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Chemical constituents of *Picea neoveitchii*

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ABSTRACT

Four flavonoids, 5,7,4′-trihydroxy-3,8,-dimethoxy-6-*C*-methylflavone (**1**), 5,8,4′-trihydroxy-3,7-dimethoxy-6-*C*-methylflavone (**2**), 7-methoxy-6-*C*-methylkaempferol (**3**) and kaempferol-7-*O*-(2″-*E*-*p*-coumaroyl)-α-L-arabinofuranoside (**4**), together with 15 known compounds, were isolated from the twigs and leaves of *Picea neoveitchii* Mast. Their structures were elucidated on the basis of analyses of spectroscopic data. Compound **4** showed strong anti-fungal activity against *Fusarium oxysporum* whereas compounds **1**–**4** were all active against *Rhizoctonia solani*.

1. Introduction

The genus *Picea* represents about 40 species of coniferous evergreen trees of the family *Pinaceae* that are distributed throughout the north temperate zone. There are 16 species and 9 varieties native to China (Delectis Florae Reipublicae Popularis Sinicae Agendae, 2000). Previous phytochemical studies on some plants of this genus have identified bioactive compounds like serratane triterpenes (Tanaka et al., 2001, 2002, 2003a,b, 2004), lignans (Kawamura et al., 1997), phenolic compounds, and alkaloids (Schneider et al., 1995; Slimestad et al., 1996; Tzong et al., 2006). *Picea neoveitchii* Mast. is a pine tree native to Gansu, Shanxi, and Sichuan provinces of China. Its pine cones have been used in traditional Chinese medicine for the relief of coughs and for reducing sputum. In an attempt to discover naturally occurring pesticides from diverse organisms, the EtOAc-soluble fraction of the methanol extract from the leaves and twigs of this plant was found to be insecticidal against larva of *Culex pipiens fatigans* and anti-fungal against *Rhizoctonia solani* and *Fusarium oxysporum*. This prompted us to investigate the bioactive constituents of this plant. The investigation led to isolation of four new flavonoids (**1–4**) (Fig. 1) and 15 known compounds (**5–19**). The present paper reports the isolation, structure characterization, and anti-fungal activities of the isolated compounds.

2. Results and discussion

Compound **1** was obtained as yellow powder. The ESI-MS gave quasi-molecular ion peaks at *m/z* 367 $[M+Na]^+$, 345 $[M+H]^+$, 343 $[M-H]^-$, consistent with the molecular formula $C_{18}H_{16}O_7$. This formula was confirmed by HRESIMS. The ^1H NMR spectrum (Table 1) indicated the presence of two aromatic methoxyl groups [δ_H 3.79 (3H, s, 3-OCH_3), 3.83 (3H, s, 8-OCH_3)], one *C*-methyl [δ_H 2.03 (3H, s, 6-CH_3)], and three hydroxyl groups [δ_H 12.65, 10.30, 10.27 (each 1H, s)]. The spectrum also exhibited two doublets at δ_H 7.96 (2H, d, J = 8.4 Hz, H-2′,6′) and 6.98 (2H, d, J = 8.4 Hz, H-3′,5′), typical of an AA′BB′ coupling system and indicating the presence of a *p*-disubstituted benzene ring. Analysis of the ^{13}C NMR spectrum (Table 1) indicated presence of a conjugated ketone carbonyl signal (δ_C, 178.2) characteristic of a flavone. These findings suggested compound **1** was a flavone with three hydroxyls, two methoxyls, and a *C*-methyl group. The ^1H NMR and ^{13}C NMR spectroscopic data of **1** were found to be similar to those of 5,7-dihydroxy-3,8,4′-trimethoxy-6-*C*-methylflavone (Wollenweber et al., 2000) except that the resonances for H-3′ (5′) and C-4′ were shifted upfield by 0.17 and 1.5 ppm, respectively, due to the presence of a hydroxyl group at C-4′ instead of a methoxyl group. This was confirmed by the HMBC correlations of C-4′ with H-2′ (6′) (δ_H 7.96), H-3′ (5′), and 4′-OH (δ_H 10.30). A downfield shifted signal at δ_H 12.65 indicated the existence of a 5-OH group, which was supported by long range correlations of the 5-OH hydrogen with C-6 (δ_C, 106.9), C-5 (δ_C, 157.9), and C-10 (δ_C, 103.7) (Fig. 2). In addition, HMBC correlations of C-6 (δ_C, 106.9) with 6-methyl hydrogens, 5-OH and 7-OH (δ_H 10.27) suggested the *C*-methyl was located at C-6 and that the hydroxyl was located at C-7 (δ_C 153.3). The methoxyl groups at C-8 (δ_C 126.8) and C-3 (δ_C

