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Research Article

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Oregonin from the stems and leaves of Korean *Alnus* species (Betulaceae)

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ABSTRACT

*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 tinctoria Sarg. 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.*

Keywords: *Alnus tinctoria*, Betulaceae, oregonin, Chemotaxonomy

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INTRODUCTION

Genus *Alnus* refers to deciduous broad-leaved trees or shrubs found in damp areas, 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 and *Alnus vermicularis* Nakai are growing in Korea[1]. The species examined in this study were *Alnus japonica* (stem, leaf), *Alnus hirsuta* (stem, leaf) (collected in Seoul National University Gwanak Arboretum, Anyang-si, Gyeonggi-do, Republic of Korea, 8 June 2009, vouchers AJS2009-01, AJL2009-01, AHS2009-01 and AHL2009-01), *Alnus tinctoria* Sarg. (stem, leaf) and *Alnus hirsuta Turcz* var. *sibirica* Fischer (stem, leaf) (collected in Korea National Arboretum, Pocheon-si, Gyeonggi-do, Republic of Korea, 5 June 2009, vouchers ATS2009-01, ATL2009-01, AHTS2009-01 and AHTL2009-01) and they were certificated by Dr. Jin (Division of Horticulture and Education, Korea National Arboretum).

Diarylheptanoids are characteristic components of the *Alnus* species[2,3]. Several interesting biological activities of diarylheptanoids including their anti-inflammatory[4-7], anti-oxidant properties[8] and anti-atopic dermatitis[16] 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*, *Alnus hirsuta* var. *sibirica*[9], *Alnus pendula*, *Alnus firma and Alnus maximowiczii*[18]. Here, as part of our continuous search for diarylheptanoids from new natural sources, we describe the isolation and identification of oregonin (**1**) from the stems and leaves of *Alnus tinctoria* Sarg. and screening of oregonin (**1**) from some other *Alnus* species [*Alnus japonica* (stem, leaf), *Alnus hirsuta* (stem, leaf) and *Alnus hirsuta Turcz* var. *sibirica* Fischer (stem, leaf)].

EXPERIMENTAL SECTION

General experimental procedure

The stationary phases for the column chromatographic isolation were performed on Sephadex LH-20 (10-25 μm, GE Healthcare Bio-Science AB, Uppsala, Sweden), MCI-gel CHP 20P (75-150 μm, Mitsubishi Chemical, Tokyo,

Japan) and ODS-B gel (40-60 μm, Daiso, Osaka, Japan). ODS-B gel was used as stationary phase on middle pressure liquid chromatography (MPLC) system. Sample injector was Waters 650E (Waters, Seoul, Korea), detector was 110UV/VIS detector (Gilson, Middleton, WI) and pump was TBP5002 (Tauto Biotech, Sanghai, China). Thin layer chromatography (TLC) was carried out using a pre-coated silica gel 60 F_{254} plate (Merck, Darmstadt, Germany) on chloroform, methanol and water (70:30:4, volume ratio). The spots were detected under UV radiation (254 nm) and by spraying with $FeCl_3$ and 10% H_2SO_4 followed by heating.

The components from the *Alnus* species were identified by several instrumental analyses. The 1 D NMR such as ^{1}H- (300 or 600MHz) and ^{13}C- (75 or 150MHz) nuclear magnetic resonance (NMR) experiments were recorded with Gemini 2000 and VNS (Varian, Palo Alto, CA, USA) at center for research facilities on Chung-Ang University. Low resolution fast atom bombardment mass spectrum (LRFAB-MS) were recorded with JMSAX505WA (JEOL, Tokyo, Japan) at National Center for Inter-University Research Facilities on Seoul National University.

