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TWO NEW MYRICETIN GLYCOSIDES FROM PINE NEEDLES OF *Cedrus deodara*

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*Two new myricetin glycosides, myricetin-3-*O*-(6″-*O*-E-p-coumaroyl)-α-D-glucocopyranoside (**1**) and 3′,5′-di-*O*-methylmyricetin-3-*O*-(6″-*O*-acetyl)-α-D-glucopyranoside (**2**), and three known flavonoids, myricetin (**3**), cedrin (**4**), and 2*R*,3*R*-dihydromyricetin (**5**), were isolated from the pine needles of* Cedrus deodara. *Their structures were elucidated on the basis of extensive spectroscopic analysis and chemical evidence.*

Keywords: pine needles of *Cedrus deodara*, myricetin glycosides.

Cedrus deodara (Roxb.) G. Don (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 *Cedrus deodara* has been used since ancient days in Indian medical practice for the treatment of inflammations and rheumatoid arthritis. It is recorded in the dictionary of Chinese Crude Drugs as an effectual herbal drug for expelling wind, removing dampness, destroying parasites, and relieving itching. Its indications are wind-cold-dampness arthralgia, traumatic injury, sleeplessness, edema, eczema, and acariasis. Previous chemical investigations have indicated the presence of terpenes, lignans, and flavonoids; the major pharmacological activities included analgesic, antispasmodic, anti-inflammatory, anticancer, antibacterial, and antivirus [1]. Pine needles from the leaves of *Cedrus deodara* is a rich source. No phytochemical work on the needles of this genus has so far been reported. The medicinal importance of *Cedrus deodara* prompted us to carry out phytochemical investigations on this genus. As one part of our efforts to study the chemical constituents of pine needles of *Cedrus deodara*, 16 compounds have been isolated from the petroleum ether extract and *n*-butanol extract of pine needles, 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 [2, 3]. The present report deals with five flavonoids that were isolated from the ethyl acetate extract of pine needles by chromatography on silica gel and Sephadex LH-20, in which there are two new myricetin glycosides identified as myricetin-3-*O*-(6″-*O*-*E*-*p*-coumaroyl)-α-D-glucopyranoside (**1**), 3′,5′-di-*O*-methylmyricetin-3-*O*-(6″-*O*-acetyl)-α-D-glucopyranoside (**2**), and three known flavonoids identified as myricetin (**3**), cedrin (**4**), and 2*R*,3*R*-dihydromyricetin (**5**).

Compound 1, obtained as a light yellow powder, responded positively to HCl-Mg and Molisch reagent. The molecular formula, $C_{30}H_{26}O_{15}$, was determined on the basis of positive HR-ESI-MS [*m/z* 649.1155 $[M + Na]^+$ (calcd for $C_{30}H_{26}O_{15}Na$, 649.1169)]. The IR spectrum showed absorption bands at 3423, 2921, 1706, 1653, 1604, and 1514 cm^{-1}, which were in agreement with hydroxy and carbonyl groups. Examination of the NMR of compound **1** and comparison with the signals in myricetin-3′-*O*-(6″-*E*-*p*-coumaroyl)-β-D-glucopyranoside reported previously [4] indicated that the structure of compound **1** was similar to myricetin-3′-*O*-(6″-*E*-*p*-coumaroyl)-β-D-glucopyranoside. The NMR spectra (Table 1) of compound **1** showed the presence of a 3,5,7,3′,4′,5′-hexasubstituted flavonoid moiety [δ 7.20 (2H, s, H-2′,6′), 6.25 (1H, br.s, H-6), 6.08 (1H, br.s, H-8)], an *E*-*p*-coumaroyl group [6.06 (1H, d, J = 16.0 Hz, H-β″), 7.36 (1H, d, J = 16.0 Hz, H-γ″), 7.27 (2H, d, J = 8.0 Hz, H-2‴, 6‴), 6.76 (2H, d, J = 8.0 Hz, H-3‴, 5‴)], and a glucopyranoside [δ 5.24 (1H, d, J = 4.0 Hz, H-1″)]. The J-value (4.0) of H-1″ in compound **1** revealed that the glucopyranoside had an α-D configuration, and the six protons of the glucopyranoside were much further downfield than in the unsubstituted sugar. The ^{13}C NMR also supported the presence of the *E*-*p*-coumaroyl group and α-D-glucopyranoside.

