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# Phenolic Compounds on the Leaves of *Betula platyphylla* var. *latifolia*

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**Abstract** □ Chemical examination of *Betula platyphylla var. latifolia* afforded a novel diarylheptanoid named betulatetraol, together with a phenylpropanoid (3,4'-dihydroxypropiophenone), flavan-3-ol [(+)-catechin] and its glycosides [(+)-catechin 5-*O*-β-D-glucopyranoside, (+)-catechin 7-*O*-β-D-glucopyranoside] and two proanthocyanidins (procyanidins B-1 and B-3).

**Keywords** □ *Betula platyphylla* var. *latifolia*. betulaceae; diarylheptanoid, phenylpropanoid, flavan-3-ols, proanthocyanidins.

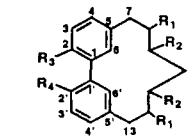
In the course of chemical studies on the phenolic compounds in the family Betulaceae[1,2], we have examined *Betula platyphylla var. latifolia* (Betulaceae), which grows in the northern parts of Korea and Japan and used medicinally for the relief of heat and cough, and also for the treatment of inflamation[3]. As regards the constituents of this plant, catechin and its glycoside and diarylheptanoid derivatives were isolated from the bark and wood[4,5]. We describe here the isolation and structural elucidation of a new diarylheptanoid named betulatetraol **1**, together with a phenylpropanoid, (+)-catechin and its two glycosides and two proanthocyanidins, from the leaves.

## RESULTS AND DISSCUSSION

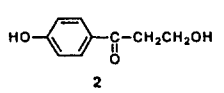
Fresh leaves of *B. platyphylla* var. *latifolia* were extracted with aqueous acetone and the extract was subjected to a combination of chromatographies over Sephadex LH-20, MCI-gel CHP 2OP, Bondapak $C_{18}$/Porasil B and TSK-gel Toyopearl HW 40F to afford a new diarylheptanoid, betulatetraol **1**, together with known phenylpropanoid (3,4'-dihydroxypropiophenone **2**[5]), flavan-3-ols ((+)-catechin **3**[6], (+)-catechin 7-*O*-β-D-glucopyranoside **4**[7] and (+)-catethin 5-*O*-β-D-glucopyranoside **5**[8]) and proanthocyanidins (procyanidins B-1 **6**[9] and B-3 **7**[9]) which were identified by comparisons of their physical and spectral data with those described in the literatures or by direct comparisons with authentic smaples.

The $^1$H-NMR spectrum of **1** exhibited signals due to methylene at δ 2.97 (d, $J$=7.8 Hz) and neighboring hydroxy-bearing methine at δ 4.40 (t, $J$=7.8 Hz), the lowfield shift of former being considered to be assignable to benzyl. Another hydroxy-bearing methine signal appeared at δ 4.08, which was found to be coupled with the singal at δ 2.22 by the $^1$H-$^1$H COSY spectrum. In the aromatic region, AMX-type signals were observed at δ 6.84, 6.88 and 7.06, indicating the presence of 1,2,4-trisubstituted aromatic ring.

The $^{13}$C-NMR specrum of **1** showed ten peaks in total, consisting of six aromatic and four aliphatic ones. Among these, the chemical shifts (δ 116.8, 126.7, 130.5, 134.8, 152.0) of the aromatic signals were consistent with a 2,4-disubstituted phenol structure.



| | $R_1$ | $R_2$ | $R_3$ | $R_4$ |
|---|---|---|---|---|
| 1 | OH | OH | OH | OH |
| 1a | OH | H | H | OH |
| 1b | OAc | OAc | OAc | OAc |
| 1c | OH | OH | OMe, OH | |



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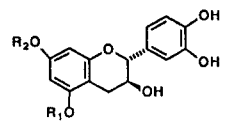
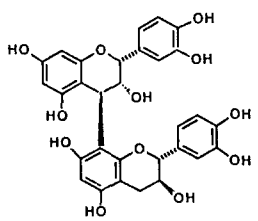


| | $R_1$ | $R_2$ |
|---|---|---|
| 3: | H | H |
| 4: | H | β-D-glc |
| 5: | β-D-glc | H |



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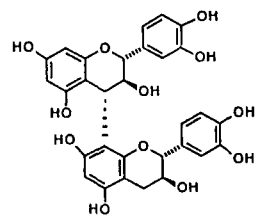

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On the other hand, the aliphatic resonances at δ 67.2 and 70.2 clearly indicated the presence of hydroxy-bearing methines and those at δ 35.8 and 40.8 showed the existence of methylenes haveing no oxygen atom.