Extraction and isolation (stems)

Fresh, chopped stems (350 g) of *Alnus tinctoria* Sarg. were extracted using 80% aqueous MeOH at room temperature for 3 days. The filtrate was concentrated and applied to a Sephadex LH-20 column (10-25 μm, GE Healthcare Bio-Science AB, Uppsala, Sweden) containing increasing proportions of MeOH (30~100%) afforded 4 fractions (1-4). Repeated column chromatography of fraction 2 on MCl-Gel CHP 20P (75-150 μm, Mitsubishi Chemical, Tokyo, Japan) using a H_2O: methanol gradient has resulted in oregonin (**1**).

Extraction and isolation (leaves)

Fresh, chopped leaves (200 g) of *Alnus tinctoria* Sarg. were extracted using 80% aqueous MeOH at room temperature for 3 days. The filtrate was concentrated and applied to a Sephadex LH-20 column (10-25 μm, GE Healthcare Bio-Science AB, Uppsala, Sweden) containing increasing proportions of MeOH (30~100%) afforded 4 fractions (1-4). Repeated column chromatography of fraction 2 on the MCl-Gel CHP 20P (75-150 μm, Mitsubishi Chemical, Tokyo, Japan) and then fraction 2-2 on ODS-B gel (40-60 μm, Daiso, Osaka, Japan) with 30%-100% methanol gradient in middle pressure liquid chromatography (MPLC) system (5 ml/min, 280 nm) has resulted in oregonin (**1**).

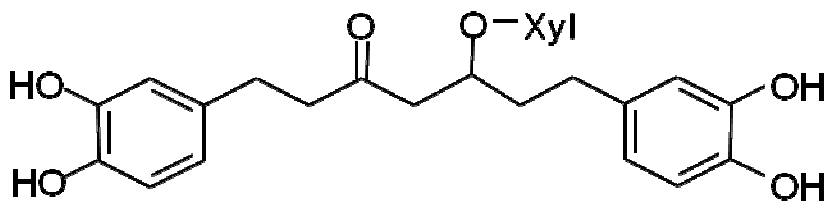
Oregonin (**1**)

Brown amorphous powder, Negative FAB MS: *m/z* 477 $[M-H]^-$, ^{1}H-NMR (600MHz, 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*=7.8Hz, 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): see Table 1[10-12].

RESULTS AND DISCUSSION

Quantitative analysis of Oregonin (1) of *Alnus tinctoria* Sarg. 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, 5μm particle) with a 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 18.54 ± 0.01 min. Oregonin (**1**) was detected in the extracts of *Alnus tinctoria* Sarg.(stem, leaf), *Alnus japonica* (stem, leaf), *Alnus hirsuta* (stem, leaf) and *Alnus hirsuta Turcz* var. *sibirica* Fischer (stem, leaf). We were able to quantify oregonin (**1**) from the stems and leaves extracts of *Alnus tinctoria* Sarg. (4.24 ± 0.002%), (0.85 ± 0.001%) using a calibration equation (y=5201.9x-47967; R^2=0.9985).



1

Fig. 1. Chemical structures of Oregonin (1) isolated from *Alnus tinctoria* Sarg.

Table 1. ^{13}C-NMR spectra of Oregonin (1)

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

** 150 MHz (DMSO-d_6 + D_2O)*

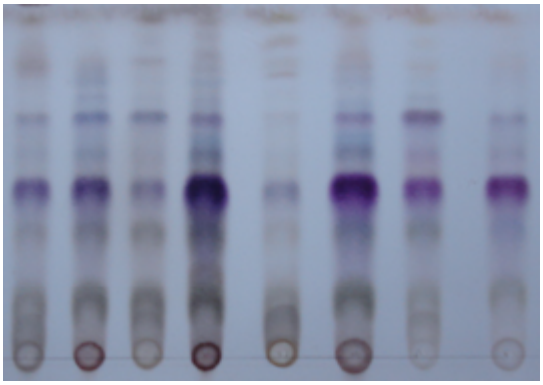
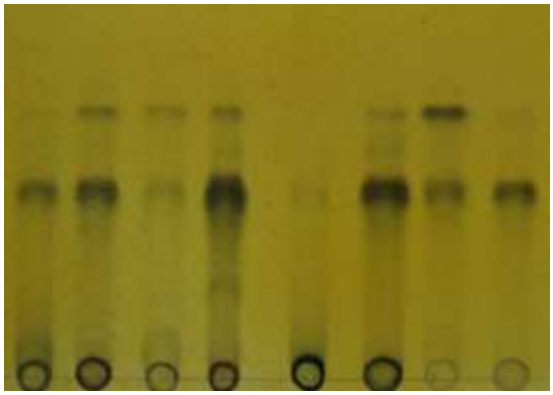


