Three New Diarylheptanoid Glycosides from *Alnus japonica*

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Two new diarylheptanoid glycosides, hirsutanonol 5-*O*-(6-*O*-galloyl)-β-D-glucopyranoside and 3-deoxohirsutanonol 5-*O*-β-D-glucopyranoside, were isolated from fresh leaves of *Alnus japonica* together with three known diarylheptanoids [hirsutanonol, hirsutanonol 5-*O*-β-D-glucopyranoside and hirsutenone], five known triterpenes [β-amyrin, 3-*O*-acetyl-β-amyrin, 3-*O*-acetyltaraxerol, glutinone and lupenone] and quercitrin. One more new diarylheptanoid glycoside, 3-deoxohirsutanonol 5-*O*-(6-*O*-β-D-apiosyl)-β-D-glucopyranoside, was also isolated from bark together with 3-deoxohirsutanonol 5-*O*-β-D-glucopyranoside, two known diarylheptanoids [hirsutoside and hirsutanonol 5-*O*-β-D-glucopyranoside] and four known triterpenes [glutinol, glutinone, lupenone and taraxerone]. Their structures were determined on the basis of spectroscopic and chemical evidence.

Key words *Alnus japonica*; diarylheptanoid glycoside; hirsutanonol; 3-deoxohirsutanonol; triterpene

Alnus japonica is a Betulaceous tree found in damp parts of forests and along river banks. From the wood of this tree, a number of diarylheptanoids and other phenolics have been isolated.[1] To compare its chemical profile with that of *Betula* species, fresh leaves and bark were further investigated, resulting in the isolation of three new diarylheptanoid glycosides. This paper deals with the structural determination of these compounds.

From the methanol extract of fresh leaves, two new diarylheptanoid glycosides, **1** and **2**, were isolated together with three known diarylheptanoids (hirsutanonol (**3**),[2] hirsutanonol 5-*O*-β-D-glucopyranoside (**4**)[2] and hirsutenone[2]), five triterpenes (β-amyrin, 3-*O*-acetyl-β-amyrin, 3-*O*-acetyltaraxerol, glutinone and lupenone) and quercitrin.

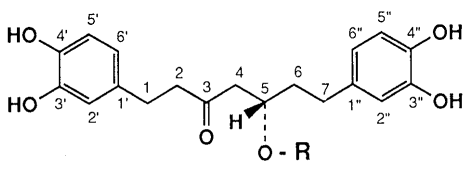
1 was formulated as $C_{32}H_{36}O_{15}$ by high-resolution (HR) FAB-MS. The ^{1}H- and ^{13}C-NMR spectra of **1** were similar to those of **4**, except for the additional signals of a galloyl group and differences in the chemical shifts around the C-6 of β-D-glucosyl which were due to the acylation shifts[3] (Table 1 and Experimental). On enzymatic hydrolysis with tannase, **1** gave **4** and gallic acid. Thus the structure of **1** was determined to be hirsutanonol 5-*O*-(6-*O*-galloyl)-β-D-glucopyranoside.

2 was formulated as $C_{25}H_{34}O_{10}$ by HR-FAB-MS. The ^{1}H- and ^{13}C-NMR spectra showed the presence of two 3,4-dihydroxyphenyl groups, a glucopyranosyl group, six methylene carbons and an oxymethine carbon (Table 1 and Experimental). Checking the sequence of the methylene and oxymethine carbons by two-dimensional shift correlation spectroscopy (2D-COSY), the structure of **2** was determined to be a 3-deoxo-derivative of **4**.

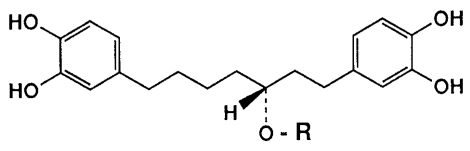
Table 1. ^{13}C-NMR Data

C	1[a]	2[b]	2a[b]	3[a]	4[a]	5[b]
1	29.5	36.2	36.3	29.5	29.4	36.1
2	46.1	33.0	33.0	45.9	45.9	32.9
3	209.1	25.6	26.3	209.7	209.1	25.6
4	48.3	34.8	38.3	51.3	48.1	35.0
5	75.2	79.4	71.8	67.4	75.1	80.1
6	38.1	38.0	40.6	40.6	38.1	38.1
7	31.4	31.8	32.4	32.0	31.1	32.0
1′	133.3	135.7	135.5	133.3	133.1	135.8
2′	116.6	116.6	116.6	116.6	116.5	116.6
3′	147.0	145.9	146.0	147.2	146.8	145.9
4′	145.1	144.0	144.1	145.2	145.0	143.9
5′	117.0	116.3	116.2	116.9	116.8	116.2
6′	119.9	120.7	120.7	119.8	119.7	120.7
1″	134.3	135.7	135.7	134.4	134.1	135.7
2″	116.6	117.0	116.6	116.6	116.5	116.7
3″	147.0	146.0	146.1	147.2	147.0	145.9
4″	145.4	144.0	144.1	145.4	145.2	143.9
5″	117.0	116.3	116.3	117.0	117.1	116.3
6″	120.2	120.9	120.7	119.9	120.0	120.9
Glc-1	103.9	103.1			103.3	103.4
Glc-2	75.4	75.3			75.0	75.3
Glc-3	78.4	78.2			78.3	78.1
Glc-4	71.3	71.8			71.5	71.6
Glc-5	76.0	77.8			78.2	76.5
Glc-6	64.5	62.9			62.6	68.4
Galloyl-1	121.3				Api-1	110.8
Galloyl-2,6	110.3				Api-2	78.0
Galloyl-3,5	147.5				Api-3	80.5
Galloyl-4	140.9				Api-4	75.0
Galloyl-7	167.4				Api-5	65.7