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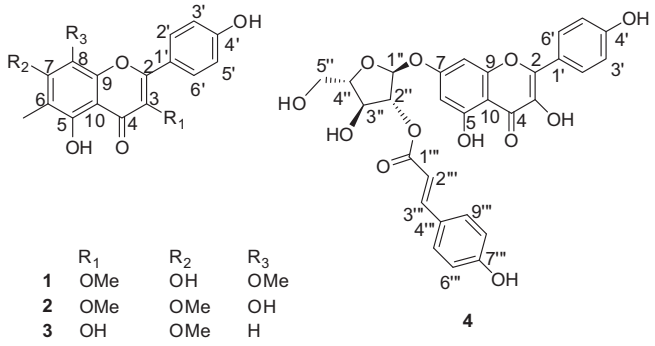

Fig. 1. Structures of compounds **1-4.**

Table 1
^{1}H and ^{13}C NMR spectroscopic data of compounds **1–3** (δ in ppm, J in Hz).

Position	**1**		**2**		**3**	
	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
2	155.2		155.3		146.7	
3	137.7		137.4		136.5	
4	178.2		178.4		176.7	
5	157.9		155.1		157.9	
6	106.9		112.3		112.4	
7	153.3		159.4		161.4	
8	126.8		137.7		93.9	6.14, s
9	154.7		146.1		153.9	
10	103.7		106.6		103.6	
1′	120.5		120.6		120.5	
2′,6′	129.9	7.96, d (8.4)	129.8	8.07, d (8.4)	130.0	7.93, d (8.4)
3′,5′	115.8	6.98, d (8.4)	115.5	6.96, d (8.4)	115.5	6.94, d (8.4)
4′	160.0		160.0		160.0	
3-OMe	59.6	3.79, s	59.6	3.79, s		
6-Me	7.6	2.03, s	7.9	2.08, s	7.8	2.09, s
7-OMe			60.3	3.86, s	59.6	3.87, s
8-OMe	61.4					
5-OH		12.65, s		12.30, s		12.93, s
4′-OH		10.30, s		10.29, s		10.34, s
3-OH						10.03, s
7-OH		10.27, s				
8-OH				10.85, s		

^{1}H (600 MHz) and ^{13}C (150 MHz) NMR in DMSO-d_6.

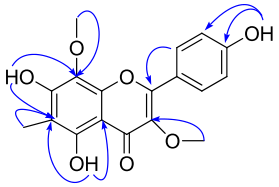

Fig. 2. Key HMBC correlations of compound **1.**

137.7) were deduced from the cross-peaks of 8-OCH_3 with the C-8 and 3-OCH_3 (δ_H 3.79) with the C-3 in the HMBC spectrum. Compound **1** was therefore identified as 5,7,4′-trihydroxy-3,8-dimethoxy-6-*C*-methylflavone.

Compound **2** was also obtained as a yellow powder. The HRESIMS gave a molecular formula of $C_{18}H_{16}O_7$, in accordance with quasi-molecular ion peaks in ESI-MS spectrum at *m/z* 367 $[M+Na]^+$, 345 $[M+H]^+$, and 343 $[M-H]^-$. The ^{1}H and ^{13}C NMR spectra of compound **2** were similar to those of **1** (Table 1) except that the carbon signals of C-7 (δ_C 159.4) and C-8 (δ_C 137.3) were shifted downfield by 6.1 and 10.7 ppm, respectively, indicating that the methoxyl group at C-8 in **1** was at C-7 in **2**. Its structure was confirmed by analysis of the HMBC spectrum (Fig. 3), in which

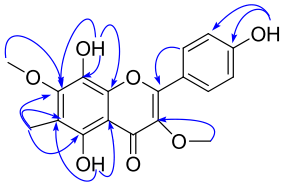

Fig. 3. Key HMBC correlations of compound **2.**

correlations were observed from C-7 to 7-OCH_3 [δ_H 3.79 (3H, s)] and 6-CH_3 [δ_H 2.08 (3H, s)], and from C-5 to 6-CH_3. Thus, compound **2** was determined to be 4′,5,8-trihydroxy-3,7-dimethoxy-6-*C*-methylflavone.

