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New Diarylheptanoid from the Barks of *Alnus japonica* Steudel

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Abstract: A new diarylheptanoid glycoside, 1,7-bis-(3,4-dihydroxyphenyl)-5-hydroxyheptane-3-O-β-D–xylopyranoside (**1**), together with nine known diarylheptanoids (**2-10**) were isolated from the fresh bark of *Alnus japonica* which is a species of the genus *Alnus* species, growing throughout Korea.

Keywords: *Alnus japonica,* Betulaceae, diarylheptanoid.

Alnus japonica Steudel (Betulaceae), which is one of the indigenous *Alnus* species in Korea and Japan, is a deciduous ovate-elliptic leaved tree found in the wet low lands[1]. The bark of the *Alnus* species is used in oriental traditional medicine as a remedies for fever, hemorrhage, diarrhea and alcoholism[2]. Several diarylhepta- noids have been isolated from the stem bark of this tree, which may be used to characterize this genus[3,4,5]. This study on the constituents of the barks of *Alnus japonica* led to the isolation of a new diarylheptanoid glycoside (**1**), together with nine known diarylheptanoids (**2-10**). The structural elucidation of **1** is described here-in.

Experimental

The ^{1}H- and ^{13}C-nuclear magnetic resonance (NMR) spectra were recorded on a Varian Unity NMR at 300 MHz, Brucker Amx 500 MHz or 600 MHz (^{1}H-NMR) and 75, 125 or 150 MHz (^{13}C-NMR), respectively. The chemical shift are shown in δ (ppm) relative to TMS. The negative FAB-MS was measured at 35 keV with a glycerol matrix. The stationary phases for the column chromatographic isolation were performed on a Sephadex LH-20 column (25-10 μm, Pharmacia), a MCI-gel CHP 20P column (75-150 μm, Mitsubishi) and YMC-gel ODS-A column (230-70 and 500-400 mesh, YMC Co.). TLC was carried out on a precoated silica gel 60 F_{254} plate (Merck). The spots were detected under UV radiation and by spraying with $FeCl_3$ and 10% H_2SO_4, followed by heating.

The bark of *A. japonica* was collected in Mt. Chung-gei, Seoul city, Korea in November 2002. A voucher specimen was deposited at the herbarium, College of Pharmacy, Chung-Ang University.

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The fresh bark (2.5 kg) was finely cut and extracted three times with 80% aqueous acetone at room temperature. After removing the acetone under vacuum, the aqueous solution was filtered. The filtrate was concentrated and applied to the Sephadex LH 20 column containing increasing proportions of MeOH, which afforded 6 fractions (**I-VI**). Column chromatography of fraction **II** on the MCI-gel CHP 20P column with a H_2O-MeOH gradient yielded **10** (300 mg). Repeated column chromatography of fraction **III** on the MCI-gel CHP20P and ODS-gel columns followed by low-pressure liquid column chromatography with a H_2O-MeOH gradient yielded **2** (1.4 g) and **3** (2 g). Repeated column chromatography of fraction **IV** on the MCI-gel CHP 20P column followed by low-pressure liquid column chromatography with a H_2O-MeOH gradient yielded **1** (80 mg) and **7** (300 mg). Column chromatography of fraction **V** over the Sephadex LH 20 column with a H_2O-MeOH gradient yielded **4** (300 mg) and **6** (130 mg). Finally, repeated column chromatography of fraction **VI** on the MCI-gel CHP 20P and, ODS-gel columns followed by low-pressure liquid column chromatography with a H_2O-MeOH gradient yielded **5** (100 mg), **9** (100 mg), and **8** (80 mg), respectively.

1,7-Bis-(3,4-dihydroxyphenyl)-3-hydroxyheptane-5-*O*-*β*-D-xylopyranoside 1

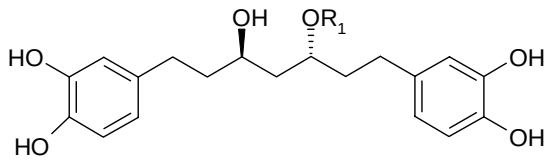
Brown amorphous powder, $[\alpha]_D^{20}$ -6.3 (*c* 1.0, MeOH). Negative FAB MS: *m/z* 479 $[M-H]^-$. ^{1}H-NMR (300 MHz, Acetone-d_6+D_2O, δ ppm): 6.75-6.70 (m, 4H in total, H-2', 2", 5', 5"), 6.57-6.52 (m, 2H in total, H-6', 6"), 4.41 (1H, d, *J*=7.2 Hz, xyl-1), 3.96 (m, 1H, H-5), 3.80 (dd, 1H, *J*=11.4, 5.4 Hz, xyl-5e), 3.53 (m, 1H, xyl-4), 2.71-2.45 (m, 4H in total, H-1, 6), 1.90-1.67 (m, 6H in total, H-2, 4, 7). ^{13}C-NMR (150 MHz, Acetone-d_6+D_2O, δ ppm): 145.9 (C-3', 3"), 144.0 (C-4', 4", 135.5 (C-1', 1", 120.6 (C-6' 6", 116.6 (C-5', 5", 116.2 (C-2', 2"), 104.7 (xyl-1), 78.1 (C-5), 78.0 (xyl-3), 75.1 (xyl-2), 71.1 (xyl-4), 67.7 (C-3), 66.7 (xyl-5), 42.4 (C-4), 41.2 (C-6), 38.7 (C-2), 32.2 (C-7), 30.7 (C-1)

Acid hydrolysis of **1** A solution of **1** (20 mg) was refluxed in 5% HCl for 1 h, and the reaction mixture was extracted with EtOAc. The organic part was concentrated and then applied to a Sephadex LH-20 column with a H_2O--MeOH in gradient to gave a white amorphous powder **1a**. The remaining aqueous solution was neutralized with Amberlite MB-3 and concentrated. TLC detected the D-xylose.