The spectra were recorded in C_5D_5N[a] or CD_3OD.[b]



1 : R = 6-*O*-galloyl-β-D-glucopyranosyl
3 : R = H
4 : R = β-D-glucopyranosyl



2 : R = β-D-glucopyranosyl
2a : R = H
5 : R = 6-*O*-β-D-apiosyl-β-D-glucopyranosyl

Chart 1

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On acid hydrolysis, **2** gave D-glucose and 3-deoxohirsutanonol (**2a**). The absolute configuration at C-5 was confirmed by application of the β-D-glucosylation shift rule.[4] When compared with the ^{13}C-NMR data of **2a**, those of **2** showed β-D-glucosylation shifts of −3.5 ppm for C-4 and −2.6 ppm for C-6. This indicated the (*R*)-configuration at C-5, which was the same stereochemistry as **3** and **4** although the symbol is different because of the change in priority of substituent groups at C-5.

From the methanol extract of dried bark, one more new diarylheptanoid glycoside, **5**, was isolated together with 3-deoxohirsutanonol 5-*O*-β-D-glucopyranoside (**2**), two known diarylheptanoids [hirsutoside,[2] hirsutanonol 5-*O*-β-D-glucopyranoside (**4**)] and four known triterpenes [glutinol, glutinone, lupenone and taraxerone].

5 was formulated as $C_{30}H_{42}O_{14}$ by HR-FAB-MS. The ^{1}H- and ^{13}C-NMR data were similar to those of **2**, except for the additional signals of an apiosyl[5] and differences in the chemical shifts around the C-6 of β-D-glucosyl which were due to the glycosylation shifts (Table 1 and Experimental). On acid hydrolysis with 3% HCl, **5** gave **2a**, D-apiose and D-glucose. Thus, the structure of **5** was determined to be 3-deoxohirsutanonol 5-*O*-(6-*O*-β-D-apiosyl)-β-D-glucopyranoside.

In this study, *A. japonica* was shown to contain as many diarylheptanoids as the *Betula* species.[6] It is noteworthy that the leaves and bark contain diarylheptanoids which have dihydroxyphenyl groups, while the wood has monohydroxyphenyl groups.[1]

Experimental

The instruments, materials and experimental conditions were the same as described in the previous paper.[7]

Isolation Fresh leaves (1.3 kg) collected in May at Noda, Chiba prefecture, were extracted twice with 4 l MeOH under reflux for 6 h. The extracts and then 10 l MeOH were passed over a column of activated charcoal (100 g). The resultant solution was concentrated to a syrup under reduced pressure. The syrup was chromatographed on silica gel by using $CHCl_3$ and MeOH. The fractions containing triterpenes were collected and chromatographed on silica gel by using *n*-hexane and EtOAc to give β-amyrin (20 mg), 3-*O*-acetyl-β-amyrin (12 mg), 3-*O*-acetyltaraxerol (8 mg), glutinone (190 mg) and lupenone (254 mg). The fractions containing polar compounds were collected and partitioned between *n*-BuOH and water. The *n*-BuOH layer was further partitioned with $CHCl_3$–MeOH–H_2O (4 : 4 : 3) and the upper layer was evaporated. The residue was chromatographed on Sephadex LH-20 by using MeOH and H_2O to give **1** (57 mg), **2** (88 mg), hirsutanonol (**3**, 170 mg), hirsutanonol 5-*O*-β-D-glucopyranoside (**4**, 1.1 g), hirsutenone (25 mg) and quercitrin (29 mg).

The air-dried bark (600 g) collected at the same time as the leaves was extracted twice, each time with 3 l MeOH under reflux for 6 h. The extracts were evaporated under reduced pressure to a syrup. The syrup was chromatographed on silica gel by using $CHCl_3$ and MeOH. The fractions containing triterpenes were collected and chromatographed on silica gel by using *n*-hexane and EtOAc and on silica gel impregnated with 20% $AgNO_3$ by using *n*-hexane and $CHCl_3$ to give glutinol (255 mg), glutinone (242 mg), lupenone (313 mg) and taraxerone (296 mg). The fractions containing diarylheptanoids were collected and chromatographed on Sephadex LH-20 by using 80% MeOH and on Lichroprep RP-8 by using MeOH and H_2O to give **2** (158 mg), **4** (248 mg), **5** (50 mg) and hirsutoside (250 mg).

