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NOTE

Chemotaxonomic Significance of Oregonin in *Alnus* Species

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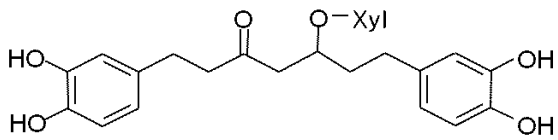
A diarylheptanoid, (5S)-1,7-*bis*-(3,4-dihydroxyphenyl)-5-hydroxyheptane-3-on-5-O-β-D-xyiopyranoside, named as oregonin (**1**), was isolated from the bark of *Alnus pendula* Matsumura which is a species of the genus *Alnus* species, growing throughout Korea. The structure elucidation was accomplished by various spectroscopic methods including negative FAB-MS, ^{1}H NMR and ^{13}C NMR techniques or comparison with authentic samples.

Key Words: *Alnus pendula*, Betulaceae, Diarylheptanoid, Chemotaxonomy.

Genus *Alnus* refers to deciduous broad-leaved trees or shrubs found in damp areas and mountains and comprises of more than 17 species, *Alnus borealis* Koidzumi, *Alnus firma* Sieb. et Zucc., *Alnus hirtella* Koidz., *Alnus hirsuta* Turcz, *Alnus hirsuta* Turcz. var. *sibirica* Fischer, *Alnus japonica* Steudel, *Alnus maritima* var. *arguta* Regel., *Alnus japonica* Thunb. var. *koreana* Callier, *Alnus japonica* Thunb. var. *reginosa* Nakai, *Alnus japonica* Thunb. var. *serrata* Nakai, *Alnus mandshurica* Callier, *Alnus mandschurica* Thunb. for. *barbinervis*, *Alnus mandschurica* Thunb. for. *pubescens* Kitagawa, *Alnus maximowiczii* Call, *Alnus mayrii* Call, *Alnus pendula* Matsum, *Alnus vermicularis* Nakai are growing in Korea[1]. The bark of *Alnus pendula* (bar code; PB 2368.2) as well as MeOH extracts of *Alnus pendula* (leaf, bar code; PB 2368.1), *Alnus firma* (fruit, stem, leaf, flower, bar code; PB 2366.1-4) and *Alnus maximowiczii* (flower, bark, leaf, bar code; PB 2364.1-3) were purchased from the Korea Plant Extract Bank in October 2008.

Diarylheptanoids are characteristic components of the *Alnus* species[2,3]. Several interesting biological activities of diarylheptanoids including their antiinflammatory[4-7] and antioxidant properties[8] have previously been reported. In a previous study conducted in our lab, quantitative analysis of diarylheptanoids including oregonin (**1**) was conducted using HPLC on *Alnus japonica*, *Alnus hirsuta* and *Alnus hirsuta* var. *sibirica*[9]. Here, as part of our continuous search for diarylheptanoids from new natural sources, we describe the isolation and identification of oregonin (**1**) from the bark of *Alnus pendula* and screening of oregonin (**1**) from some other *Alnus* species [*Alnus firma* (fruit, stem, leaf, flower) and *Alnus maximowiczii* (flower, bark, leaf)].



Chemical structures of compound **1** isolated from *Alnus pendula* Matsumura

Extraction and isolation: The dried and powdered bark (300 g) of *Alnus pendula* was extracted using 80 % aqueous acetone at room temperature for 3 days. The filtrate was concentrated and applied to a Sephadex LH-20 column (25-100 μm, 8 × 150 cm, Pharmacia, Uppsala, Sweden) containing increasing proportions of MeOH (60-100 %) afforded 5 fractions (1-5). Repeated column chromatography of fraction 2 on the MCl-Gel CHP 20P (75-150 μm, 5 × 80 cm, Mitsubishi Chemical Co., Tokyo, Japan) and then fraction 2-2 on Disogel (40-60 μm, 3 × 50 cm, Daiso CO., Osaka, Japan) with 30-80 % methanol gradient in middle pressure liquid chromatography (MPLC) system (5 mL/min, 280 nm) resulted in oregonin (**1**).

Oregonin (1): Brown amorphous powder, Negative FAB MS: m/z 477 $[M-H]^-$, ^{1}H NMR (600 MHz, DMSO-d_6 + D_2O): δ 6.67-6.60 (4H in total, H-2',2",5',5"), 6.48-6.45 (2H in total, H-6", 6'), 4.19 (1H, br d, J = 7.8 Hz, xyl-1), 4.03 (1H, m, H-5), 3.76 (1H, dd, J = 11.4, 6Hz xyl-5e), 3.35 (1H, m, xyl-4), 3.08-2.56 (8H in total, H-1,2,4,7), 1.74-1.68 (2H in total, m, H-6)[10-12]. ^{13}C NMR (150 MHz, DMSO-d_6 + D_2O) (Table-1)[10-12].

TABLE-1
^{13}C NMR SPECTRA OF COMPOUND **1**

Carbon No.	Compound **1**	Carbon No.	Compound **1**
C-1	28.7	C-3"	145.1
C-2	45.1	C-4'	143.2
C-3	209.6	C-4"	143.4
C-4	47.5	C-5'	116.0
C-5	76.9	C-5"	116.0
C-6	39.7	C-6'	119.2
C-7	30.5	C-6"	119.3
C-1'	132.4	Xyl-1	102.8
C-1"	133.3	Xyl-2	74.7
C-2'	115.8	Xyl-3	77.0
C-2"	115.8	Xyl-4	69.8
C-3'	145.1	Xyl-5	66.0

*150 MHz (DMSO-d_6 + D_2O)

Quantitative analysis of oregonin (1) of *Alnus pendula* using high pressure liquid chromatography (HPLC): HPLC was used for the quantitative analysis of the oregonin (**1**) contents. An Waters 600 series HPLC system (Milford, MA, USA), was employed and equipped with a vacuum degasser, a binary pump, a UV detector and column compartment. Oregonin (1) was separated on Kromasil 100-5 C18 (4.6 × 250 mm, 5 μm particle) with an linear gradient water:acetonitrile = 90:10 to 60:40 for 24 min. The column temperature was maintained at room temperature and the flow rate was 1.0 mL/min. The system was monitored at 280 nm (λ_{max} of 1) eluting at 19.09 min. Oregonin (**1**) was detected in the extracts of only the bark of *Alnus pendula* but not in the leaf extracts of *Alnus pendula*, as well as the extracts of *Alnus firma* (fruit, stem, leaf, flower) and *Alnus maximowiczii* (flower, bark, leaf). We were able to quantify oregonin (**1**) from the bark extract of *Alnus pendula* (7.69 ± 0.079 %) using a calibration equation (y = 5201.9x - 47967; R^2 = 0.9985).

Chemotaxonomic significance: This is report on the isolation and identification of oregonin (**1**) from *Alnus pendula*. Since the initial isolation of oregonin (**1**) from *Alnus rubra*[13] and oregonin (**1**) has only been distributed among *Alnus hirsuta*[3], *Alnus cordata, Alnus incana, Alnus virdis* and *Alnus glutinosa*[14], *Alnus japonica*[15], *Alnus serrulatoides*[11,12] and *Pinus flexilis*[10].

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