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TABLE 1. NMR Spectroscopic Data for Compounds **1** and **2** (CD_3OD, δ, mult, J/Hz)

Position	**1**		**2**	
	δ_H	δ_C	δ_H	δ_C
2		159.1		158.8
3		135.3		135.5
4		179.4		179.3
5		162.9		163.0
6	6.25 (br.s)	99.9	6.40 (br.s)	99.9
7		165.9		166.1
8	6.08 (br.s)	94.7	6.18 (br.s)	94.8
9		158.3		158.4
10		106.2		106.3
1′		122.0		121.8
2′, 6′	7.20 (s)	110.0	7.48 (s)	108.3
3′, 5′		146.3		148.7
4′		140.1		138.0
1″	5.24 (d, J = 4.0)	103.6	5.24 (d, J = 4.0)	104.1
2″	3.52 (m)	75.9	3.43 (m)	75.9
3″	3.43 (m)	78.1	3.43 (m)	77.9
4″	3.34 (m)	71.7	3.28 (m)	71.3
5″	3.43 (m)	75.6	3.36 (m)	75.6
6″	4.29 (d, J = 4.0)	64.4	4.10 (d, J = 4.0)	64.1
	4.19 (m)		4.09 (m)	
OCH_3			3.90 (s)	57.1
CH_3			1.79 (s)	20.4
O-CO (α‴)		169.0		172.5
β‴	6.06 (d, J = 16.0)	114.7		
γ‴	7.36 (d, J = 16.0)	146.5		
1‴		127.1		
2‴, 6‴	7.27 (d, J = 8.0)	131.2		
3‴, 5‴	6.76 (d, J = 8.0)	116.7		
4‴		161.1		

All of these assignments were made on the basis of heteronuclear multiple bond connectivity (HMBC) correlations (Fig. 1). The HMBC correlations between δ 5.24 (1H, d, J = 4.0 Hz, H-1″) and δ 135.3 (C-3) indicated that the glucopyranoside group was located at C-3. The HMBC correlations between δ 4.19 (1H, m, H-6″) and δ 169.0 indicated that the *E*-*p*-coumaroyl group was located at C-6″. On the basis of the above evidence, compound **1** was elucidated as myricetin-3-*O*-(6″-*O*-*E*-*p*-coumaroyl)-α-D-glucopyranoside.

Compound 2, obtained as a light yellow powder, responded positively to HCl-Mg and Molisch reagent. The molecular formula, $C_{25}H_{26}O_{14}$, was determined on the basis of positive HR-ESI-MS [*m/z* 573.1210 $[M + Na]^+$ (calcd for $C_{25}H_{26}O_{14}Na$, 573.1220)]. The IR spectrum showed absorption bands at 3420, 2911, 1704, 1652, 1603, and 1512 cm^{-1}, which were in agreement with hydroxy and carbonyl groups. Compared with the signals of compound **1**, the NMR spectra (Table 1) of compound **2** also showed the presence of a 3,5,7,3′,4′,5′-hexasubstituted 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 J-value (4.0) of H-1″ in compound **2** revealed that the glucopyranoside had an α-D configuration, and the six protons of the glucopyranoside were much further downfield than in the unsubstituted sugar. Additionally, the NMR spectra of compound **2** showed the presence of two methoxyl groups [δ_H 3.9 (6H, s), δ_C 57.1] and an acetyl group [δ_H 1.79 (3H, s), δ_C 20.4, 172.5]. The ^{13}C NMR also supported the presence of the methoxyl group, acetyl group, and α-D-glucopyranoside. The ^1H and ^{13}C NMR signals (Table 1) were assigned with the aid of heteronuclear multiple bond connectivity (HMBC) correlations (Fig. 1). The HMBC correlations between δ 5.24 (1H, d, J = 4.0 Hz, H-1″) and δ 135.5 (C-3) indicated that the glucopyranoside group was located at C-3. The HMBC correlations between δ_H 3.90 (6H, s, CH_3) and δ_C 148.7 (C-3′,5′) indicated that the two methoxyl groups were located at C-3′,5′. The HMBC correlations between 4.09 (1H, m, H-6″) and 172.5 indicated that the acetyl group was located at C-6″. On the basis of the above evidence, compound **2** was elucidated as 3′,5′-di-*O*-methylmyricetin-3-*O*-(6″-*O*-acetyl)-α-D-glucopyranoside.