1,7-Bis-(3,4-dihydroxyphenyl)-3,5-dihydroxyheptane 1a

White amorphous powder, $[\alpha]_D^{20}$ -7.9(c 1.0, MeOH), Negative FAB MS: *m/z* 347 $[M-H]^-$. ^{1}H -NMR (300 MHz, Acetone-d_6+D_2O, δ ppm): 6.70-6.68 (m, 4H in total, H-2', 2", 5', 5"), 6.50-6.47 (m, 2H in total, H-6', 6"), 2.64-2.41 (m, 4H, in total, H-2, 6), 1.75-1.54(m, 6H in total, H-1, 7). ^{13}C -NMR (75 MHz, Acetone-d_6+ D_2O, δ ppm): 145.6 (C-3', 3"), 143.7 (C-4', 4"), 135.1 (C-1', 1"), 120.3 (C-6', 6"), 116.3 (C-5', 5"), 116.0 (C-2', 2"), 68.3 (C-3, 5), 44.6 (C-4), 40.7 (C-2, 6), 31.9 (C-1, 7)

Figure 1 The structure of **1** and **1a**



	R_1
1	Xyl
1a	H

Results and Discussion

The fresh bark of *A. japonica* was extracted with aqueous acetone and the extract was subjected to a combination of Sephadex LH-20, MCI-gel CHP 20P and YMC-gel ODS-A chromatography to afford a new diarylheptanoid glycoside **1,** including nine known diarylheptanoids, 1,7-bis-(3,4-dihydroxyphenyl)-heptane-3-O-β-D-glucopyranosyl (1→3)-β-D-xylopyranoside **2,** 1,7-bis-(3,4-dihydroxyphenyl)-heptane-3-O-β-D-apiofuranosyl (1→6)-β-D-glucopyranoside **3**[5], 1,7-bis-(3,4-dihydroxyphenyl)-heptane-5-O-β-D-glucopyranoside **4**[6], 1,7-bis-(3,4-dihydroxyphenyl)-5-hydroxylheptane **5**[7], 1,7-bis-(3,4-dihydroxyphenyl)-heptane-3-one-5-O-β-D-glucopyranoside **6**[7], oregonin **7**, hirsutanonol **8**, hirsutenone **9**[8], platyphylloside **10**[9]**.** The structures of the known compounds were identified by a direct comparison either with the authentic samples or with the reported spectral and physical data (co-TLC, IR, MS, NMR). **1** gave a green coloration with ferric chloride. The ^{1}H-NMR spectrum of **1** showed the presence of three methylene groups at δ 1.67-1.90 and another two methylene groups at δ 2.45-2.71, two methine groups at δ 3.85-4.15 and two pairs of 1,3,4-trisubstituted aromatic ring at δ 6.52-6.75. The ^{13}C-NMR spectrum of **1** showed five methylene groups at δ 30.7 (C-1), 32.2 (C-7), 38.7 (C-2), 41.2 (C-6), 42.4 (C-4) and two oxygen bearing methines at δ 67.7 (C-3) and 78.1 (C-5) and two 3,4-dihydroxyphenyl groups at δ 145.9 (C-3', 3" and, 144.0 (C-4', 4"), and one β-D-xylopyranosyl moiety at δ 104.7 (xyl-1), 75.1 (xyl-2), 78.0 (xyl-3), 71.1 (xyl-4), 66.7 (xyl-5). Acid hydrolysis of **1** gave **1a** and D-xylose. The ^{13}C-NMR spectrum of **1a** showed, the presence of a free hydroxy, bearing methine (C-5) at δ 68.3, which was upfield-shifted compared with **1** (δ 78.1), indicating the linkage of heptanoid with sugar moiety is at C-5. The ^{13}C-NMR spectrum of **1a** also showed a symmetrical figure which had three peaks for five methylene groups at δ 31.9 (C-1, 7), 40.7 (C-2, 6), 44.6 (C-4) and one peak for two oxygen bearing methine groups at δ 68.3 (C-3, 5). In order to demonstrate the absolute configuration of C-3 and 5, the heptane moiety of **1a** was compared with 1,7-diphenylheptane-(3R),(5R)-diol, and good agreement was found in the ^{13}C-NMR data[10]. The negative FAB MS spectrum of **1** exhibited a prominent $[M-H]^-$ peak at *m/z* 479. Based on the these findings, the structure of **1** was determined to be (3R)-(5R) 1,7-bis-(3,4-dihydroxyphenyl)-3-hydroxyheptane-5-O-β-D-xylopyranoside.

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