Diarylheptanoids from the leaves of *Alnus hirsuta* Turcz

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Diarylheptanoids, (5*S*)-1,7-bis-(3,4-dihydroxyphenyl)-5-hydroxyheptane-3-one (**1**, hirsutanonol), (5*S*)-1,7-bis-(3,4-dihydroxyphenyl)-heptane-3-one-5-O-β-D-xylopyranoside (**2**, oregonin), (5*R*)-1,7-bis-(3,4-dihydroxyphenyl)-heptane-5-O-β-D-xylopyranoside (**3**), and (5*R*)-1,7-bis-(3,4-dihydroxyphenyl)- heptane-5-O-β-D-glucopyranoside (**4**) were isolated from the leaves of *Alnus hirsuta* Turcz. The structures of these compounds were identified based on the spectral and physicochemical data.

Key words: *Alnus hirsuta* Turcz, Betulaceae, Diarylheptanoid

INTRODUCTION

Alnus hirsuta Trucz, one of the indigenous *Alnus* species growing in Korea, is deciduous broad-leaved tree found in damp place and the bark of *Alnus* species have been used for the oriental traditional medicine as the remedies for fever, hemorrhage, diarrhea, and alcoholism (Lee, 1966). Recently, some pharmacological effects by diarylheptanoids of this plant, such as inhibitory effect on nitric oxide production (Lee *et al.*, 1998a) and cytotoxicity against B-16 F10 mouse melanoma cell line (Lee *et al.*, 1998b) were reported. From the stem bark of this tree, several diarylheptanoids and their glycosides were isolated as the constituents which may be characteristic to this genus (Terazawa *et al.*, 1974 and Sasaya *et al.*, 1985). In this paper, we describe the structure elucidation of diarylheptanoids from the fresh leaves of *Alnus hirsuta*.

MATERIALS AND METHODS

General

^{1}H- and ^{13}C-NMR spectra were recorded on a Varian Unity at 300 MHz or on a Brucker Amx 500 MHz (^{1}H-NMR) Chemical shift are given in δ (ppm) scale with TMS as internal standard. Negative FAB-MS was mea-sured at 35 keV with glycerol matrix and ESIMS at 3.5 kV. Column chromatographic isolation were carried out on Sephadex LH-20 (25~10 μm, Pharmacia), MCI-gel CHP 20P(75~150 μm, Mitsubishi), and YMC-gel ODS-A (230-70 and 500-400 mesh, YMC Co.). TLC was conducted on precoated silica gel 60 F_{254} plate (Merck). Spots were detected under UV radiation and by spraying with $FeCl_3$ and 10% H_2SO_4, followed by heating.

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Plant material

Leaves of *A. hirsuta* were collected in Mt. Chung-gei, Seoul, Korea in August of 1998. A voucher specimen is deposited at the herbarium, College of Pharmacy, Chung-Ang University.

Extraction and isolation

Fresh leaves (3 kg) were finely cut and extracted with 80% aqueous Me_2CO at room temperature for three days. After a removal of Me_2CO in *vacuo*, the aqueous solution was filtered. The filtrate was concentrated and then applied to a column of Sephadex LH-20 to afford 3 fractions, I (50 g), II (30 g) and III (40 g). Repeated column chromatography of fraction I on YMC-gel ODS-A with a H_2O-MeOH gradient and Sephadex LH-20 with 60% MeOH yielded oregonin (**2**) (2 g). Column chromatography of fraction II over Sephadex LH-20 with EtOH-H_2O-Me_2CO and MCI-gel with H_2O-MeOH furnished 1,7-bis-(3,4-dihydroxy-phenyl)-heptane-5-O-β-D-xylopyranoside (**3**) (700 mg) and 1,7-bis-(3,4-dihydroxyphenyl)-heptane-5-O-β-D-glucopyranoside (**4**) (900 mg). Sephadex LH-20 column chromatography of fraction III with EtOH led to isolation of hirsutanonol (**1**) (200 mg).

hirsutanonol (1)

