



## Oregonin from the barks and xylems of Chinese *Alnus* species

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### ABSTRACT

A diarylheptanoid, (5*S*)-1,7-bis-(3,4-dihydroxyphenyl)-5-hydroxyheptane-3-on-5-*O*-β-*D*-xylopyranoside, named as oregonin (**1**), was isolated from the barks and xylems of *Alnus ferdinandi-coburgii* C.K. Schneid. which is a species of the genus *Alnus* species, growing throughout China. The structure elucidation was accomplished by various spectroscopic methods including Negative FAB-MS, <sup>1</sup>H-NMR and <sup>13</sup>C-NMR techniques or comparison with authentic samples.

**Keywords:** *Alnus ferdinandi-coburgii*, Betulaceae, diarylheptanoid, Chemotaxonomy

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### INTRODUCTION

Genus *Alnus* refers to deciduous broad-leaved trees or shrubs found in damp areas and mountains and comprises of more than 10 species, *A. ferdinandi-coburgii*, *A. cremastogyne*, *A. lanata*, *A. nepalensis*, *A. henryi*, *A. mandshurica*, *A. hirsuta*, *A. formosana*, *A. japonica*, *A. trabeculosa* are growing in China[1]. The barks and xylems of Chinese *Alnus* species as well as 95% EtOH extracts of *A. nepalensis* (bar code; FBM021-002), *A. japonica* (bar code; FBM068-027) and *A. ferdinandi-coburgii*(bar code; FBM071-014) were purchased from the International Biological Material Research Center.

#### previous work

Diarylheptanoids are characteristic components of the *Alnus* species[2,3]. Several interesting biological activities of diarylheptanoids including their anti-inflammatory[4-7] and anti-oxidant properties[8] and anti-atopic dermatitis[19] 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], *Alnus pendula*, *Alnus firma*, *Alnus maximowiczii*[10], *Alnus tinctoria* Sarg., *Alnus japonica* (stem, leaf), *Alnus hirsuta* (stem, leaf) and *Alnus hirsuta* Turcz var. *sibirica* Fischer (stem, leaf)[11].

Here, as part of our continuous search for diarylheptanoids from new natural sources, we describe the isolation and identification of oregonin (**1**) from the barks and xylems of *A. ferdinandi-coburgii* and screening of oregonin (**1**) from some other *Alnus* species *A. nepalensis* and *A. japonica*.

### EXPERIMENTAL SECTION

#### General experimental procedure

Thin layer chromatography (TLC) was carried out using a pre-coated silica gel 60 F<sub>254</sub> 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<sub>3</sub> and 10% H<sub>2</sub>SO<sub>4</sub> followed by heating.

The components from the *Alnus* species were identified by several instrumental analyses. The 1D NMR such as  $^1\text{H}$ - (300 or 600MHz) and  $^{13}\text{C}$ - (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.

#### Quantitative analysis of Oregonin (1) of Chinese *Alnus* species using high pressure liquid chromatography (HPLC) :

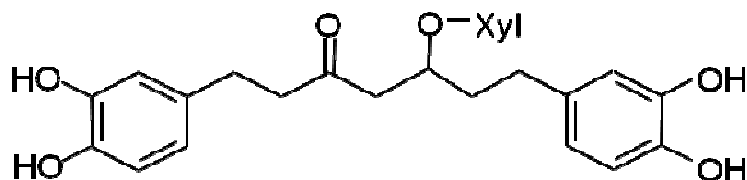
HPLC was used for the quantitative analysis of the oregonin contents. An Waters 600 series HPLC system (Milford, MA, USA), was employed and equipped with a vacuum degasser, a binary pump, a UV detector and column compartment. Oregonin (1) was separated on Kromasil 100-5 C18 ( $4.6 \times 250$  mm,  $5\mu\text{m}$  particle) with an linear gradient water: acetonitrile = 90:10 to 60:40 for 30 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 ( $\lambda_{\text{max}}$  of 1) eluting at 18.64 min. Oregonin was detected in all extracts. We were able to quantify oregonin (1) from the barks and xylems extract of *A. ferdinandi-coburgii* ( $0.70 \pm 0.001\%$ ), *A. nepalensis* ( $0.71 \pm 0.002\%$ ) and *A. japonica* ( $0.70 \pm 0.002\%$ ) using a calibration equation ( $y=5201.9x-47967$ ;  $R^2=0.9985$ ).