Compound **3** was obtained as a yellow powder as well. Its ESI-MS gave quasi-molecular ion peaks at 337 $[M+Na]^+$, 315 $[M+H]^+$, and 313 $[M-H]^-$, consistent with a molecular formula of $C_{17}H_{14}O_6$. This was confirmed by HRESIMS. The ^{1}H NMR spectrum (Table 1) exhibited signals for a methoxyl [δ_H 3.87 (3H, s, 7-OCH_3)], a methyl [δ_H 2.09 (3H, s, 6-CH_3)], three hydroxyl groups [δ_H 12.93, 10.34, 10.03 (each 1H, s)], and a *p*-substituted benzene ring [δ_H 7.93 (2H, d, J = 8.4 Hz, H-2′,6′), 6.94 (2H, d, J = 8.4 Hz, H-3′,5′)]. These data suggested that compound **3** was also a 6-*C*-methylflavonol with a structure closely related to compound **2**. However, in the ^{1}H NMR spectrum, an additional aromatic hydrogen singlet at δ_H 6.14 (1H, s, H-8) was observed, which was correlated to C-7 (δ_C 161.4), C-8 (δ_C 93.9), and C-9 (δ_C 153.9) in the HMBC spectrum, indicating that C-8 was unsubstituted. The only methoxyl group was located at C-7, as deduced by analysis of the HMBC correlations (Fig. 4) of C-7 with 7-OCH_3 and 6-CH_3. The 3-OH group was assigned based on the chemical shifts of C-2 (δ_C 146.7) and C-3 (δ_C 136.5). Thus, compound **3** was elucidated as 7-*O*-methyl-6-*C*-methylkaempferol.

Compound **4**, obtained as a yellow solid, had the molecular formula $C_{29}H_{24}O_{12}$ as determined from analyses of HRESIMS, ^{13}C NMR, and DEPT spectroscopic data. The ^{1}H and ^{13}C NMR spectra showed resonance characteristics of a flavonoid (Iinuma et al., 1980; Yang et al., 2010) with a pentose moiety and a cinnamoyl fragment. In the ^{1}H NMR spectrum, signals at δ_H 8.02 (2H, d, J = 8.4 Hz, H-2′,6′), 6.80 (2H, d, J = 8.4 Hz, H-3′,5′), 6.52 (1H, d, J = 1.8 Hz, H-6), δ_H 6.86 (1H, d, J = 1.8 Hz, H-8), and 12.93 (1H, s) indicated that the flavone moiety of **4** was kaempferol (Monika and Maria, 2001). The hydrogen signals at δ_H 6.91 (2H, d, J = 8.4 Hz, H-6‴,8‴), 7.59 (2H, d, J = 8.4 Hz, H-5‴,9‴), 7.63 (1H, d, J = 15.6 Hz), and 6.46 (1H, d, J = 15.6 Hz), along with a α,β-unsaturated carbonyl carbon signal at δ_C 165.4 (C-1‴) in the ^{13}C NMR spectrum, suggested the existence of a cinnamoyl fragment. The presence of an L-arabinofuranose moiety was indicated by the ^{13}C NMR signals at δ_C 105.3 (C-1‴), 85.9 (C-4″), 83.7 (C-2″), 74.8 (C-3″), and 60.1 (C-5″). In addition, acidic hydrolysis yielded an L-arabinose that was confirmed by co-TLC with an authentic sample. The α-anomeric configuration for the arabinose was determined based on the broad singlet of the anomeric hydrogen at δ_H 5.80 (1H, brs, H-1″) in the ^{1}H NMR spectrum (Monika and Maria, 2001). The HMBC correlations of H-1″ with C-7 and H-2″ with C-1‴ (Fig. 5) indicated that the arabinose moiety is attached to C-7 through a glycosidic bond and that the *E*-*p*-hydroxycinnamic

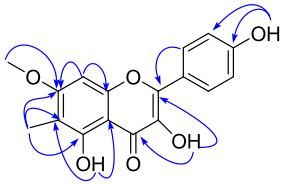

Fig. 4. Key HMBC correlations of compound **3.**