Two Acylated Diarylheptanoid Glycosides from Red Alder Bark

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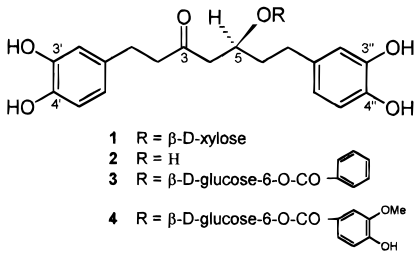
Received March 6, 1998

Two novel acylated diarylheptanoid glycosides, oregonosides A and B (**3**, **4**, respectively), were isolated from red alder bark and their structures established by spectrometric techniques. The compounds were identified as (5*S*)-1,7-bis(3,4-dihydroxyphenyl)-5-*O*-(6-*O*-benzoyl-*β*-D-glucopyranosyl)-heptan-3-one and (5*S*)-1,7-bis(3,4-dihydroxyphenyl)-5-*O*-(6-*O*-vanilloyl-*β*-D-glucopyranosyl)-heptan-3-one.

Red alder (*Alnus rubra*, Bong.) (Betulaceae) is the major hardwood species of the Pacific Northwest coastal forests. The tree receives its name from the reddish orange color that rapidly develops on the surface of freshly exposed wood and bark. Oregonin, a diarylheptanoid xyloside (**1**), was shown to be a precursor to this staining phenomenon.[1] Recently, oregonin and its aglycon hirsutanonol (**2**) were shown to exhibit antibiotic activity.[2,3] Indigenous peoples of the region have long used the bark of this tree for both its colorant and medicinal properties.[4−6] When used as a coloring material, the red-orange color was stabilized by mixing the crushed bark with fish eggs, and acceleration of color formation was accomplished by mixing with an iron-rich clay.[6] As a medicine, the bark was often used to treat a variety of ailments from colds to heart pain. In an investigation of additional diarylheptanoid glycosides of red alder bark, two novel acylated glycosides were isolated, and their structures were elucidated by spectrometric techniques.

Oregonosides A (**3**) and B (**4**) were isolated as minor components from a methanol extract of fresh red alder bark after chromatography over Sephadex LH-20. Additional chromatography over Toyopearl HW-40S with ethanol and Sephadex LH-20 with ethanol−water[7] gave homogeneous samples of **3** and **4** as amorphous powders. Hirsutanonol (**2**) was later isolated from red alder bark in order to compare its chemical shifts with those of the three glycosides.[3,8] ^{1}H and ^{13}C NMR spectra (Table 1) indicated that **3** and **4** had the same aglycon moiety (hirsutanonol) as oregonin but a different sugar moiety and an acyl group as well.



The ^{13}C NMR signals for the glycosidic moiety of **3** indicated a *β*-D-glucopyranosyl group with acyl-induced shifts of glucose C-5 and C-6 signals.[10] The carbon signal at *δ* 103.6 is indicative of the *β*-glucosyl linkage that was confirmed by the proton doublet at *δ* 4.32 with $J = 7.7$ Hz. The proton signal at *δ* 4.32 showed connectivity to the carbon signal at *δ* 103.6 in the HETCOR spectrum, which further confirmed this to be the anomeric proton signal. The HMBC spectrum confirmed attachment of the glucosyl moiety to the diarylheptanoid C-5 position by showing connectivity of the anomeric proton signal (*δ* 4.32) to the diarylheptanoid C-5 carbon signal at *δ* 76.6 and connectivity of diarylheptanoid C-5 proton signal at *δ* 4.10 (1H, m) to the glucosyl anomeric carbon signal at *δ* 103.6.

Acylation of the glucosyl group at C-6 is indicated by the downfield shift of 2.4 ppm for the C-6 signal and an upfield shift of 2.5 ppm of the C-5 signal in the ^{13}C NMR spectrum compared to the reported signals for a diarylheptanoid 5-*O*-*β*-D-glucopyranoside, which was also recorded in CD_3OD.[10,11] The glucosyl C-6 proton signals at *δ* 4.45 (H-6a) and *δ* 4.69 (H-6b) exhibited downfield shifts of ca. 0.8 ppm, respectively, from those reported for an unsubstituted *β*-D-glucosyl moiety, further substantiating acylation at the glucosyl C-6 in **3**. Finally, the HMBC spectrum also showed connectivity of the acyl carbonyl signal at *δ* 168.0 to the glucosyl C-6 proton signals at *δ* 4.45 and *δ* 4.69.

