Phenolic Glycosides from Salix lasiandra

Paul B. Reichardt, Howard M. Merken, Thomas P. Clausen, and Jiejun Wu

J. Nat. Prod., **1992**, 55 (7), 970-973• DOI:
10.1021/np50085a022 • Publication Date (Web): 01 July 2004

Journal of Natural Products
Vol. 55, No. 7, pp. 970-973, July 1992

PHENOLIC GLYCOSIDES FROM *SALIX LASIANDRA*

PAUL B. REICHARDT,* HOWARD M. MERKEN, THOMAS P. CLAUSEN,

Department of Chemistry, University of Alaska Fairbanks, Fairbanks, Alaska 99775-0520

and JIEJUN WU

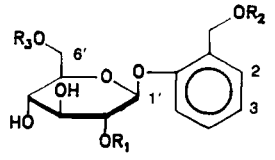
Department of Chemistry, University of California Irvine, Irvine, California 92717

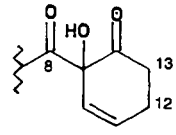
ABSTRACT.—The major phenolic glycosides of *Salix lasiandra* (Salicaceae) consist of three known metabolites (salicortin [**1**], 2′-*O*-acetylsalicortin [**2**], and 2′-*O*-acetylsalicin [**4**]) as well as one previously unknown substance, lasiandrin [2′-*O*-acetylsalicortin-6′-*O*-(1-hydroxy-6-oxo-2-cyclohexene-1-carboxylate)] [**3**].

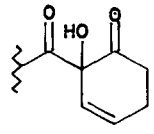
Phenolic glycosides are commonly found as secondary metabolites of willows. Within the *Salix* genus these compounds have been used as taxonomic markers (1) and, in some cases, have been demonstrated to play important roles in the ecology of plant/animal interactions (2,3). As part of our effort to understand the ecological significance of the diverse array of willows in high latitude ecosystems, we have begun to examine the phenolic glycoside content of Alaskan willows. This note reports the structures of the major phenolic glycosides of the Pacific willow, *Salix lasiandra* Benth. (Salicaceae), a pioneer species often found along Alaskan rivers (4).

An Me_2CO extract of fresh *S. lasiandra* leaves and twigs provided, after chromatography on Si gel, three major fractions containing phenolic glycosides. Tlc and ^{13}C-nmr spectroscopy indicated that two of the fractions were individual compounds **3** and **2** and that the third was a mixture. The latter fraction was separated into two substances, **1** and **4**, by reversed-phase flash chromatography.

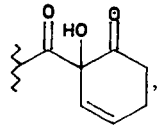
One of these substances was easily identified as salicortin [**1**] by comparison of its chromatographic mobility and

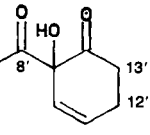


1 $R_1=R_3=H$, $R_2=$ 

2 $R_1=Ac$, $R_2=$ 

, $R_3=H$

3 $R_1=Ac$, $R_2=$ 

, $R_3=$ 

4 $R_1=Ac$, $R_2=R_3=H$

^{13}C-nmr spectrum (Table 1) with those of a standard sample

Cursory inspection of the ^{13}C-nmr spectrum of **2** (Table 1) revealed that it was an acetylated derivative of **1**, an assignment consistent with the molecular formula of $C_{22}H_{26}O_{11}$ as determined by hrfabms. Begining with assignment of the ^{13}C signal at 100.0 ppm to the anomeric carbon, analyses of the HETCOR and COSY spectra of **2** allowed complete assignments of the ^{13}C and ^{1}H signals associated with the glucopyranosyl fragment. When these assignments were compared to analogous ones reported for glucose and simple glucosides (5), it became clear that the signal for H-2′ of **2** (5.01 ppm) is shifted 1.5–2 ppm downfield from the position of the corresponding signal in each reference compound, indicating that **2** is 2′-*O*-acetylsalicortin. The placement of the acetyl group on the 2′ oxygen was confirmed by an nOe experiment in which irradiation of the signal for the acetyl Me group (2.065 ppm) gave rise to enhanced signals for H-1′ (5.13 ppm), H-2′ (5.01 ppm), and H-10 (5.79 ppm). This substance has been previously reported as a metabolite of other willow species (1,6) and has been chemically characterized, but its spectral properties have not previously been reported in the available literature. However, Dr. Beat Meier has informed us that **2** has been fully characterized and is described in a thesis written by C.P. Egloff at the ETH in Zurich.

TABLE 1. ^{13}C-nmr Chemical Shifts (ppm) for Compounds **1–4**.