Brown oil, $[\alpha]_D^{20}$: -15.7° (c = 1.0, Me_2CO). IR ν_{max}^{KBr} cm^{-1}: 3394(broad, OH), 1700 (C=O), 1606, 1521(Aromatic C=C). Negative FAB MS: m/z 345 $[M-H]^-$. ^{1}H-NMR (Me_2CO-d_6, 300 MHz) δ : 1.64-1.71 (2 H in total, m, H-6), 2.47-2.76 (8 H in total, m, H-1,2,4,7), 4.05 (1 H, tt, J=6.0Hz, H-5), 6.49-6.53 (2 H in total, m, H-6' and 6"), 6.70-6.74 (4 H in total, m, H-2',2",5' and 5"). ^{13}C-NMR (Me_2CO-d_6, 75MHz) δ : 211.2 (C-3), 145.9 (C-3"), 145.8 (C-3'), 144.1 (C-4"), 143.9 (C-4'), 134.8 (C-1"), 133.9 (C-1'), 120.4 (C-6"), 120.3 (C-6'), 116.4 (C-5"), 116.3 (C-5'), 116.1 (C-2"), 116.1 (C-2'), 67.8 (C-5), 50.8 (C-4), 45.9 (C-2), 40.1 (C-6), 31.8 (C-7), 29.4 (C-1).

oregonin(2)

Brown amorphous powder, $[\alpha]_D^{20}$:-17.5° (c=1.0, Me_2 CO). IR ν_{max}^{KBr} cm^{-1} : 3269 (OH), 1700 (C=O), 1606, 1525 (Aromatic C=C). Negative FAB MS: m/z 477 [M-H] $^-$. ^{1}H-NMR (300 MHz, Me_2CO-d_6 +D_2O) δ : 1.75-1.82 (2 H in total, m, H-2), 2.52-2.86 (8 H in total, H-1,4,6 and 7), 3.21 (1 H, dd, J=9.0, 7.8 Hz, xyl-2), 3.23 (1 H, dd, J=11.4, 11.2 Hz, xyl-5a), 3.48 (1H, dd, J=9.0, 9.0 Hz, xyl-3), 3.56 (1 H, m, xyl-4), 3.90 (1 H, dd, J=11.4, 6.1 Hz, xyl-5eq), 4.14 (1 H, m, H-5), 4.32 (1 H, d, J=7.8 Hz, xyl-1), 6.52 (2 H in total, dd, J=8.1, 2.1 Hz, H-6' and 6"), 6.71-6.75 (4H in total, H-2',2",5' and 5"). ^{13}C-NMR (75 MHz, Me_2CO-d_6 +D_2O) δ : 211.0 (C-3), 145.7 (C-3"), 145.3 (C-3'), 144.1 (C-4"), 143.8 (C-4'), 134.8 (C-1"), 133.8 (C-1'), 120.4 (C-6"), 120.4 (C-6'), 116.4 (C-5"), 116.4 (C-5'), 116.2 (C-2"), 116.1 (C-2'), 103.8 (xyl-1), 77.3 (xyl-3), 76.1 (C-5), 74.5 (xyl-2), 70.6 (xyl-4), 66.4 (xyl-5), 48.1 (C-4), 46.0 (C-2), 38.1 (C-6), 31.3 (C-7), 29.2 (C-1).

1,7-bis-(3,4-dihydroxyphenyl)-heptane-5-O-β-D-xylopyranoside (3)

Brown amorphous powder, $[a]_D^{20}$: -60.0° (c = 1.0, Me_2 CO). IR ν_{max}^{KBr} cm^{-1} : 3392 (broad, OH), 1616, 1533 (Aromatic C=C). ESI MS: m/z 464 $[M]^+$, 332 $[M\text{-xylose}]^+$. ^{1}H-NMR (500 MHz, pyridine-d_5) δ : 1.50-1.80 (6 H in total, m, H-3,2 and 4), 1.95-2.15 (2H in total, m, H-6), 2.51-2.54 (2 H in total, m, H-1), 2.80-3.15 (2 H in total, m, H-7), 3.66-3.70 (2H in total, m, Xyl-5a), 3.95-4.00 (2H in total, m, H-5 and xyl-2), 4.1 4 (1 H, , t, J=8.7 Hz, xyl-3), 4.20 (1 H, m, xyl-4), 4.32 (1 H, dd, J=5.1, 11.2 Hz, xyl-5eq), 4.79 (1 H, d, J=7.5 Hz, xyl-1), 6.74 (1 H, dd, J=1.9, 7.9 Hz, H-6'), 6.86 (1 H, dd, J=1.9, 7.9 Hz, H-6"), 7.16 (1 H, d, J=1.9Hz, H-2'), 7.19-7.21 (2H in total, over-lapped with pyridine-d_5, H-5',5"), 7.28 (1 H, d, J=1.9 Hz, H-2"). ^{13}C-NMR (75 MHz, Me_2CO-d_6 +D_2O) : Table I.

Acid hydrolysis of 3. Compound 3 (300 mg) was hydrolyzed with 60% dioxane-2N H_2SO_4 (8 ml) under reflux for 1 h. The reaction mixture was extracted with EtOAc. The EtOAc layer was washed with water and concentrated to a syrup, which was subjected to Sephadex LH-20 column chromatography. Elution with Me OH afforded 3a [1,7-bis-(3,4-dihydroxyphenyl)-heptane-5-ol, 15 mg]. The aqueous layer, after neutralization with Ag_2CO_3, was confirmed the presence of xylose (co-TLC).

