-2-cyclohexen-1-carboxylate, 2-hydroxyphenylmethanol, 6′-*O*-(1-hydroxy-6-oxo-2-cyclohexen-1-carboxylate) salicin, and 6-hydroxycyclohexen-2-one units.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Analytical tlc was performed on pre-coated Merck aluminum sheets (Si gel 60 F_{254}, 0.2 mm) with CH_2Cl_2-MeOH (85:15) as eluent. Hplc analyses were performed using a Varian 9010 instrument, with a column LiChrospher 100 RP-18 endcapped (5 μm) (length, 125 mm; i.d., 4 mm). The mobile phase consisted of H_2O and MeOH with the following gradient: % H_2O: 0 min, 100%; 20 min, 0%; % MeOH: 0 min, 0%; 20 min, 100%. Flow rate=1.5 ml/min, uv detection at 220 nm. ^{1}H-nmr spectra, DMSO-d_6 and DMSO-d_6-D_2O with TMS as internal standard (AC-300 MHz Bruker). ^{13}C-nmr spectra, MeOH-d_4 with TMS as internal standard (AC-200 MHz Bruker). Mass spectroscopy was performed on a Nermag R10-10-C instrument in NH_3.

PLANT MATERIAL.—Leaves were sampled from *Populus deltoides* on an experimental plantation at the INRA Research Center of Orléans (France). In July 1992, we collected the five apical leaves, on four branches of ten 3-year-old trees pruned in the spring. Samples were immediately weighed, immersed in liquid nitrogen, freeze-dried and stored *in vacuo* in the dark in a freezer at −20° until used. The percent H_2O content of *P. deltoides* leaves was about 75%.

EXTRACTION AND ISOLATION.—A sample of lyophilized leaves (3 g) was powdered and extracted twice for 2 h at room temperature with 90% aqueous Me_2CO. After filtration, the extract was concentrated at 40°. The residue, diluted in H_2O (100 ml) and 40% $(NH_4)_2SO_4$ (90 ml), was depigmented with petroleum ether (×3) and finally extracted with EtOAc (×3). The combined EtOAc extracts were dried on $MgSO_4$, filtered and evaporated *in vacuo*, yielding 0.484 g (16%) of dried extract containing phenolic glycosides.

The qualitative analysis by hplc of the final residue showed the existence of four principal components. Identification of salicortin, tremulacin, and populoside was performed by comparison of their retention times with standard samples. Compound **1**, along with salicortin, tremulacin, and populoside, was isolated from *P. deltoides* leaves by flash chromatography with CH_2Cl_2-MeOH (95:5) as eluent. Chromatographic fractions were analyzed by tlc and hplc, combined, and finally evaporated to give the four crystalline products in yields of 58.5, 27, 145.8, and 235.8 mg, respectively, for salicortin, tremulacin, populoside, and **1**.

Salicortin-6′-O-(1-hydroxy-6-oxo-2-cyclohexen-1-carboxylate) [**1**].—Mp 75°; $[\alpha]^{25}D$ −205.5° (*c*=1, CH_2Cl_2); ^{1}H nmr (300 MHz, DMSO-d_6-D_2O) δ 2.4–2.8 (8H, m, H-13a, H-13b, H-13′a, H-13′b, H-12a, H-12b, H-12′a, H-12′b), 3.1–3.7 (4H, m, H-2′, H-3′, H-4′, H-5′), 4.05 (1H, dd, $J_{6'a,6'b}$=10.6 Hz, $J_{6'a,5'}$=4.8 Hz, H-6′a), 4.42 (1H, dd, $J_{6'a,6'b}$=10.58 Hz, $J_{6'b,5'}$=4.8 Hz, H-6′b), 4.85 (1H, d, $J_{1',2'}$=7.2 Hz, H-1), 5.20 (2H, 2d, J_{gem}=13.4 Hz, H-7a, H-7b), 5.65 (1H, dt, $J_{10',11'}$=9.97 Hz, H-10′), 5.71 (1H, dt $J_{10,11}$=9.97 Hz, H-10), 6.05 (1H, dt, $J_{11',10'}$=10 Hz, $J_{11',12'}$=3.73 Hz, H-11′), 6.10 (1H, dt, $J_{11,10}$=10 Hz, $J_{11,12}$=3.73 Hz, H-11), 7.0–7.4 (4H, m, H-ar); ^{13}C nmr (200 MHz, MeOH-d_4) δ 25.3 (C-12), 28.1 (C-12′), 37.6 (C-13, C-13′), 65.2 (C-7), 66.9 (C-6′), 72.2 (C-4′), 75.6 (C-2′), 76.1 (C-3′), 78.5 (C-5′), 79.9 (C-9, C-9′), 103.7 (C-1′), 118.1 (C-6), 124.7 (C-4), 127.6 (C-2), 130.2 (C-10′), 131.5 (C-10), 132 (C-3), 132.8 (C-5), 134.1 (C-11), 134.2 (C-11′), 157.7 (C-1), 172.2 (C-8′), 172.3 (C-8), 207.9 (C-14′), 208.2 (C-14). Ms *m/z* 580 (49), 442 (18), 173 (74), 124 (37), 113 (100).

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