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STRUCTURALLY INTRIGUING GLUCOSIDES FROM ALASKAN LITTLETREE WILLOW (*SALIX ARBUSCULOIDES*)

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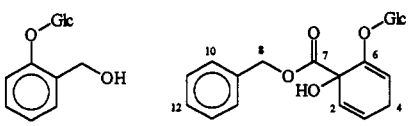
ABSTRACT.—Nmr and chemical analysis of the major glycosidic metabolites of *Salix arbusculoides* has yielded two new isomeric glucosides, **3** and **4**. Both **3** and **4** are structurally similar to salicortin, a common glucoside present in most *Populus* and *Salix* species. Unlike salicortin, however, neither **3** nor **4** possesses the phenolic functionality that is characteristic of the majority of glucosides found in these species. Compound **4** was found to exist in solution as an equilibrium mixture of keto and enol tautomers.

The dominant chemical characteristic of willows (*Salix* spp.) is the widespread occurrence of phenolic glycosides (1). Salicin [**1**] and its derivatives, such as salicortin [**2**], are commonly found in willows and are natural product prototype molecules for the therapeutically widely used aspirin (2). One structural feature common to all the salicin-based glycosides reported to date is the glycosylation of phenolic oxygen of the aglycone. We report here two aromatic glucosides isolated from an Alaskan willow, littletree willow [*Salix arbusculoides* Anderss. (Salicaceae)], which are unusual in that glycosylation is not at the phenolic oxygen.

Fresh twigs of *S. arbusculoides* were extracted with MeOH at room temperature. Separation of the extract by flash chromatography yielded **3** and **4**, both having the formula $C_{20}H_{24}O_9$ (hrfabms). Acidic hydrolyses of **3** and **4** with sulfonic acid resin (H^+ type) gave benzyl salicylate (in excess of 30% yield), along with D-glucose (3) as the major products. Traces of phenol and benzyl alcohol were also observed.



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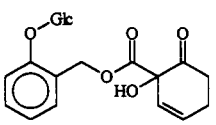

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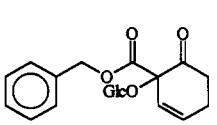

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The results of these experiments demonstrate that **3** and **4** possess a common carbon skeleton. In addition, both are shown to be *O*-D-glucosides, and the presence of a benzyl ester was evident.

In determining the structures of **3** and **4**, **2** proved to be an excellent model (4). The ^{1}H-nmr spectrum of **4** is very similar to that of **2**, with the exception of the aromatic region. The ^{13}C-nmr spectrum of **4** differed from **2** in that the phenolic carbon resonance (δ 154.3) of **2** was absent in the spectrum of **4**. In addition, **4** displayed only four aromatic carbon signals, which along with the ^{1}H-nmr spectrum indicated a monosubstituted benzene ring. The absence of a phenolic carbon, the requirement for an *O*-glucoside, and the similarities between the nmr spectra of **4** and **2**, thus required the placement of the glucosidic linkage at the position indicated in **4**.

However, an additional set of signals not assigned to **4** was also present in the nmr spectra of this sample. Assignment of the extra signals to the enol tautomer of **4** is consistent with the observed experimental results. ^{1}H-Nmr studies in various solvents established that the relative ratios of the signals assigned to H-3

of the keto (δ 6.42) and enol (δ 6.03) forms of **4** were solvent-dependent. In the three solvents tested, we estimated the mole fractions of enol to be 0.33 (Me_2CO-d_6), 0.23 (MeOH-d_4), and 0.10 (D_2O). Close inspection of the olefinic region of the ^{1}H-nmr spectrum of **4** revealed three signals (δ 6.03, 5.93, 5.52), which were assigned to H-3, H-5, and H-2, of the enol form. Those ^{1}H-nmr signals which were clearly resolved from those of ketone **4** are assigned to the enol form (see Experimental). Signals not observed for the enol are assumed to overlap those of the ketone.

Like **4**, **3** also displayed ^{1}H- and ^{13}C-nmr signals consistent with the presence of a monosubstituted benzene ring. However, the ^{1}H-nmr spectrum of **3** contained one more olefinic proton (δ 5.49) than did **2**. The ^{13}C-nmr spectrum of **3** lacked a ketone signal (ca. δ 204), as well as a methylene resonance (ca. δ 30), both shared by **4** and **2**, but exhibited additional signals at δ 151.9 and δ 101.6. Assignment of these signals to adjacent alkene carbons is consistent with the presence of an enol ether (5,6). Because this substance was not identical with the enol form of **4**, the only possible position for the glucosidic linkage is at the enol ether oxygen, giving the enol glycoside **3**. The structure indicated for **3** was further corroborated by 2D nmr spectroscopy. The COSY spectrum verified the coupling of the olefinic protons (δ 6.02, 5.64, 5.49) with the methylene protons (δ 2.84). The ^{1}H-^{13}C correlation identified the ^{13}C-nmr resonances of the methylene (δ 26.8) and the adjacent enolic carbon (δ 101.6).