Table 1. ^{13}C NMR (*δ*, 100 MHz) Data for Oregonin (**1**), Hirsutanonol (**2**), and Oregonosides A and B (**3**,**4**)

position	**1**	**2**	**3**	**4**
1	30.1	30.2	30.0	30.1
2	46.4	46.5	46.3	46.4
3	211.9	212.2	211.8	211.8
4	48.4	51.4	48.9	48.9
5	76.4	68.4	76.6	76.5
6	38.6	40.5	38.4	38.6
7	31.8	32.3	31.8	31.9
1′,1″	134.1, 135.2	135.1, 134.2	134.0, 135.1	134.1, 135.3
2′,2″	116.6, 116.2	116.7, 116.6	116.4, 116.5	116.6, 116.6
3′,3″	146.2, 146.1	146.3, 146.2	146.1, 145.9	146.1, 146.0
4′,4″	144.5, 144.2	144.6, 144.6	144.3, 144.0	144.4, 144.1
5′,5″	116.4, 116.3	116.5, 116.5	116.5, 116.2	116.4, 116.3
6′,6″	120.6, 120.7	120.8, 120.7	120.7, 120.6	120.6, 120.8
acyl moiety				
1			131.2	122.5
2			130.6	113.7
3			129.5	148.7
4			134.2	152.8
5			129.5	115.9
6			130.6	125.2
−CO_2			168.0	168.1
OMe				56.4
glycosyl	xylose		glucose	
1	104.3		103.6	103.6
2	75.1		75.2	75.3
3	77.9		77.8	77.9
4	71.3		71.9	72.0
5	66.0		75.2	75.5
6			65.2	64.9

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10.1021/np980083l CCC: $15.00
Published on Web 08/15/1998

The acyl moiety was indicated to be a benzoyl group in **3** by the broad two proton triplet at δ 7.37 (J = 7.7 Hz) for H-3x and H-5x, a one-proton broad triplet at δ 7.53 (J = 7.6 Hz) for H-4x, and a broad two-proton doublet at δ 7.96 (J = 7.4 Hz) for H-2x and H-6x. FABMS data for **3** indicated a molecular weight of 612 by an intense $[M - H]^-$ ion peak at m/z 611.

Assignment of the (*S*)-configuration to the C-5 position of **3** was accomplished as follows. Acid hydrolysis of **3** gave the aglycon **2**, which was found to be identical ($[\alpha]_D$, NMR) to the aglycon (hirsutanonol) that was obtained by hydrolysis of oregonin. Compounds **1** and **2** have been previously established as having an (*S*)-configuration at C-5 by both X-ray data and application of the ^{13}C NMR glycosylation shift rule.[8,12]

Examples for application of the glycosylation shift rule to assign chirality to secondary alcohols has been primarily shown for D-glucopyranosides, however, oregonin[8] and, later, a 2-tetralol xyloside (asplenoside) showed the applicability of this rule to xylopyranosides as well.[13] Also, such experiments have been primarily carried out in C_5D_5N; however, Speranza et al.[14] demonstrated that the glycosylation shifts for O-glucosyl 1-methyltetralins (feroxins A and B) recorded in CD_3OD were consistent with glycosylation shift rules and the assignment of chirality.