The negative FAB MS exhibited a prominent $[M-H]^-$ peak at *m/z* 345. Thus, taking into account the above $^1H$ and $^{13}C$-NMR data, the structure of compound **1** was considered to be symmtrical, possessing a biphenolic heptanoid skeleton. Comparisons of the chemical shifts of the $^{13}C$-NMR spectrum of **1** with that of known diarylheptanoid, alnusdiol **1a**[10], indeed showed a close resemblance, in particular, the chemical shifts of the aromatic resonances being in good agreement. Based on these observations, the aromatic substitution system in **1** was concluded to be the same as that of **1a**.

Acetylation of **1** with acetic anhydride and pyridine yielded the acetate **1b**, whose FD MS exhibited the $[M]^+$ peak at *m/z* 598. This mass number indicated that six acetyl group are introduced. The $^1H$-NMR spectrum of **1b** showed three acetoxyl signals at δ 2.05, 2.12 and 2.23 (each s), together with two methine signals at δ 5.12 and 5.84, the low field shifts, thus confirming the presence of hydroxy-bearing methines in **1**.

Methylation of **1** with etheral diazomethane furnished unexpectedely monomethyl ether **1c** [EI MS *m/z* 360], but this finding was consistent with the earlier findings[11,12] that owing to the steric hindrance of biphenyl group in the molecule, complete phenol methylation with diazomethane is impossible, giving only monomethyl ether. In addition, the observation of a prominent EI MS peak at *m/z* 211 as the base peak, resulting from the cleavage at the benzylic position, supported the structure **1c**.

On the basis of these finding, the structure of betulatetraol was represented by the formula **1**. Further study including x-ray examination is underway to establish the absolute stereo structure.

## EXPERIMENTAL METHODS

General. NMR spectra were recorded at 100 and 270 MHz ($^1H$-NMR), and 25.05 ($^{13}C$-NMR). Chemical shifts are given in δ (ppm) scale with TMS as int. std. Negative FAB MS were measured 1.5 kV (accelerating voltage) with $Me_2CO$-glycerol as matrix. EIMS at 30 eV and FDMS at the accerating voltage of 3 kV, emitter current of 17 mA. CC was carried out on Sephadex LH-20 (25-100 μm, Pharmacia), MCI-gel CHP 2OP (75-150, Mitsubishi), Bondapak $C_{18}$/Porasil B (37-75 μm, Waters), TSK-gel Toyopearl HW 40F (30-60 μm, Tosoh) and Kieselgel 60 (70-230 mesh, Merck). TLC was conducted on precoated silica gel 60 $F_{254}$ (Merck) and precoated cellulose $F_{254}$ plates (Merck). Spots were detected under UV and by spraying with $FeCl_3$ (for phenolics) and dil $H_2SO_4$, followed by heating (for phenolics, acetate and me ether).

Plant material. Leaves of *B. platyphylla* var. *latifolia* were collected in Mt. Kuan-ack near Seoul City, Korea. A voucher specimen is deposited at the Herbarium, Faculty of Pharmaceutical Sciences, Kyushu University.

Extraction and isolation. Fresh leaves (5.5 kg) were extracted with 80% aq. $Me_2CO$ at room temp. After removal of $Me_2CO$ *in vacuo*, the aq. soln was filtered. The filtrate was concd and then applied to a column of Sephadex LH-20. Elution with $H_2O$ containing increasing proportions of MeOH afforded 3 frs, I (150g), II (225g) and III (320g). Repeated CC of fr. I on MCI-gel CHP 2OP with an $H_2O$-MeOH gradient system gave 3,4′-dihydroxypropiophenone (**2**, 5g). CC of fr. II over MCI-gel, Bondapak $C_{18}$/Porasil B with an $H_2O$-MeOH gradient sys-

tem furnished (+)-catechin 5-*O*-β-D-glucopyranoside (**5**, 10 mg) and Sephadex LH-20 with EtOH yielded (+)-catechin 7-*O*-β-D-glucopyranoside (**4**, 45 mg), betulatetraol (**1**, 400 mg), CC of fr. III over MCI-gel, TSK-gel Toyopearl HW 40F with an $H_2O$-MeOH gradient system furnished procyanidin B-1 (**6**, 11 mg) and Sephadex LH-20 with EtOH afforded (+)-catechin (**3**, 1g) and procyanidin B-3 (**7**, 100 mg).