Conclusive evidence for the stereochemistry of the glucosidic linkage was provided by comparison of the ^{13}C-nmr carbohydrate resonances of **3** and **4** with those of various β-D-glucose derivatives (7), as well as the magnitudes (ca. 7 Hz) of the $J_{H1'-H2'}$ coupling constants (8) in the ^{1}H-nmr spectra of **3** and **4**. These observations indicated that both **3** and **4** are β-D-glucosides.

Attempts to hydrolyze the glucose moieties of **3** and **4** with almond β-glucosidase were unsuccessful in both cases. Although a sample of **1** hydrolyzed within 30 min, neither **3** nor **4** exhibited any observable evidence of reaction even when the reaction time was extended to five days. The failure of almond β-glucosidase to cleave the glucose moieties of these compounds is surprising given what is known (9) about the enzyme's ability to hydrolyze a broad array of glucosides of varying aglycone structure.

The discovery of **3** and **4** has extended significantly the structural range of known willow glycosides. Most of the examples described to date have been phenolic glycosides, but **3** and **4** do not belong to this class of substances.

Furthermore, the aglycone of **3** is equivalent to that of the enol form of **4**. Thus one can imagine that **3** and **4** are formed by glucosylation of a single aglycone or that one is formed from the other by enzyme-mediated transfer of glucose. Even though the enol form of **4** does not benefit from the normal stabilization associated with β-ketoesters, **4** does exist in solution as a mixture of keto and enol tautomers.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Optical rotations were measured on a Perkin-Elmer 141 polarimeter. Nmr spectra were recorded at 500 MHz or 90 MHz in $(CD_3)_2CO$; chemical shifts are given in ppm relative to TMS as internal standard.

Multiplicities of ^{13}C-nmr peaks were determined from DEPT data. Two-dimensional ^{1}H-^{1}H and ^{13}C-^{1}H correlations were made using standard pulse sequences. Hrfabms was done on a Kratos MS-50 instrument. Product identification/quantification was accomplished with gc by co-injection of authentic materials using a Perkin-Elmer 8410 gc instrument equipped with a FID detector and a 25 m×0.53 mm i.d. capillary column with polydiphenyldimethylsiloxane stationary phase (*Alltech RSL-200*). All solvents used were reagent grade unless otherwise indicated. Supports for flash chromatography were Baker bonded phase-octadecyl (C_{18}) or Baker Si gel.

PLANT MATERIAL.—*Salix arbusculoides* L.

(Salicaceae) was collected in December 1989 near Fairbanks, Alaska. A voucher specimen has been deposited at the Museum, University of Alaska, Fairbanks.

EXTRACTION AND ISOLATION.—Fresh twigs of *S. arbusculoides* (50 g) were cut into approximately 5-cm lengths and extracted with 500 ml MeOH for 48 h at room temperature. The extract was then filtered, evaporated *in vacuo*, and lyophilized to yield 3.3 g of a solid. This residue was taken up in MeOH, mixed with 10 g of Si gel, and the solvent removed under high vacuum. The sample-impregnated Si gel was applied to the top of a 50-mm×20-cm i.d. Si gel column and subjected to flash chromatography with a gradient of increasing proportions of MeOH in $CHCl_3$: MeOH-$CHCl_3$ (250 ml, 15:85) followed by MeOH-$CHCl_3$ (250 ml, 17:83), MeOH-$CHCl_3$ (250 ml, 19:81), and MeOH-$CHCl_3$ (500 ml, 23:77). Appropriate fractions were combined to afford 0.27 g crude **4** and 1.14 g crude **3** as glasses.

Further purification of **3** was accomplished by repeated flash chromatography on reversed-phase C_{18} support using an eluent of H_2O-Me_2CO (72:28). Fractions containing pure **3** were freed of Me_2CO by flash evaporation, then lyophilized. Compound **4** was similarly purified using an eleunt of H_2O-Me_2CO (75:25).