Because we have found it is more convenient to use CD_3OD in most of NMR analyses on natural phenols, we decided to test application of the glycosylation shift rule to the diarylheptanoid glycosides **1** and **3**, as both have established chirality of the C-5 positions by other means. From Table 1, it was found that the glycosylation shift at C-4 is −2.6 ppm, while the shift at C-6 is −1.9 ppm when the spectrum of oregonin is compared with that of its aglycon **2**. Application of the rule for secondary alcohols having two methylene groups assigns the pro-(*S*) carbon to the more shielded carbon on glycosylation (−2.6 ppm) and pro-(*R*) to the less shielded carbon (−1.9 ppm). This result predicts a configuration of (*S*) at C-5 for oregonin. When comparing the shifts for **3** and **2**, we find again the shift for C-4 (−2.5 ppm) to be larger than the shift for C-6 (−2.1 ppm), and therefore C-5 is designed as (*S*) and in agreement with chemical data noted above. The structure of **3** was assigned (5*S*)-1,7-bis(3,4-dihydroxyphenyl)-5-*O*-(6-*O*-benzoyl-β-D-glucopyranosyl)-heptan-3-one.

Oregonoside B (**4**) also was indicated to be a β-D-glucopyranoside acylated at the C-6 position from the ^{13}C NMR signals (Table 1). In the ^1H NMR spectrum of **4** the anomeric proton signal appeared as a doublet (J = 7.6 Hz) at δ 4.31, confirming the conformation of the glycoside linkage. Again, the site of acylation is indicated by the downfield shift of the C-6 carbon signal and upfield shift of the C-5 signal compared to an unsubstituted glucopyranoside.[10,11] As expected, the glucosyl C-6 proton signals are also shifted downfield to δ 4.44 and 4.65. In final confirmation, an HMBC spectrum exhibited connectivity of the glucosyl C-6 protons to the acyl group carbonyl at 168.1 ppm. The remaining ^1H and ^{13}C NMR signals for the acyl group were consistent with a vanilloyl structure.[15,16]

Application of the ^{13}C NMR glycosylation shift rule showed that the shift was larger for C-4 (−2.5 ppm) than for C-6 (−1.9 ppm) when the spectrum of **4** is compared to its aglycon **2**. Therefore, the C-5 position is assigned the (*S*)-configuration as in oregonin and oregonoside A (**3**). The FABMS of **4** showed an intense $[M - H]^-$ ion at m/z 657, confirming a molecular weight of 658. The structure of **4** was assigned as (5*S*)-1,7-bis(3,4-diyhdroxyphenyl)-5-*O*-(6-*O*-vanilloyl-β-D-glucopyranosyl)-heptan-3-one.

The non-acylated counterpart to **3** and **4**, (5*S*)-1,7-bis-(3,4-dihydroxyphenyl)-5-*O*-(β-D-glucopyranosyl)-heptan-3-one has been found to occur in *Alnus serrulatoides* flowers along with oregonin.[8,17] This, however, is the first example of this compound and diarylheptanoids existing in nature as acyl derivatives.

Experimental Section

General Experimental Procedures. NMR spectra were obtained in CD_3OD using a Bruker model AM400 spectrometer. FABMS experiments were performed on a Kratos MS-50TC mass spectrometer. Optical rotations were obtained using a JASCO model DIP-370 polarimeter.

Plant Material. *Alnus rubra* bark was collected in July 1993, from McDonald State Forest, Corvallis, Oregon. A voucher specimen is deposited at the Oregon State University Herbarium (no. 139987).

Extraction and Isolation. Red alder bark (1928 g) was extracted (3 ×) with MeOH at room temperature. The combined MeOH extracts were reduced in volume and extracted with hexane (3 ×) to remove waxy material (10.5 g). Evaporation of the MeOH-solution gave 187 g of tar-like material. A portion (5 g) of the MeOH soluble material was dissolved in a minimum amount of MeOH−H_2O (1:1) and applied to a column of Sephadex LH-20 (5 × 60 cm). The column was eluted with H_2O to give a carbohydrate fraction (0.86 g), MeOH−H_2O (1:1) to give a glycoside fraction (3.74 g), and Me_2CO−H_2O (7:3) to give a tannin fraction (0.4 g).[7] The glycoside fraction was further chromatographed over Toyopearl HW-40S in EtOH and then Sephadex LH-20 using EtOH−H_2O (1:1) to give a fraction rich in **1** and **2** (3.35 g), **3** (0.05 g), and **4** (0.008 g).

