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Anti-adipogenic diarylheptanoids from *Alnus hirsuta* f. *sibirica* on 3T3-L1 cells

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ABSTRACT

A new diarylheptanoid, (5*S*)-hydroxy-1-(3,4-dihydroxyphenyl)-7-(4-hydroxyphenyl)-hepta-1*E*-en-3-one (**1**), was isolated along with seventeen known diarylheptanoids (**2**–**18**) from the methanol extract of *Alnus hirsuta* f. *sibirica* leaves using bioactivity-guided fractionation. Among the isolated compounds, compounds **1** and **2** and **4**–**12** reduced lipid accumulation dose-dependently in 3T3-L1 preadipocytes. Of the compounds active in the present assay system, the most potent compound **7**, platyphyllonol-5-*O*-β-D-xylopyranoside, significantly suppressed the induction of peroxisome proliferator activated receptor γ (PPARγ and CCAAT/enhancer binding protein α (C/EBPα) protein expression, and inhibited adipocyte differentiation induced by troglitazone, a PPARγ agonist. It was demonstrated that compound **7** has anti-adipogenic activity mediated by the regulation of PPARγ dependent pathways.

Obesity causes or aggravates many health problems, both independently and in association with several pathological disorders, including Type II diabetes, hypertension, atherosclerosis, and cancer.[1] Obesity is known to be associated with an excessive growth of adipocyte mass tissue by increasing the number and size of adipocytes differentiated from preadipocytes.[2–4] Adipogenesis is the development of mature fat cells from preadipocytes. The cellular and molecular mechanisms of adipocyte differentiation have been extensively studied using preadipocyte culture systems. Committed preadipocytes undergo alteration of cell shape, growth arrest, clonal expansion, and subsequent terminal differentiation into adipocytes, as well as a complex sequence of changes in gene expression and the storage of lipids.[5] Therefore, 3T3-L1 cells were used as a screening tool to determine the anti-adipogenic activity of the isolated compounds. Adipogenesis is mediated by adipogenic factors including PPARγ and C/EBPα, which are at the center of this network and oversee the entire terminal differentiation process.[6]

Genus *Alnus* has been used for hemorrhage, burn injuries, antipyretic fever, diarrhea, and alcoholism in traditional Korean medicine. The stem of these plants come into the oriental herb market as a health tea for treating alcoholism in Korea. The evidence from pharmacological studies has suggested that these have anti-inflammatory, anti-tumor, anti-obesity, and anti-oxidative effects.[7] The various types of plant secondary metabolites such as flavonoids, triterpenoids, tannin, phenols, steroids, and diarylheptanoids have been isolated from the plants of the genus *Alnus*.[7] Recently, there is growing interest in searching for anti-adipogenic compounds from natural products. In the course of searching for anti-adipogenic molecules from the genus *Alnus* using 3T3-L1 cells, it was found that the MeOH extract of *Alnus hirsuta* Turcz. f. *sibirica* (Spach) H. Ohba leaves (Betulaceae) showed significant inhibitory activity on adipocyte differentiation. *A. hirsuta* f. *sibirica*, an indigenous *Alnus* species found in Korea, is a deciduous broad-leaved tree growing in the damp areas of mountain valleys. In Korean traditional medicine, the bark of this plant has been used as an antipyretic and as a health tea for alcoholism.[8] Regarding pharmacological studies of diarylheptanoids isolated from this plant, the inhibitory activities of cyclooxygenaxe-2 expression and melanogenesis have been reported.[8,9] To date, there have been no previous studies on the anti-adipogenic activity of *A. hirsuta* f. *sibirica*. Thus, in the present study, we have attempted to isolate the anti-adipogenic constituents from *A. hirsuta* f. *sibirica* and further explore the active mechanism of the isolated diarylheptanoids on 3T3-L1 preadipocytes.

