A New Flavonoid in Pine Needles of *Cedrus deodara*

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Abstract: **Objective** To study the chemical constituents of flavonoids in pine needles of *Cedrus deodara*. **Methods** Flavonoids were isolated and purified from ethyl acetate extract of pine needles by chromatography on silica gel and Sephadex LH-20. Their structures were identified on the basis of spectroscopic analysis and chemical evidence. **Results** Five flavonoids were isolated and purified. Their structures were identified as cedrusone A (**1**), myricetin (**2**), 2*R*,3*R*-dihydromyricetin (**3**), quercetin (**4**), and 2*R*,3*R*-dihydroquercetin (**5**). **Conclusion** Compound **1** is a new compound. Compounds **2**－**5** are isolated from pine needles of this genus for the first time.

Key words: cedrusone A; flavonoids; myricetin; pine needles of *Cedrus deodara*; quercetin

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Introduction

Cedrus deodara (Roxb) G. Don from Pinaceae which consists of *C. deodara, C. libani*, *C. brevifolia*, and *C. atlantica* is an evergreen tree growing extensively on the slopes of the Himalayas. The wood of *C. deodara* has been used for the treatment of inflammations and rheumatoid arthritis since ancient times in Indian medical practice. Previous chemical investigations have indicated the presence of terpenes, lignans, and flavonoids. Major pharmacological activities included analgesic, spasm, anti-inflammatory, anticancer, antibacterial, and antivirus effects (Zhang, Shi, and Fan, 2009). As one part of our efforts to investigate the chemical constituents of pine needles of *C. deodara*, 16 compounds had been isolated from petroleum ether extract and *n*-butanol extract of pine needles, such as 1-[3-(4-hydroxyphenyl)-2-propenoate]-α-*D*-glucopyranoside, (+)-(6*S*,9*R*)-9-*O*-β-*D*-glucopyranosyloxy-6-hydroxy-3-oxo-α-ionol, 10-nonacosanol, ferulic acid β-*D*-glucopyranoside, and so on (Zhang *et al*, 2010a; 2010b). No phytochemical work on ethyl acetate fraction from pine needles of this genus has so far been reported. With the efforts to search for the novel bioactive constituents from natural source, we investigated the chemical constituents of ethyl acetate fraction from pine needles of *C. deodara*. In the present paper, the isolation and structure elucidation of a new flavonoid, as well as four known flavonoids were described.

Materials and methods

NMR spectra were recorded on the INOVA—400 spectrometer (^{1}H-NMR: 400 MHz and ^{13}C-NMR: 100 MHz) with TMS as internal standard. HR-ESI-MS was recorded by APEX II FT—ICR mass spectrometer. Column chromatography was carried out with Sephadex LH-20 (Pharmacia Biotech Company, Sweden) and silica gel (100－200 and 200－300 mesh, Qingdao Marine Chemical Co., Ltd., China). TLC was prepared with silica gel 60 F_{254} (Qingdao Marine Chemical Co., Ltd., China).

The pine needles of *Cedrus deodara* (Roxb) G. Don were collected from Lanzhou (Gansu, China) in June 2008. The plant sample was identified by Prof. HE Fu-jiang, in Gansu Academy of Medical Science.

The air dried pine needles of *C. deodara* (5.5 kg) were extracted with 95% ethanol (14 times volume) for three times to afford ethanol extract (700 g), and then was suspended in water and extracted with petroleum ether, ethyl acetate, and *n*-butanol, successively. The ethyl acetate extract (105 g) was subjected to silica gel column and gradiently eluted with methylene chloride-

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methanol (36:1→0:100) to give 23 fractions. Fraction 8 (2.65 g) was then applied on silica gel column and Sephadex LH-20 column to give compounds **4** (25.9 mg) and **5** (16.9 mg). Similarly, fraction 12 (3.95 g) was then applied on silica gel column and Sephadex LH-20 column to give compounds **1** (9.8 mg), **2** (105 mg), and **3** (50.8 mg).

Results

Compound **1**: light yellow powder, responded positively to HCl-Mg and Molish reagent, indicating the characteristic of flavonoid glycoside. The molecular formula, $C_{25}H_{26}O_{14}$, was determined on the basis of positive HR-ESI-MS *m/z*: 573.1210 $[M + Na]^+$ (calculated for $C_{25}H_{26}O_{14}Na$, 573.1220). The NMR spectra (Table 1) of compound **1** showed the presence of 5,7,3′,4′,5′-quiquesubstituted flavonoid moiety [δ 7.48 (2H, s, H-2′, 6′), 6.40 (1H, br s, H-8), 6.18(1H, br s, H-6)] and a glucopyranoside [δ 5.24 (1H, d, J = 4.0 Hz, H-1″)]. The data mentioned above revealed that the glycopyranosidic bond was α-*D* configuration, and the six protons of the sugar part were downfield than in the unsubstituted sugar. Additionally, NMR spectra of compound **2** showed the presence of two methoxyl groups [δ 3.9 (s, 6H), 57.1] and an acetyl group [δ 1.79 (s, 3H), 20.4, 172.5]. ^{1}H-NMR and ^{13}C-NMR signals (Table 1) were assigned with the aid of HMBC correlations. The HMBC correlations between δ 5.24 (1H, d, J = 4.0 Hz, H-1″) and 135.5 (C-3) indicated that the glucopyranoside group was located at C-3. The HMBC correlations between δ 3.90 (6H, s, -CH_3) and 148.7 (C-3′, 5′) indicated that the two methoxyl groups were located at C-3′, 5′. The HMBC correlations between δ 4.09 (1H, m, H-6″) and 172.5 indicated that the acetyl group was located at C-6″. On the basis of above evidences, the structure of compound **1** was established as 3′,5′-dimethoxy-myricetin-3-*O*-(6″-*O*-acetyl)-α-*D*-glucopyranoside, named cedrusone A (Fig. 1).

Table 1 NMR spectroscopic data for compound 1 in CD_3OD

No.	δ_H	δ_C	No.	δ_H	δ_C	No.	δ_H	δ_C
2		158.4	10		106.3	2″	3.42 (m)	75.9
3		135.5	1′		121.8	3″	3.42 (m)	77.9
4		179.3	2′	7.48 (s)	108.3	4″	3.31 (m)	71.3
5		163.0	3′		148.7	5″	3.36 (m)	75.6
6	6.18 (br s)	99.9	4′		140.1	6″-a	4.10 (d, 4.0)	64.1
7		166.1	5′		148.7	6″-b	4.09 (m)	
8	6.40 (br s)	94.8	6′	7.48 (s)	108.3	-OCH_3	3.90 (s)	57.1
9		158.8	1″	5.23 (d, 4.0)	104.1	-CH_3	1.79 (s)	20.4
—C(=O)—		172.5						

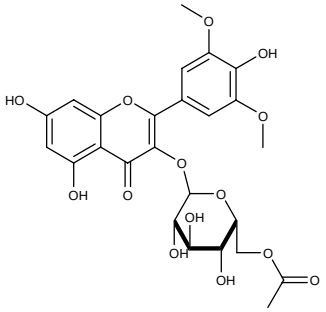

Fig. 1 Structure of compound 1

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