3a. Yellow oil, ^{1}H-NMR (500 MHz, Me_2CO-d_6) δ : 1.44-1.47 (4 H in total, m, H-3 and 2), 1.52-1.55 (2 H in total, m, H-4), 1.63-1.66 (2 H in total, m, H-6), 2.43-2.61 (4 H in total, m, H-1,7), 3.53-3.54 (1 H in total, m, H-5), 6.48-6.52 (2 H in total, m, H-6' and 6"), 6.67-6.72 (4 H in total, m, H-2',2",5' and 5"). ^{13}C-NMR (125 MHz, Me_2CO-d_6) δ : Table I.

1,7-bis-(3,4-dihydroxyphenyl)-heptane-5-O-β-D-glucopyranoside (4)

Brown amorphous powder, $[a]_D^{20}$: -30.0° (C=1.0, Me_2 CO). IR ν_{max}^{KBr} cm^{-1}: 3467 (broad, OH), 1606, 1523 (Aromatic C=C), ESI MS : m/z 494 $[M]^+$. ^{1}H-NMR (500 MHz, pyridine-d_5) δ : 1.48-1.80(6 H in total, m, H-3,2 and 4), 1.90-2.10 (2 H in total, m, H-6), 2.49-2.52 (2 H, m, H-1), 2.86-2.90 (2 H in total, m, H-7), 3.96 (1 H, m, glc-5), 4.01-4.06 (2 H in total, m, glc-2 and H-5), 4.19-

Table I. ^{13}C-NMR data of compounds **3**, **3a**, **4**, and **4a**

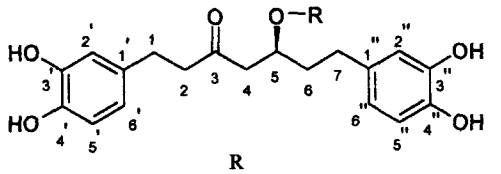
carbon number	3*	3a(4a)**	4*
C-1	35.7	36.4	35.7
C-2	32.6	33.2	32.5
C-3	25.1	26.6	25.1
C-4	34.4	38.7	34.4
C-5	76.2	71.5	77.1
C-6	37.7	41.0	37.5
C-7	31.5	32.6	31.1
C-1'	135.1	135.7	135.2
C-1"	135.2	135.7	135.2
C-2'	116.0	116.4	116.0
C-2"	116.0	116.5	116.0
C-3'	145.7	146.2	145.5
C-3"	145.7	146.2	145.7
C-4'	143.8	144.3	143.8
C-4"	144.8	144.3	143.8
C-5'	116.3	116.8	116.3
C-5"	116.4	116.8	116.9
C-6'	120.3	120.8	120.4
C-6"	120.3	120.8	120.5
xyl-1	103.8		
xyl-2	74.5		
xyl-3	77.3		
xyl-4	70.7		
xyl-5	66.2		
glc-1			102.7
glc-2			74.9
glc-3			81.0
glc-4			71.4
glc-5			78.4
glc-6			62.7

*Measeured in Me_2CO-d_6+D_2O at 75 MHz
** Measured in Me_2CO-d_6 at 125 MHz

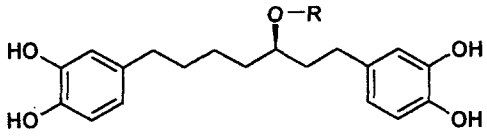
4.24 (2 H in total, m, glc-3 and 4), 4.38 (1 H, dd, J=11.7, 5.4 Hz, glc-6), 4.58 (1 H, dd, J=11.7, 2.5 Hz, glc-6), 4.93 (1 H, d, J=7.7 Hz, glc-1), 6.73 (1 H, dd, J=2.1, 7.9 Hz, H-6'), 6.85 (1H, dd, J=2.0, 8.0 Hz, H-6"), 7.15 (1 H, d, J=2.1 Hz, H-2'), 7.18-7.20 (overlaped with pyridine-d_5, H-2" and H-5'), 7.21 (1 H, d, J=8.0Hz, H-5"). ^{13}C-NMR (75 MHz, Me_2CO-d_6 + D_2O) δ : Table 1.