Carbon	Compound			
	1[a]	**2**[b]	**3**[b]	**4**[c]
C-1′	101.0	100.0	100.1	100.6
C-2′	73.4	74.3	74.1	75.2
C-3′	77.2	75.6	75.3	76.0
C-4′	69.9	71.4	71.2	71.6
C-5′	76.6	78.0	74.9	78.3
C-6′	60.9	62.3	65.6	62.4
C-1	155.1	155.8	155.7	155.8
C-2	124.6	125.8	126.0	131.8
C-3	129.5	129.4	129.4	128.9
C-4	121.7	123.3	123.6	123.7
C-5	128.4	130.4	130.5	129.5
C-6	115.1	116.4	116.6	116.0
C-7	62.3	63.0	63.0	60.0
C-8	170.2	170.3	170.7[d]	—
C-9	77.5	78.7	78.6[e]	—
C-10	131.6	132.4	132.3[f]	—
C-11	128.9	129.2	129.1[g]	—
C-12	26.0	27.1	27.1	—
C-13	35.7	36.0	36.0	—
C-14	206.1	206.2	206.6	—
C-8′	—	—	170.7[d]	—
C-9′	—	—	78.7[e]	—
C-10′	—	—	132.4[f]	—
C-11′	—	—	129.4[g]	—
C-12′	—	—	27.1	—
C-13′	—	—	36.0	—
C-14′	—	—	206.2	—
C=O	—	170.7	170.2	172.0
Me	—	21.0	21.0	21.0

[a]In DMSO-d_6.
[b]In Me_2CO-d_6.
[c]In MeOH-d_4.
[d–g]Signal assignments with same letter may be interchanged.

A third metabolite, lasiandrin [**3**], was found to have the formula $C_{29}H_{32}O_{14}$ (hrfabms). The most conspicuous feature of its ^{1}H-nmr spectrum, in addition to its similarity to the spectrum of **1**, was a set of signals (5.7–6.2 ppm) which indicated the presence of two -CH=CH-CH_2- moieties, an observation which led to the recognition that all the signals associated with the esterifying carboxylic acid group in **1** appeared as "duplicates" in **3**, i.e., either duplicate signals were observed or signal intensities were approximately doubled. An acetyl Me group (2.08 ppm) was also apparent. Mapping of the nmr signals of the glucopyranosyl moiety by HETCOR and COSY in the same manner as with **2** led to the conclusion that the oxygens at C-2′ (signal for H-2′ at 5.02 ppm) and C-6′ (signals for H_a-6′ and H_b-6′ at 4.63 and 4.26 ppm) were esterified. The attachment of the acetyl group to the oxygen at C-2′ was assigned from the results of an nOe experiment in which irradiation of the acetyl Me group produced enhancements of the signals due to H-1′ (5.16

ppm), H-2′, H-3′ (3.74 ppm), and H-10 (5.80 ppm). With the site of acetylation securely established, only structure **3** fits the remaining data for this new phenolic glycoside.

The remaining glycoside, **4**, has a formula of $C_{15}H_{20}O_8$ (hrfabms). Its ^{13}C-nmr spectrum (Table 1) is essentially the same as that of salicin (7), with additional signals (172.0 and 21.0 ppm) indicative of an acetyl group. Thus, **4** appeared to be an acetylated form of salicin. Mapping of the nmr signals associated with the glucopyranosyl ring by the process used for **2** and **3** clearly indicated that the oxygen attached to C-2′ was acylated (H-2′ signal at 5.02 ppm). An nOe experiment in which irradiation of the acetyl Me signal (2.12 ppm) led to enhanced signals for H-1′ (5.06 ppm), H-2′ (5.02 ppm), and H-3 (7.39 ppm) allowed placement of the acetyl group at C-2′. Thus, **4** is 2′-*O*-acetylsalicin, a phenolic glycoside which has been reported from European *Salix pentandra* (6) and which has been fully characterized and described in a thesis by Y. Shao (B. Meier, personal communication).

There are three significant points associated with the results reported here. First, this is the first report of the characterization of **3** from any source and the first report of **2** and **4** as metabolites of North American woody plants. Second, the concentrations of **1–3** in *S. lasiandra* tissue (ca. 1–3% of dry wt) are rather high compared to the concentrations of phenolic glycosides found in many *Salix* species. For example, Julkunen-Tiitto found phenolic glycoside concentrations in leaves (8) and twigs (9) of northern Salicaceae species generally to be two to

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—^{13}C- and ^1H-nmr spectra were recorded at 125 and 500 MHz, respectively, and chemical shifts are reported in ppm relative to TMS. Peak assignments for the spectra of **2–4** are all consistent with information obtained from COSY and HETCOR spectra obtained from standard pulse sequences. Analytical tlc was performed on Si gel F_{264} plates (Merck), and R_f's are reported for elution with MeOH-$CHCl_3$ (15:85).