Benzyl-6-O-β-D-glucopyranosyl-1,6-dihydroxy-2,5-cyclohexadienyl carboxylate [**3**].—Amorphous powder; $[\alpha]_D$ −63° (*c*=0.012, H_2O); ^{1}H nmr δ 7.35 (4H, m, H-10, H-11), 7.29 (1H, m, H-12), 6.02 (1H, dtd, *J*=9.9, 2.3, and 1.1 Hz, H-3), 5.64 (1H, dt, *J*=9.9 and 2.0 Hz, H-2), 5.49 (1H, dt, *J*=1.1 and 2.5 Hz, H-5), 5.21 (1H, d, *J*=11.0 Hz, H-8), 5.15 (1H, d, *J*=11.0 Hz, H-8), 5.08 (1H, br s, OH), 4.84 (1H, br s, OH), 4.70 (1H, d, *J*=7.0 Hz, H-1′), 4.40 (1H, br s, OH), 4.32 (1H, br s, OH), 3.88 (1H, m, H-6′), 3.70 (2H, m, H-6′), OH), 3.47 (1H, m, H-5′), 3.44 (1H, m, H-3′), 3.40 (1H, m, H-4′), 3.33 (1H, m, H-2′), 2.95 (1H, s, H_2O), 2.84 (2H, m, H-4); ^{13}C nmr δ 172.70 (s, C-7), 151.92 (s, C-6), 136.66 (s, C-9), 128.55 (d, C-11), 128.11 (d, C-3), 127.95 (d, C-12), 127.39 (d, C-10), 126.21 (d, C-2), 102.14 (d, C-1′), 101.59 (d, C-5), 77.20 (d, C-3′), 76.78 (d, C-5′), 74.06 (d, C-2′), 72.11 (s, C-1), 70.71 (d, C-4′), 66.67 (t, C-8), 62.10 (t, C-6′), 26.75 (t, C-4); hrfabms (LiI matrix) *m/z* 415.1594 ($C_{20}H_{24}O_9Li$ requires *m/z* 415.1580).

Benzyl 1-O-β-D-glucopyranosyl-1-hydroxy-6-oxo-2-cyclohexenyl carboxylate [**4**].—Glass; $[\alpha]_D$ 0° (*c*=0.004, H_2O); ^{1}H nmr δ 7.34 (5H, m, H-10, H-11, H-12), 6.42 (1H, dtd, *J*=9.9, 3.7, and 0.9 Hz, H-3), 5.83 (1H, dt, *J*=9.9 and 1.6 Hz, H-2), 5.10 (1H, d, *J*=11.7 Hz, H-8), 5.06 (1H, d, *J*=11.7 Hz, H-8), 4.70 (1H, d, *J*=7.3 Hz, H-1′), 4.56 (1H, br s, OH), 4.35 (1H, br s, OH), 4.28 (1H, br s, OH), 3.76 (1H, m, H-6′), 3.54 (2H, m, H-6′, OH), 3.36 (1H, m, H-5′), 3.29 (1H, m, H-2′), 3.26 (1H, m, H-4′), 3.21 (1H, m, H-3′), 2.99 (3.5H, s, H_2O), 2.83 (1H, m, H-5), 2.62 (1H, m, H-4), 2.55 (1H, m, H-5), 2.49 (1H, m, H-4); [signals assigned to enol form of **4**: 6.03 (1H, dtd, H-3), 5.93 (1H, br s, H-5), 5.52 (1H, dt, H-2), 5.33 (1H, d, H-8), 5.27 (1H, d, H-8), 4.66 (1H, d, H-1′)]; ^{13}C nmr δ 204.06 (s, C-6), 168.88 (s, C-7), 136.23 (d, C-3), 136.00 (s, C-9), 128.65 (d, C-11), 128.32 (d, C-12), 127.92 (d, C-10), 125.06 (d, C-2), 98.91 (d, C-1′), 80.38 (s, C-1), 77.24 (d, C-3′), 77.21 (d, C-5′), 73.90 (d, C-2′), 70.67 (d, C-4′), 67.16 (t, C-8), 62.11 (t, C-6′), 36.17 (t, C-5), 25.25 (t, C-4); hrfabms (LiI matrix) *m/z* 415.1580 ($C_{20}H_{24}O_9Li$ requires *m/z* 415.1580).

RESIN HYDROLYSIS OF **3** AND **4**.—Approximately 1-mg samples of **3** or **4** were dissolved in 250 μl of deionized H_2O and added to a 0.25-ml volume of Dowex 50W-X8 sulfonic acid cation-exchange resin (H^+ form). After 3 days at room temperature, the solutions were diluted with 250 μl of CH_3CN containing 0.524 mg/ml camphor (internal standard). The resulting solutions were analyzed by gc (column 75° for 10 min, 75–230° at 17.5°/min, 230° for 2 min, injector/detector 300°, carrier gas He 8.0 ml/min) for phenol (4.5 min, trace), benzyl alcohol (6.3 min, trace), and benzyl salicylate (18.5 min, 30%).

ENZYMATIC HYDROLYSIS WITH ALMOND β-GLUCOSIDASE.—Solutions of **3**, **4**, and **1** (20 mM each) were prepared in 0.1 M NaOAc/HOAc buffer at pH 5.0. A solution of β-glucosidase (ICN 100348) was made up in the same buffer having an approximate activity of 2 units per ml (one unit of activity is defined as the amount of enzyme which will hydrolyze 1 μM of salicin per min). Equal volumes of the glucoside solutions and enzyme solution were combined. As controls, equal volumes of the glucoside solutions and buffer were combined. All six solutions were placed in small sample vials equipped with teflon cap liners and incubated at 37°. Periodically, samples were removed and analyzed by tlc (Si gel, MeOH-$CHCl_3$ 1:9).

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