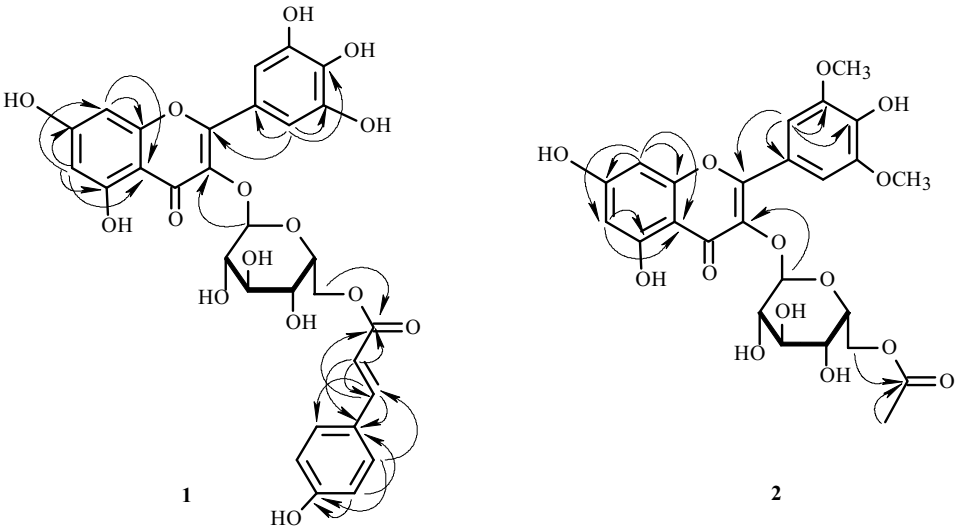

Fig. 1. Key HMBC correlations of **1**, **2**.

Furthermore, three known flavonoids, **3**, **4**, **5**, were identified as myricetin, cedrin, and 2*R*,3*R*-dihydromyricetin, respectively, by comparison of their spectroscopic data with those reported in the literature [5–7].

EXPERIMENTAL

General Experimental Procedures. NMR spectra were recorded on an INOVA-400 spectrometer (^{1}H: 400 MHz and ^{13}C: 100 MHz) with TMS as internal standard. UV spectra were obtained on a Shimadzu UV-160 spectrophotometer. HR-ESI-MS were recorded by an 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).

Plant Material. The pine needles of *Cedrus deodara* were collected from Lanzhou City of Gansu Province of China in June 2008. The plant sample was identified by Prof. Fu-jiang He at Gansu Academy of Medical Science.

Extraction and Isolation. The air-dried pine needles of *Cedrus deodara* (5.5 kg) were extracted with 95% ethanol (14 times volume) three times to afford an ethanol extract (700 g), which was suspended in water and extracted with petroleum ether, ethyl acetate, and *n*-butanol, separately. The ethyl acetate extract (105 g) was subjected to silica gel column and gradiently eluted with methylene chloride–methanol (36:1~0:100) to give 23 fractions. Fraction 12 (3.95 g) was then applied on a silica gel column and a Sephadex LH-20 column to give compounds **1** (9.8 mg), **2** (13.3 g), **3** (105 mg), **4** (10.8 mg), and **5** (50.8 mg).