PLANT MATERIAL.—*S. lasiandra* leaves and twigs were collected near the University of Alaska Fairbanks campus in Fairbanks, Alaska. A voucher specimen, number V105399, has been deposited in the herbarium of the University of Alaska Museum.

EXTRACTION AND ISOLATION OF PHENOLIC GLYCOSIDES.—A sample of lyophilized minced leaves and twigs of *S. lasiandra* (47.4 g) was extracted in a Soxhlet apparatus with Me_2CO for 15 h. The extract was evaporated under reduced pressure to give a green oil (7.7 g) which was separated by flash chromatography (Si gel, 40 mm diameter column, MeOH/$CHCl_3$, 7–20% $CHCl_3$). Chromatographic fractions were analyzed by tlc, and appropriate fractions were combined to give three samples. The most mobile component was further purified by reversed-phase flash chromatography [C_{18}, Me_2CO-H_2O (1:1)] to give **3** (0.89 g) as an off-white amorphous powder. Purification of the component with intermediate mobility by the same method gave **2** (1.37 g) as a white powder. Reversed-phase flash chromatography of the most polar fraction (step gradient of Me_2CO/H_2O; 15, 50, and 90% Me_2CO) gave **1** (0.43 g) and **4** (0.06 g), which could be further purified by recrystallization from Me_2CO.

Salicortin [**1**].—R_f 0.30; ^1H nmr (Me_2CO-d_6, 500 MHz) essentially identical to that reported by Lindroth *et al.* (10); ^{13}C nmr (DMSO-d_6, 125 MHz) see Table 1.

2′-O-Acetylsalicortin [**2**].—White amorphous powder: mp 175–185°; R_f 0.47; fabms *m/z* $[M+H]^+$ 467.1557 ($C_{22}H_{27}O_{11}$ requires 467.1553); ^1H nmr (ppm, Me_2CO-d_6, 500 MHz) 7.29 (1H, d, J = 8.0 Hz, H-3), 7.27 (1H, dt, J = 8.0, 1.5 Hz, H-5), 7.21 (1H, d, J = 8.0 Hz, H-6), 7.04 (1H, dt, J = 7.5, 0.9 Hz, H-4), 6.13 (1H, dt, J = 9.7, 3.8 Hz, H-11), 5.79 (1H, dt, J = 9.5, 1.7 Hz, H-10), 5.15 (1H, d, J = 13 Hz, H_a-7), 5.13 (1H, d, J = 8.4 Hz, H-1′), 5.12 (1H, d, J = 12.8 Hz, H_b-7), 5.01 (1H, dd, J = 9.5, 8.0 Hz, H-2′), 4.69 (1H, dd, J = 4.8, 2.4 Hz, 6′-OH), 4.58 (1H, d, J = 2.75 Hz, 3′-OH), 3.91 (1H, m, H_b-6′), 3.74 (2H, m, H-3′, H_b-6′), 3.57 (2H, m, H-4′, H-5′), 3.03 (2H, s, OH), 2.89 (1H, m, H_a-13), 2.66 (1H, m, H_b-12), 2.56 (1H, m, H_a-13), 2.49 (1H, m, H_a-12), 2.065 (3H, s, Me); ^{13}C nmr (125 MHz, Me_2CO-d_6) see Table 1.

2′-O-Acetylsalicortin-6′-O-(1-hydroxy-6-oxo-2-cyclohexene-1-carboxylate) (lasiandrin) [**3**].—Buff-colored amorphous powder: mp 170–175°; R_f 0.63; fabms *m/z* $[M+Na]^+$ 627.1706 ($C_{29}H_{32}O_{14}Na$ requires 627.16898); ^1H nmr (ppm, Me_2CO-d_6, 500 MHz) 7.37 (1H, dt,