Acid Hydrolysis. Compounds **3** and **4** were hydrolyzed with HCl.[13] The hydrolysates were freeze-dried and extracted with EtOAc to afford aglycons. The chirality of sugar moieties was determined by GC of their trimethylsilated (−)-2-butyl glycoside derivatives.[18]

Oregonin (1): $[\alpha]_D$ −18.6° (MeOH; *c* 0.6); negative ion FABMS $[M - H]^-$ m/z 477; ^1H and ^{13}C NMR data in Me_2CO-d_6 + D_2O and C_5D_5N see, Ohta et al.[8] and Lee et al.[9]

Hirsutanonol (2): $[\alpha]_D$ −2.0° (MeOH; *c* 0.31), negative ion FABMS $[M - H]^-$ m/z 345; ^1H and ^{13}C NMR data (CD_3OD) consistent with published data for C_5D_5N.[3,8]

Oregonoside A (3): $[\alpha]_D$ − 7.45° (MeOH; *c* 0.07); negative ion FABMS $[M - H]^-$ m/z 611; ^1H NMR (CD_3OD) δ 1.71 (2H, m, H-6), 2.39 (1H, m, H-7a), 2.46 (1H, m, H-7b), 2.50 (1H, dd, J = 5.1, 16.6 Hz, H-4a), 2.66 (4H, m, H-1, H-2), 2.76 (1H, dd, J = 7.1, 16.7 Hz, H-4b), 3.19 (1H, m, glH-2), 3.40 (2H, m, glH-3, glH-4), 3.58 (1H, m, glH-5), 4.10 (1H, m, H-5), 4.32 (1H, d, J = 7.7 Hz glH-1), 4.45 (1H, dd, J = 6.3, 11.8 Hz glH-6a), 4.69 (1H, dd, J = 2, 11.8 Hz, glH-6b), 6.36 (1H, dd, J = 1.7, 8.1 Hz H-6), 6.46 (1H, dd, J = 1.8, 8.0 Hz, H-6), 6.54 (1H, d, J = 1.8 Hz, H-2), 6.56 (1H, d, J = 7.9 Hz, H-5), 6.60 (1H, d, J = 2 Hz, H-2), 6.64 (1H, d, J = 8.1 Hz, H-5), 7.37 (2H, br t, J = 7.7 Hz, H-3x, H-5x), 7.53 (1H, br t, J = 7.6 Hz, H-4x), 7.96 (2H, brd, J = 7.4 Hz H-2x, H-6x).

Oregonoside B (4): $[\alpha]_D$ −14.9° (MeOH; *c* 0.07); negative ion FABMS, $[M - H]^-$ m/z 657; ^1H NMR (CD_3OD) δ 1.70 (2H, m, H-6), 2.38 (1H, m, H-7a), 2.46 (1H, m, H-7b), 2.52 (1H, dd, J = 5.3, 16.6 Hz, H-4a), 2.68 (4H, m, H-1,H-2), 2.77 (1H, dd, J = 7.0, 16.6 Hz, H-4b), 3.17 (1H, m, glH-2), 3.37 (2H, m, glH-3, glH-4), 3.59 (1H, m, glH-5), 3.76 (3H, s, OMe), 4.11 (1H, m, H-5), 4.31 (1H, d, J = 7.6 Hz, glH-1), 4.44 (1H, dd, J = 6.2, 11.8 Hz, glH-6a), 4.65 (1H, dd, J = 2.0, 11.8 Hz, glH-6b), 6.38 (1H, dd, J = 2.0, 8.0 Hz, H-6″), 6.47 (1H, dd, J = 2.0, 8.0 Hz, H-6), 6.54 (1H, d, J = 2.1 Hz, H-2″), 6.64 (1H, d, J = 8.0 Hz, H-5′), 6.56 (1H, d, J = 8.0 Hz, H-5″), 6.60 (1H, d, J = 1.8 Hz, H-2′), 6.79 (1H, d, J = 8.1 Hz, H-5x), 7.51 (1H, d, J = 1.6 Hz, H-2x), 7.55 (1H, dd, J = 1.6, 8.1 Hz, H-6x).

Acknowledgment. The first author acknowledges leave permission from Instituto Tecnológico de Durango and financial support from the Mexican Council of the Technological Education National System (SEP-COSNET).

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NP980083L