Betulatetraol **1**, White amorphous powder, $[\alpha]_D^{19}$ = −8.6° (MeOH; c 1.1). Negative FAB-MS *m/z*; 345 $[M-H]^-$ (98), 327 $[M-H_2O]^-$ (3). $^1$H-NMR ($Me_2CO$-$d_6$+$D_2O$): δ 2.22 (2H, dd, *J*=4.8, 7.8 Hz, H-10), 2.97 (4H, d, *J*=7.8 Hz, H-7, H-13), 4.08 (2H, dd, *J*=4.8, 7.8 Hz, H-9, H-11), 4.40 (2H, t, *J*=7.8 Hz, H-8, H-12), 6.84 (2H, d, *J*=2 Hz, H-6, H-6′), 6.88 (2H, d, *J*=8 Hz, H-3, H-3′), 7.06 (2H, dd, *J*=2, 8 Hz, H-4, H-4′).

$^{13}$C-NMR ($Me_2CO$-$d_6$+$D_2O$): δ 35.8 (C-7, C-13), 41.8 (C-10), 67.2, 70.5 (C-8, C-12, C-9, C-11), 116.8 (C-3, C-3′), 126.7 (C-1, C-1′), 130.3 (C-4, C-4′), 130.5 (C-5, C-5′), 134.8 (C-6, C-6′), 152.0 (C-2, C-2′). (Found: C, 65.19; H, 6.31, $C_{19}H_{22}O_6$ require: C, 65.88; H, 6.40%).

Acetylation of **1**. a solution of **1** (20 mg) in pyridine-$Ac_2O$ (1:1, 1 m*l*) was allowed to overnight at room temperature. The reaction mixture was poured into ice water to give white precipitates which were subjected to silica gel cc. Elution with $CHCl_3$: $Me_2CO$ (10:1) afforded the hexacetate of **1** (**1b**, 10 mg) as an white amorphous powder, $[\alpha]_D^{2.5}$ = + 26° ($CHCl_3$; c 0.1). FDMS *m/z*: *598* $[M]^+$. $^1$H-NMR ($CDCl_3$): δ 2.05, 2.12, 2.23 (each 6H, s, OAcx6), 2.76 (2H, dd, *J*=10, 16 Hz, H-7, H-13), 3.26 (2H, dd, *J*=4, 16 Hz, H-7, H-13), 5.12 (2H, m, H-9, H-11), 5.84 (2H, m, H-8, H-12), 6.95-7.17 (6H in total, m, aromatic H). (Found: C, 61.99; H, 5.89, $C_{31}H_{34}O_{12}$ require: C, 62.20; H, 5.72%).

Methylation of **1**, a solution of **1** (20 mg) in 80% aquous acetone was treated with etheral diazomethane at room temperature for 5 hr. After removal of solvent *in vacuo*, the residue was chromatographed over silica gel with $CHCl_3$-MeOH (5:1) to give the mono-*O*-methylether of **1** (**1c**, 10 mg) as a white powder. $[\alpha]_D^{25}$ = −4° (MeOH; c 0.6). EIMS *m/z*: 360 $[M]^+$ (39), 342 $[M-H_2O]^+$ (17), 213 (54), 211 (100). $^1$H-NMR ($Me_2CO$-$d_6$+$D_2O$): δ 2.18 (2H, dd, *J*=4.6, 7.5 Hz, H-10), 2.95-3.06 (4H in total, m, H-7, H-13), 4.00 (3H, s, OMe), 4.35 (2H in total, m, H-8, H-12), 6.74-7.22 (6H in total, m, aromatic H). (Found: C, 64.83; H, 6.67. $C_{20}H_{24}O_6$ 1/2$H_2O$ require: C, 65.02; H, 6.55%).