J = 9.5, 1.3 Hz, H-5), 7.32 (1H, dd, J = 9.5, 1.3 Hz, H-3), 7.21 (1H, d, J = 9.5 Hz, H-6), 7.08 (1H, dt, J = 9.5, 1.3 Hz, H-4), 6.14 (1H, dt, J = 12.3, 4.8 Hz, H-11), 6.09 (1H, dt, J = 12.3, 4.8 Hz, H-11'), 5.80 (1H, dt, J = 12.3, 2.1 Hz, H-10), 5.74 (1H, dt, J = 12.3, 2.1 Hz, H-10'), 5.21 (1H, s, OH), 5.17 (1H, d, J = 13.0 Hz, H_a-7), 5.16 (1H, d, J = 8.2 Hz, H-1'), 5.15 (1H, d, J = 13.0, H_b-7), 5.02 (1H, dd, J = 9.6, 8.2 Hz, H-2'), 4.86 (1H, d, J = 4.8 Hz, 4'-OH), 4.83 (1H, d, J = 5.5 Hz, 3'-OH), 4.63 (1H, dd, J = 10.9, 1.8 Hz, H_b-6'), 4.26 (1H, dd, J = 9.6, 4.8 Hz, H_a-6'), 3.85 (1H, ddd, J = 7.5, 4.8, 1.5 Hz, H-5'), 3.74 (1H, m, H-3'), 3.52 (1H, m, H-4'), 2.87 (2H, m, H_b-13, H_b-13'), 2.66 (4H, m, H-12, H-12'), 2.53 (2H, m, H_a-13, H_a-13'), 2.08 (3H, s, Me); ^{13}C nmr (Me_2CO-d_6, 125 MHz) see Table 1.

2'-O-Acetylsalicin [**4**].—White crystals: mp 189–191° from Me_2CO; R_f 0.30; fabms *m/z* $[M + H]^+$ 329.1240 ($C_{15}H_{21}O_8$ requires 329.1236); ^{1}H nmr (ppm, MeOH-d_4, 500 MHz) 7.39 (1H, d, J = 7.5 Hz, H-3), 7.24 (1H, t, J = 7.3 Hz, H-5), 7.14 (1H, d, J = 7.4 Hz, H-6), 7.05 (1H, t, J = 7.5 Hz, H-4), 5.06 (1H, d, J = 8.0 Hz, H-1'), 5.02 (1H, t, J = 9.4 Hz, H-2'), 4.59 (1H, d, J = 13.6 Hz, H_a-7), 4.54 (1H, d, J = 13.6 Hz, H_b-7), 3.92 (1H, d, J = 11.7 Hz, H_a-6'), 3.73 (1H, dd, J = 12.0, 4.7 Hz, H_b-6'), 3.64 (1H, m, H-3'), 3.49 (2H, m, H-4', H-5'), 2.12 (3H, s, Me); ^{13}C nmr (MeOH-d_4, 125 MHz) see Table 1.

ACKNOWLEDGMENTS

The work at the University of Alaska Fairbanks was supported by the National Science Foundation (Grant No. BSR-8702629). Nmr spectra were recorded at the University of California Irvine NMR facility on an instrument purchased with the assistance of an NSF Shared Instrument Grant (Grant No. CHE-8805314). Ms determinations were performed by the Midwest Center for Mass Spectrometry, an NSF Regional Instrumentation Facility (Grant No. CHE-8620177).

LITERATURE CITED

1. B. Meier, R. Julkunen-Tiitto, J. Tahvanainen, and O. Sticher, *J. Chromatogr.*, **442**, 175 (1988).
2. P.B. Reichardt, T.P. Clausen, and J.P. Bryant, in: "Handbook of Natural Toxins: Toxicology of Plant and Fungal Compounds." Ed. by R.F. Keeler and A.T. Tu, Marcel Dekker, New York, 1991, Vol. 6, pp. 313–333.
3. M. Rowell-Rahier and J.M. Pasteels, in: "Insect-Plant Interactions." Ed. by E.A. Bernays, CRC Press, Boca Raton, Florida, 1990, Vol. 2, pp. 75–110.
4. L.A. Viereck and E.L. Little, "Alaska Trees and Shrubs," Forest Service, United States Department of Agriculture, Washington, D.C., 1972, pp. 125–126.
5. K. Bock and H. Thogersen, *Ann. Rev. NMR Spectrosc.*, **13**, 1 (1982).
6. Y. Shao, M.F. Lahloub, B. Meier, and O. Sticher, *Planta Med.*, **55**, 617 (1989).
7. R.A. Domisse, L. Van Hoof, and A.J. Vlietinck, *Phytochemistry*, **25**, 1201 (1986).
8. R. Julkunen-Tiitto, *Phytochemistry*, **25**, 663 (1986).
9. R. Julkunen-Tiitto, *J. Chromatogr.*, **324**, 129 (1985).
10. R.L. Lindroth, M.T.S. Hsia, and J.M. Scriber, *Biochem. Syst. Ecol.*, **15**, 667 (1987).

Received 14 October 1991