The leaves of *A. hirsuta* f. *sibirica* were collected in the Nambu forest of Seoul Beagwoon Mountain, Gwangyang, Jeollanam-do, Korea and authenticated by Dr. Jong Hee Park, professor of Pusan National University. A voucher specimen (SNU 753) has been deposited at the Herbarium of the Medicinal Plant Garden, College

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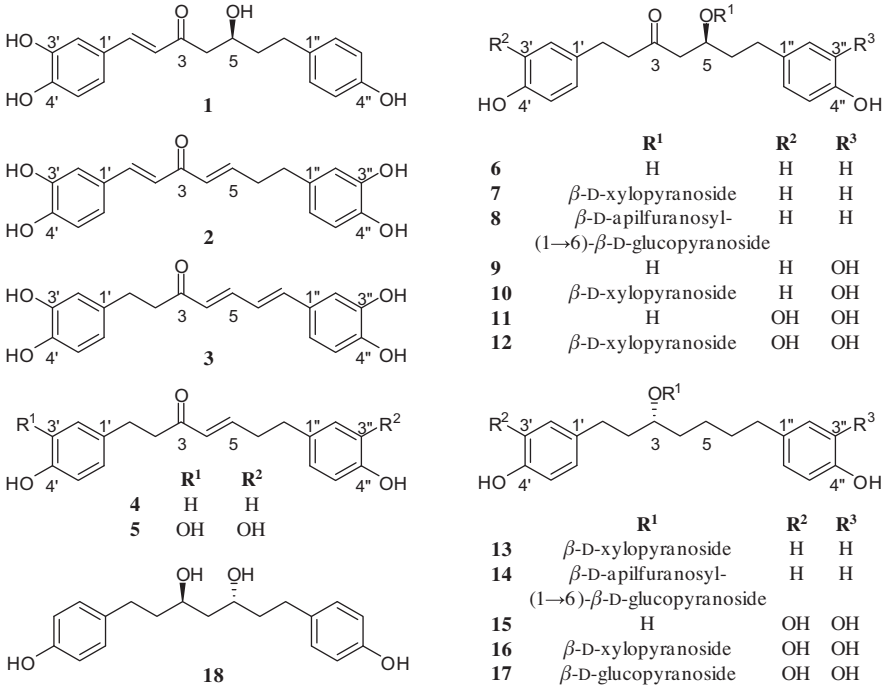

	R^1	R^2	R^3
6	H	H	H
7	β-D-xylopyranoside	H	H
8	β-D-apilfuranosyl-(1→6)-β-D-glucopyranoside	H	H
9	H	H	OH
10	β-D-xylopyranoside	H	OH
11	H	OH	OH
12	β-D-xylopyranoside	OH	OH

	R^1	R^2	R^3
13	β-D-xylopyranoside	H	H
14	β-D-apilfuranosyl-(1→6)-β-D-glucopyranoside	H	H
15	H	OH	OH
16	β-D-xylopyranoside	OH	OH
17	β-D-glucopyranoside	OH	OH

Figure 1. Structures of the isolated compounds from *A. hirsuta* f. *sibirica*.

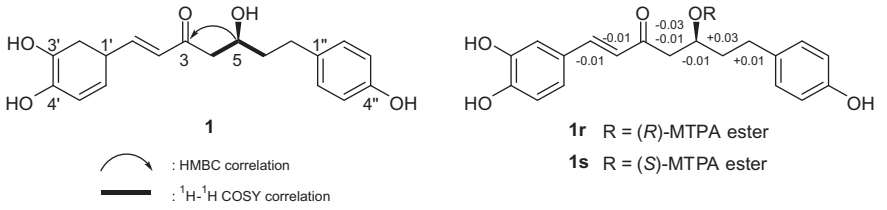

Figure 2. Key 2D NMR correlations and $\Delta\delta$ ($\delta S - \delta R$) values of the MTPA esters of compound **1**.