3,4′-dihydroxypropiophenone **2**. White amorphous powder, $[\alpha]_D^{25}$ = −0.7° (MeOH; c 1.1). $^1$H-NMR ($Me_2CO$-$d_6$): δ 3.14 (2H, t, *J*=5 Hz, H-2), 3.65 (1H, br-s, -$CH_2OH$), 3.91 (2H, m, H-1), 6.92 (2H, d, *J*=9 Hz, aromatic H), 7.92 (2H, d, *J*=9 Hz, aromatic H). $^{13}$C-NMR ($Me_2CO$-$d_6$+$D_2O$): δ 41.4 (C-2), 58.5 (C-1), 115.9 (C-3′), 130.0 (C-1′), 131.3 (C-2′), 162.9 (C-4′).

(+)-Catechin **3**. White amorphous powder, $[\alpha]_D^{25}$ = +12.5° (MeOH; c 0.8). $^1$H-NMR ($Me_2CO$-$d_6$): δ 2.52 (1H, dd, *J*=8, 16. Hz, H-4), 2.89 (1H, dd, *J*=6, 16 Hz, H-4), 3.90-4.08 (2H, m, H-3, OH), 4.56 (1H, d, *J*=8 Hz, H-2), 5.87 (1H, d, *J*=2 Hz, H-6), 6.02 (1H, d, *J*=2 Hz, H-8), 6.73 (1H, dd, *J*=2, 8 Hz, H-6′), 6.83 (1H, d, *J*=8 Hz, H-5′), 6.90 (1H, d, *J*=2 Hz, H-2′).

(+)-Catechin-7-*O*-β-D-glucopyranoside **4**. White amorphous powder, $[\alpha]_D^{25}$ = −47.0° (MeOH; c 1.1). $^1$H-NMR (MeCO-$d_6$+$D_2O$): δ 2.54 (1H, dd, *J*=8, 16 Hz, H-4), 2.93 (1H, dd, *J*=6, 16 Hz, H-4), 3.2-4.0 (6H in total, m, glc-H), 4.08 (1H, m, H-3), 4.59 (1H, d, *J*=8 Hz, H-2), 4.83 (1H, d, *J*=8 Hz, anomeric H), 6.06, 6.23 (each 1H, d, *J*=2 Hz, H-6, H-8), 6.73 (1H, dd, *J*=2, 8 Hz, H-6′), 6.83 (1H, d, *J*=8 Hz, H-5′), 6.91 (1H, d, *J*=2 Hz, H-2′).

(+)-Catechin-5-*O*-β-D-glucopyranoside **5**. White amorphous powder, $[\alpha]_D^{25}$ = −27.0° (MeOH; c 1.1). $^1$H-NMR ($Me_2CO$-$d_6$+$D_2O$): δ 2.61 (1H, dd, *J*=8, 16 Hz, H-4), 3.08 (1H, dd, *J*=6, 16 Hz, H-4), 3.5-4.0 (6H in total, m, glc-H), 4.20 (1H, m, H-3), 4.56 (1H, d, *J*=8 Hz, H-2), 4.86 (1H, d, *J*=8 Hz, anomeric H), 6.01, 6.33 (each 1H, d, *J*=2 Hz, H-6, H-8), 6.72 (1H, dd, *J*=2, 8 Hz, H-6′), 6.82 (1H, d, *J*=8 Hz, H-5′), 6.90 (1H, d, *J*=2 Hz, H-2′).

Procyanidin B-1 **6**. Light brown amorphous powder, $[\alpha]_D^{25}$ = +30.2° ($Me_2CO$; c 1.1). $^1$H-NMR ($Me_2CO$-$d_6$+$D_2O$): δ 2.54-2.86 (2H in total, m, H-4′), 3.98 (1H, s, H-3), 4.06 (1H, m, H-3′), 4.65 (1H, s, H-4), 4.76 (1H, d, *J*=8 Hz, H-2′), 5.08 (1H, s, H-2), 5.93-6.08 (3H in total, m, A-ring H), 6.65-7.00 (6H in total, m, B-ring H).

Procyanidin B-3 **7**. Light brown amorphous powder, $[\alpha]_D^{25}$ = −210.0° ($Me_2CO$; c 1.1). $^1$H-NMR ($Me_2CO$-$d_6$+$D_2O$): δ 2.38-3.10 (2H in total, m, H-4′), 3.98-4.75 (5H in total, m, H-2, 3, 4, 2′, 3′), 5.80-6.30 (3H in total, m, A-ring H), 6.45-7.06 (6H in

total, m, B-ring H).

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