Hirsutanonol 5-*O*-(6-*O*-Galloyl)-β-D-glucopyranoside (1) Colorless amorphous powder, $[\alpha]_D$ −18° (*c* = 1.0, MeOH). UV (MeOH) λ_{max} nm (log ε): 280 (4.52), 216 (4.95). ^{1}H-NMR (C_5D_5N) δ: 2.14 (2H, m), 2.80—3.01 (8H), 4.64 (1H, quintet, *J* = 6.0 Hz), 4.95 (1H, d, *J* = 7.6 Hz), 6.79, 6.87 (each 1H, dd, *J* = 2.0, 8.1 Hz), 7.19, 7.20 (each 1H, d, *J* = 8.1 Hz), 7.24 (2H, d, *J* = 2.0 Hz), 7.91 (2H, s). HR-FAB-MS (negative mode) *m/z*: 659.197 $[M-H]^-$, Calcd for $C_{32}H_{35}O_{15}$: 659.198.

Enzymatic Hydrolysis of 1 A mixture of **1** (25 mg) and tannase (50 mg) in water (10 ml) was stirred at room temperature for 5 h. The mixture was subjected to column chromatography on Sephadex LH-20 by using acetone and water to yield **4** (6 mg) and gallic acid (1.2 mg). The products were identified by direct comparison with authentic samples.

3-Deoxohirsutanonol 5-*O*-β-D-Glucopyranoside (2) Colorless amorphous powder, $[\alpha]_D$ −25° (*c* = 1.0, MeOH). UV (MeOH) λ_{max} nm (log ε): 283 (3.86), 220 (4.19). ^{1}H-NMR (CD_3OD) δ: 1.36 (2H, m), 1.54 (3H, m), 1.65 (1H, m), 1.72 (1H, m), 1.80 (1H, m), 2.44 (2H, t, *J* = 7.6 Hz), 2.55 (2H, t, *J* = 7.6 Hz), 3.70 (1H, quintet, *J* = 6.4 Hz), 3.72 (1H, dd, *J* = 5.5, 11.9 Hz), 3.90 (1H, dd, *J* = 2.4, 11.9 Hz), 4.30 (1H, d, *J* = 7.9 Hz), 6.47 (1H, dd, *J* = 2.0, 8.0 Hz), 6.51 (1H, dd, *J* = 2.0, 8.0 Hz), 6.60 (1H, d, *J* = 2.0 Hz), 6.66 (2H, d, *J* = 8.0 Hz), 6.71 (1H, d, *J* = 2.0 Hz). HR-FAB-MS (negative mode) *m/z*: 493.206 $[M-H]^-$, Calcd for $C_{25}H_{33}O_{10}$: 493.207.

Acid Hydrolysis of 2 **2** (15 mg) was hydrolyzed with 3% HCl (5 ml) under reflux for 1 h. The reaction mixture was extracted with EtOAc. The EtOAc layer was washed with water, dried over Na_2SO_4 and evaporated. The residue was chromatographed on Sephadex LH-20 by using 80% MeOH to obtain **2a** (4 mg), $[\alpha]_D$ −5° (*c* = 0.4, MeOH). The water layer was concentrated under reduced pressure and chromatographed on silica gel by using 10% MeOH in $CHCl_3$ to obtain D-glucose (3.1 mg), $[\alpha]_D$ +50° (*c* = 0.3, H_2O). Its trimethylsilyl ether was identified by comparison with an authentic sample on GLC.

3-Deoxohirsutanonol 5-*O*-(6-*O*-β-D-Apiosyl)-β-D-glucopyranoside (5) Colorless amorphous powder, $[\alpha]_D$ −52° (*c* = 1.0, MeOH). UV (MeOH) λ_{max} nm (log ε): 283 (3.81), 220 (4.16). ^{1}H-NMR (CD_3OD) δ: 1.44 (2H, m), 1.55 (4H, m), 1.75 (2H, m), 2.42 (2H, m), 2.54 (2H, m), 3.63 (1H, quintet, *J* = 7.0 Hz), 4.27 (1H, d, *J* = 7.6 Hz), 5.02 (1H, d, *J* = 2.0 Hz), 6.47 (1H, dd, *J* = 1.7, 8.1 Hz), 6.53 (1H, dd, *J* = 1.7, 8.1 Hz), 6.61 (1H, d, *J* = 1.7 Hz), 6.65 (1H, d, *J* = 1.7 Hz), 6.66 (1H, d, *J* = 8.1 Hz), 6.68 (1H, d, *J* = 8.1 Hz). HR-FAB-MS (negative mode) *m/z*: 625.250 $[M-H]^-$, Calcd for $C_{30}H_{41}O_{14}$: 625.250.

Acid Hydrolysis of 5 **5** (30 mg) was hydrolyzed in the same manner as **2** to obtain **2a** (3 mg), $[\alpha]_D$ −5° (*c* = 0.3, MeOH), D-glucose (2.7 mg), $[\alpha]_D$ +45° (*c* = 0.3, H_2O) and D-apiose (1.8 mg), $[\alpha]_D$ +8° (*c* = 0.2, H_2O).

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