**Oregonin (1)** : Brown amorphous powder, Negative FAB MS:  $m/z$  477  $[\text{M}-\text{H}]^-$ ,  $^1\text{H}$ -NMR (600MHz,  $\text{DMSO}-d_6+\text{D}_2\text{O}$ ):  $\delta$  6.74-6.71 (4H in total, H-2',2'',5',5''), 6.53-6.50 (2H in total, H-6'', 6'), 4.31 (1H, br d,  $J=7.8\text{Hz}$ , xyl-1), 4.14 (1H, m, H-5), 3.86 (1H, dd,  $J=11.4, 6\text{Hz}$  xyl-5e), 3.54(1H, m, xyl-4), 2.83-2.52 (8H in total, H-1,2,4,7), 1.80-1.76 (2H in total, m, H-6) [12-14].  $^{13}\text{C}$ -NMR (150 MHz,  $\text{DMSO}-d_6 + \text{D}_2\text{O}$ ): see Table 1[12-14].

Table 1.  $^{13}\text{C}$ -NMR spectra of Compound 1

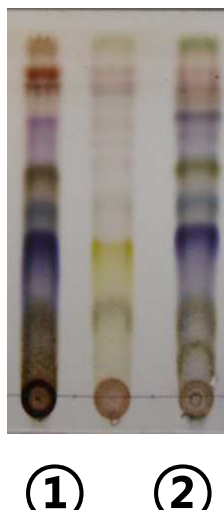
Carbon No.	Compound 1
C-1	29.7
C-2	46.1
C-3	210.6
C-4	48.2
C-5	76.1
C-6	38.3
C-7	31.4
C-1'	133.9
C-1''	134.9
C-2'	116.1
C-2''	116.2
C-3'	145.9
C-3''	145.9
C-4'	144.0
C-4''	144.3
C-5'	116.4
C-5''	116.5
C-6'	120.5
C-6''	120.4
Xyl-1	104.0
Xyl-2	74.6
Xyl-3	77.5
Xyl-4	70.8
Xyl-5	66.6

\* 150 MHz ( $\text{DMSO}-d_6 + \text{D}_2\text{O}$ )



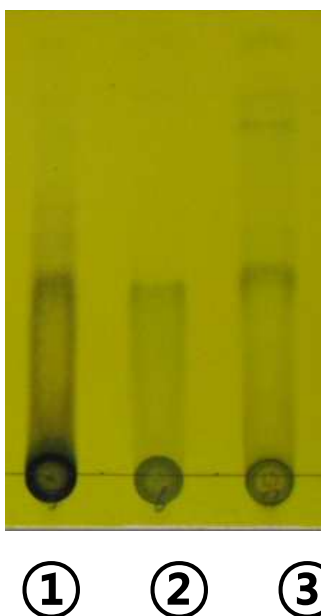
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Fig. 1. Chemical structures of compound 1 isolated from *Alnus ferdinandi-coburgii*



**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 °C, 5min), ① *A. japonica*, ② *A. nepalensis*, ③ *A. ferdinandi-coburgii*



**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), ① *A. japonica*, ② *A. nepalensis*, ③ *A. ferdinandi-coburgii*

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

Material	Retention time (min)
	Oregonin (1)
Standard	18.64 ± 0.01
<i>A. japonica</i> (barks and xylems)	18.74 ± 0.15
<i>A. nepalensis</i> (barks and xylems)	18.63 ± 0.02
<i>A. ferdinandi-coburgii</i> (barks and xylems)	18.65 ± 0.03

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

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

Material	Contents (%) Oregonin (1)
<i>A. japonica</i> (barks and xylems)	0.70 ± 0.002
<i>A. nepalensis</i> (barks and xylems)	0.71 ± 0.002
<i>A. ferdinandi-coburgii</i> (barks and xylems)	0.70 ± 0.001

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

## RESULTS AND DISCUSSION

### Chemotaxonomic significance

This is a report on the isolation and identification of oregonin (1) from *Alnus ferdinandi-coburgii*. Since the initial isolation of oregonin (1) from *Alnus rubra*[14], and oregonin (1) has only been distributed among *Alnus hirsuta*[3], *Alnus cordata*, *Alnus incana*, *Alnus viridis* and *Alnus glutinosa*[15] *Alnus japonica*[16], *Alnus serrulatooides*[12,13], *Pinus flexilis*[17], *Alnus pendula*[10,18] and *Alnus tinctoria*[11].

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