Acid hydrolysis of 4. Compound **4** (300 mg) was hydrolyzed as same manner of **3**. The reaction mixture was extracted with EtOAc. The EtOAc layer was washed with water and concentrated to a syrup, which was subjected to Sephadex LH-20 column chromatography. Elution with MeOH afforded **4a** [1,7-bis-(3,4-dihydroxyphenyl)-heptane-5-ol, 15 mg]. The aqueous layer, after neutralization with Ag_2CO_3, was confirmed the presence of glucose (co-TLC).

4a(3a). Yellow oil, ^{1}H-NMR (500 MHz, Me_2CO-d_6) δ : same as **3a**. ^{13}C-NMR (125 MHz, Me_2CO-d_6) δ : same as **3a**.



R
1: H
2: Xylose



R
3: Xylose
4: Glucose
3a(4a): H

RESULTS AND DISCUSSION

Fresh leaves of *A. hirsuta* were 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 four known diarylheptanoids (**1**-**4**).

Compound **1** was identified as hirsutanonol; (5*S*)-1,7-bis-(3,4-dihydroxyphenyl)-5-hydroxyheptane-3-one (Terazawa *et al*, 1973), and **2** was identified as oregonin; (5*S*)-1,7-bis-(3,4-dihydroxyphenyl)-heptane-3-one-5-O-β-D-xylopyranoside (Terazawa *et al.*, 1984 and Lee *et al.*, 1992) by comparison with those of reported data.

Compound **3** gave greenish blue coloration with ferric chloride on TLC. The ^{1}H-NMR spectrum of **3** showed the presence of four methylenes over δ 1.50~2.15 and another two methylenes over δ 2.51~3.15 and two pairs of 1,3,4-trisubstituted aromatic rings over δ 6.74~7.28. The ^{1}H-NMR spectrum of **3** also showed an anomeric proton signal at δ 4.79 (d, J=7.5). These spectral data indicated that **3** was a bis-(3,4-dihydroxyphenyl) heptane glycoside. The ^{13}C-NMR spectrum of **3** showed six methylenes, one oxygen bearing methine and two 3,4-dihydroxyphenyl groups and one β-D-xylopyranosyl moiety (Table I). On acid hydro-lysis, **3** gave **3a** and D-xylose. The ^{1}H-NMR and ^{13}C-NMR spectrum of **3a** showed the presence of six methylene, two 3,4-dihydroxyphenyl groups and a hydroxy bearing methine group (Table I). Comparing ^{13}C-NMR data of a glycoside (**3**) with those of its aglycone (**3a**), the downfield shift of C-5 signal (+7.7 ppm) at δ 79.2 and the upfield shift of C-4 (-4.3 ppm) at δ 34.4 which is larger than that of C-6 (-3.3 ppm) at δ 41.0 by its glycosilation shift indicate that xylose is linked to C-5 of heptanoid and the configuration of C-5 of the glycoside (**3**) is assigned to be *R* (Ohta *et al.*, 1984). Assignments of each proton and carbon were confirmed by ^{1}H-^{1}H COSY, HMQC and HMBC spectra. The ESIMS spectrum of **3** exhibited a prominent M^+ peak at *m/z* 464 and [M-xylose]$^+$ peak at *m/z* 332. Thus, the structure of **3** was identified as (5*R*)-1,7-bis-(3,4-dihydroxyphenyl)-heptane-5-O-β-D-xylopyranoside (Gonzalez *et al.*,1999).

Compound **4** gave greenish blue coloration with spraying of ferric chloride on TLC. The ^{1}H- and ^{13}C-NMR specta of **4** were very similar to those of **3** except the presence of glucose moiety instead of xylose (Table I). On acid hydrolysis of **4** gave **4a** and D-glucose. The ^{1}H- and ^{13}C-NMR spectral data of **4a** were same as **3a**. The connectivity of glucose to heptane moiety also confirmed by comparing glycoside (**4**) with its aglycone (**4a**). The downfield shift of C-5 (+5.7 ppm) at δ 77.2 and the shift of C-4 (-4.3 ppm) shown at δ 34.4 which is larger than that of C-6 (-3.5 ppm) at δ 37.5 owing to glycosilation shift indicate that glucose is linked at C-5 of heptanoid and the configuration of C-5 of the glycoside (**4**) is assigned to be *R* (Ohta *et al.*, 1984). Assignments of each proton and carbon were confirmed by ^{1}H-^{1}H COSY, HMQC and HMBC spectra. The ESIMS spectrum of **4** exhibited a prominent $[M]^+$ peak at *m/z* 494. Thus, the structure of **4** was identified as (5*R*)-1,7-bis-(3,4-dihydroxyphenyl)-heptane-5-O-β-D-glucopyranoside (Wada *et al.*, 1998). These compounds (**3** and **4**) have not been previously isolated from this plant.

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