Fig. 2. Comparative result of TLC chromatogram (10% sulfuric acid test)

TLC conditions: stationary phase (Silica gel 60 F_{254}), developing solvent[($CHCl_3$/MeOH/H_2O(70:30:4)] and detection (diluted sulfuric acid test solution for spraying 105 ℃, 5min)

①A. japonica (leaf), ②A. japonica (stem), ③ A. hirsuta (leaf), ④ A. hirsuta (stem), ⑤ A. tinctoria Sarg. (leaf), ⑥ A. tinctoria Sarg. (stem), ⑦ A. hirsuta Turcz var. sibirica Fischer (leaf) and ⑧ A. hirsuta Turcz var. sibirica Fischer (stem)



① ② ③ ④ ⑤ ⑥ ⑦ ⑧

Fig. 3. Comparative result of TLC chromatogram ($FeCl_3$ test)

TLC conditions: stationary phase (Silica gel 60 F_{254}), developing solvent[$CHCl_3$/MeOH/H_2O(70:30:4)] and detection ($FeCl_3$ test solution for spraying, dry)

①A. japonica (leaf), ②A. japonica (stem), ③ A. hirsuta (leaf), ④ A. hirsuta (stem), ⑤ A. tinctoria Sarg. (leaf), ⑥ A. tinctoria Sarg. (stem), ⑦ A. hirsuta Turcz var. sibirica Fischer (leaf) and ⑧ A. hirsuta Turcz var. sibirica Fischer (stem)

Table 2. Retention time of Oregonin (1) from *Alnus* species

Material	Retention time (min)
	Oregonin (1)
Standard	18.54 ± 0.01
A. japonica (leaf)	18.22 ± 0.15
A. japonica (stem)	18.17 ± 0.02
A. hirsuta (leaf)	18.37 ± 0.15
A. hirsuta (stem)	18.60 ±0.15
A. tinctoria Sarg. (leaf)	18.71 ± 0.15
A. tinctoria Sarg. (stem)	18.84 ± 0.15
A. hirsuta Turcz var. *sibirica* Fischer (leaf)	18.33 ± 0.15
A. hirsuta Turcz var. *sibirica* Fischer (stem)	18.31 ± 0.15

The results are expressed as means ±S.D (n=3).

Table 3. Contents of Oregonin (1) from *Alnus* species

Material	Contents (%)
	Oregonin (1)
A. japonica (leaf)	1.37 ± 0.002
A. japonica (stem)	5.13 ± 0.001
A. hirsuta (leaf)	2.20 ± 0.001
A. hirsuta (stem)	3.56 ± 0.001
A. tinctoria Sarg. (leaf)	0.85 ± 0.001
A. tinctoria Sarg. (stem)	4.24 ± 0.002
A. hirsuta Turcz var. *sibirica* Fischer (leaf)	1.27 ± 0.002
A. hirsuta Turcz var. *sibirica* Fischer (stem)	3.44 ± 0.001

The results are expressed as means ±S.D (n=3).

CONCLUSION

Chemotaxonomic significance

This is a report on the isolation and identification of oregonin (**1**) from *Alnus tinctoria* Sarg. 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], *Pinus flexilis*[10] *and Alnus pendula*[17,18].

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