of Pharmacy, Seoul National University, Koyang, Korea. The dried plant material (119 g) was pulverized and then extracted with 80% methanol (2 L, 3 h × 3) by ultrasonication at room temperature. The methanolic extract was concentrated in vacuo to give a crude extract (35 g). This methanolic extract was suspended in H_2O and partitioned successively with $CHCl_3$ (2 L), EtOAc (2 L) and *n*-butanol (2 L), giving a solid residue of 2.8, 8.7, and 9.9 g, respectively. Of these fractions, the EtOAc-soluble and *n*-BuOH-soluble extracts with significant inhibitory effect on adipocyte differentiation (17.7 ± 3.3% and 56.9 ± 7.5% at a concentration 100 μg/mL, respectively) were used for the follow-up isolation work. The EtOAc-soluble extract (8.7 g) was subjected to a silica gel column chromatography eluted with a gradient mixture of $CHCl_3$/CH_3OH/H_2O to yield 13 fractions (E1–E13). E3 was subjected to preparative HPLC (C_{18}, 500 × 20 mm, CH_3CN/H_2O, 38:62, 7 ml/min) to yield two fractions. Compound **9** (3.4 mg, t_R 37.64 min) obtained from E3-1 (t_R 25.4 min) was purified through semi-preparative HPLC (YMC J'Sphere, C_{18}, 250 × 10 mm, CH_3OH/H_2O, 45:55, 2 ml/min). E4 yielded compound **3** (4.3 mg, t_R 72.36 min), **2** (3.0 mg, t_R 98.88 min), and **18** (6.9 mg, t_R 103.28 min) through preparative HPLC (C_{18}, 500 × 20 mm, CH_3OH/H_2O, 45:55, 6 ml/min), respectively. Compound **1** (2.3 mg, t_R 58.24 min) from E5, and compound **15** (4 mg, t_R 31.18 min) and **11** (34.5 mg, t_R 45.13 min) from E6 were isolated using preparative HPLC (C_{18}, 500 × 20 mm, CH_3OH/H_2O, 55:45, 6 ml/min). E7 was further subjected to preparative HPLC (C_{18}, 500 × 20 mm, CH_3OH/H_2O, 48:52, 6 ml/min) to yield six fractions. Compound **6** (2.3 mg, t_R 17.44 min) and compound **7** (6 mg, t_R 22.75 min) was purified from E7-4 and E7-2 through semi-preparative HPLC (YMC J'Sphere, C_{18}, 250 × 10 mm, CH_3OH/H_2O, 50:50, 2 ml/min), respectively. Compound **8** (2.4 mg) was obtained from E8 by column chromatography on Sephadex LH-20 (MeOH). The *n*-butanol fraction was chromatographed on a silica gel column eluting with a gradient of $CHCl_3/CH_3OH/H_2O$ to yield nine fractions (B1–B9). B4 afforded compound **10** (10.6 mg, t_R 20.01 min) through semi-preparative HPLC (YMC J'Sphere, C_{18}, 250 × 10 mm, CH_3OH/H_2O, 50:50, 2 ml/min). Compound **12** (126.5 mg, t_R 73.92 min), compound **17** (6.7 mg, t_R 127.68 min), compound **16** (5.9 mg, t_R 152.92 min), compound **5** (3.4 mg, t_R 202.56 min) and compound **4** (3.8 mg, t_R 232.32 min) were isolated from B7 preparative HPLC (C_{18}, 500 × 20 mm, CH_3OH/H_2O, 60:40, 4 ml/min). Compound **13** (4.9 m) and compound **14** (3.9 mg) were obtained from B9 by column chromatography on Sephadex LH-20 (CH_3OH).

Compound **1** was obtained as brownish syrup, and its molecular formula of $C_{19}H_{20}O_5$ was established by the observed pseudomolecular ion peak at *m*/*z* 327.1225 $[M-H]^-$ (calcd for $C_{19}H_{19}O_5$ 327.1232) in the negative HRFABMS. The IR spectrum showed the presence of hydroxyl (3266 cm^{-1}), carbonyl (1710 cm^{-1}), and *trans* double bond (1024 cm^{-1}) functions. The ^1H NMR spectrum revealed an 1,3,4-trisubstituted aromatic ring [δ_H 7.07 (1H, d, J = 2.0 Hz, H-2′), 6.98 (1H, dd, J = 8.4, 2.0 Hz, H-6′), and 6.78 (1H, d, J = 8.4 Hz, H-5′)] and an 1,4-disubstituted aromatic ring [δ_H