Myricetin-3-*O*-(6″-*O*-*E*-*p*-coumaroyl)-α-D-glucocopyranoside (1): light yellow powder. UV (MeOH, λ_{max}, nm): 256, 359 (sh). IR (KBr, ν_{max}, cm^{-1}): 3423, 2921, 1706, 1653, 1604, 1514. (+) HR-ESI-MS data: *m/z* 649.1155 $[M + Na]^+$, 1276.2691 $[2M + Na + H]^+$. ^{1}H NMR (400 MHz, CD_3OD) and ^{13}C NMR (100 MHz, CD_3OD), see Table 1.

3′,5′-Di-*O*-methylmyricetin-3-*O*-(6″-*O*-acetyl)-α-D-glucopyranoside (2): light yellow powder. UV (MeOH, λ_{max}, nm): 255, 357 (sh). IR (KBr, ν_{max}, cm^{-1}): 3420, 2911, 1704, 1649, 1603, 1512. (+) HR-ESI-MS data: *m/z* 551.1409 $[M + H]^+$, 573.1210 $[M + Na]^+$, 1123.2837 $[2M + Na]^+$. ^{1}H NMR (400 MHz, CD_3OD) and ^{13}C NMR (100 MHz, CD_3OD), see Table 1.

Myricetin (3): light yellow powder.^{1}H NMR (400 MHz, CD_3COCD_3, δ, ppm, J/Hz): 12.16 (1H, s, 5-OH), 7.43 (2H, s, H-2′,6′), 6.51 (1H, d, J = 2.0, H-8), 6.26 (1H, d, J = 2.0 Hz, H-6). ^{13}C NMR (100 MHz, CD_3COCD_3, δ): 176.7 (C-4), 165.2 (C-7), 162.4 (C-5), 157.9 (C-9), 147.1 (C -2), 146.5 (C-3′, 5′), 137.0 (C-3), 136.5 (C-4′), 122.9 (C-1′), 108.4 (C-2′,6′), 103.5 (C-10), 99.3 (C-6), 94.6 (C-8).

Cedrin (4): light yellow powder. ^{1}H NMR (400 MHz, CD_3COCD_3, δ, ppm, J/Hz): 11.96 (1H, s, 5-OH), 6.64 (2H, s, H-2′, 6′), 1.97 (3H, s, 6-CH_3), 6.01 (1H, s, H-8), 4.92 (1H, d, J = 11.2, H-2), 4.55 (1H, d, J = 11.2, H-3). ^{13}C NMR (100 MHz, CD_3COCD_3, δ): 198.3 (C-4), 165.7 (C-7), 165.2 (C-5), 162.3 (C-9), 146.4 (C-3′, 5′), 134.3 (C-4′), 129.3 (C-1′), 107.8 (C-2′,6′), 101.7 (C-10), 95.5 (C-8), 84.8 (C-2), 73.5 (C-3), 7.2 (C-6).

2*R*,3*R*-Dihydromyricetin (5): light yellow powder. ^{1}H NMR (400 MHz, CD_3COCD_3, δ, ppm, J/Hz): 11.72 (1H, s, 5-OH), 6.63 (2H, s, H-2′, 6′), 5.96 (1H, d, J = 2.0, H-6), 6.00 (1H, d, J = 2.0, H-8), 4.94 (1H, d, J = 11.2, H-2), 4.57 (1H, d, J = 11.2, H-3). ^{13}C NMR (100 MHz, CD_3COCD_3, δ): 198.2 (C-4), 168.0 (C-7), 164.3 (C-5), 161.8 (C-9), 146.4 (C-3′, 5′), 134.3 (C-4′), 129.3 (C-1′), 107.8 (C-2′,6′), 101.5 (C-10), 97.2 (C-6), 96.2 (C-8), 84.8 (C-2), 73.4 (C-3).

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