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A BIFLAVONOID FROM STEMS AND LEAVES OF *Lonicera macranthoides*

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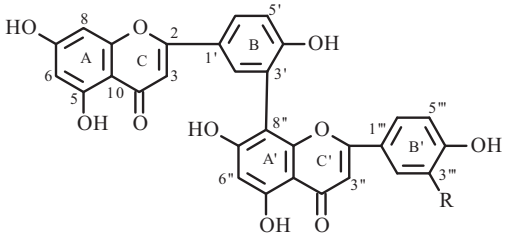
*A new biflavonoid, 3‴-*O*-methylamentoflavone (**1**), as well as a known biflavonoid, amentoflavone (**2**), was isolated from the stems and leaves of* Lonicera macranthoides *Hand.-Mazz. Their structures were established on the basis of 1D, 2D NMR (HSQC and HMBC), and ESI-TOF-MS spectroscopic methods and chemical evidence.*

Keywords: *Lonicera macranthoides*, stems and leaves, 3‴-*O*-methylamentoflavone.

Lonicera macranthoides Hand.-Mazz., a plant of the genus *Lonicera* of the Caprifoliaceae family, is commonly used in traditional Chinese medicine in the southwest of China. Its flower buds have been listed in the PRC Pharmacopoeia since the 2005 edition as a newly added species, which forms the item *Shanyinhua*, together with *L. hypoglauca* Miq. and *L. confuse* DC. Although the chemical constituents of the flower buds of *L. macranthoides* Hand.-Mazz. have been well documented [1–3], there has been no report on the chemical composition from its stems and leaves. We, therefore, report here the structure elucidation of two biflavonoids from the stems and leaves of this plant.

Compound **1** was obtained as a yellow amorphous powder. HR-ESI-MS exhibited $[M + H]^+$ of **1** at *m/z* 569.1145 (calcd for $C_{31}H_{21}O_{11}$, 569.1077). The ^1H NMR and ^{13}C NMR (Table 1) spectra of **1** revealed the presence of 31 carbon signals, among which 11 were methines and 19 were quaternary carbons, including two carbonyls (δ 181.4 and 181.3). The ^1H NMR spectrum displayed signals for one methoxy and 11 aromatic protons. Moreover, the ^1H NMR spectra showed two singlets at δ 13.05 and 13.20, which are characteristic of hydrogen-bonded hydroxyl groups (5-OH and 5″-OH). From the analysis of the ^1H–^1H COSY and HMBC spectra of **1**, it was deduced that the aromatic protons were distributed among one 1,3,4-trisubstituted ring (B ring) at δ_H 8.24 (1H, s, H-2′), 6.86 (1H, d, J = 8.45 Hz, H-5′), and 7.83 (1H, s, J = 8.45 Hz, H-6′), another 1,3,4-trisubstituted ring (B′ ring) at 7.28 (1H, s, H- 2‴), 6.54 (1H, d, J = 8.25 Hz, H-5‴), and 7.35 (1H, d, J = 8.25 Hz, H-6‴), one 5,7,9,10-tetrasubstituted ring (A ring) at 6.13 (1H, d, J = 2.1 Hz, H-6) and 6.21 (1H, d, J = 2.1 Hz, H-8), and one pentasubstituted ring (A′ ring) at 5.97 (1H, s, H-6″), indicating that **1** was a biflavonoid.



1, 2

1: R = OCH_3

2: R = H

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TABLE 1. ^{1}H and ^{13}C NMR for **1** and **2** (500 and 125 MHz, DMSO-d_6, δ, ppm, J/Hz)

C atom	**1**			**2**	
	δ_C	δ_H	HMBC	δ_C	δ_H
2	164.6		H-3	164.0	
3	101.6	6.69 s		102.5	6.80 s
4	181.3		H-3	181.6	
5	161.3		H-6	161.3	
6	98.7	6.13 (d, J = 2.1)	H-8	98.7	6.18 (d, J = 2.1)
7	163.9		H-8	163.9	
8	93.7	6.21 (d, J = 2.1)	H-6	93.9	6.41 (d, J = 2.1)
9	157.2		H-8	157.3	
10	103.4		H-3,6	103.5	
1′	117.3		H-5′	119.8	
2′	131.2	8.24 s	H-6′	131.3	8.08 s
3′	123.9		H-2′, 5′	121.1	
4′	165.3		H-2′, 6′	161.1	
5′	119.6	6.86 (d, J = 8.45)		117.1	7.07 (d, J = 8.60)
6′	126.0	7.83 (d, J = 8.45)	H-2′	127.3	7.96 (d, J = 8.60)
2″	162.3		H-3, 2‴, 6‴	163.3	
3″	102.4	6.78 s		102.4	6.74 s
4″	181.4			181.8	
5″	160.3		H-6″	160.4	
6″	101.8	5.97 s		99.6	6.28 s
7″	160.3		H-6″	161.3	
8″	107.2		H-6″	104.9	
9″	154.6			154.5	
10″	101.4		H-3″, H-6″	102.9	
1‴	121.9		H-3″, H-5″	121.4	
2‴	110.0	7.28 s	H-6‴	128.4	7.61 (d, J = 8.60)
3‴	147.7		H-2‴, 5‴, 3‴-OCH_3	115.5	6.67 (d, J = 8.60)
4‴	150.1		H-2‴	160.8	
5‴	115.3	6.54 (d, J = 8.25)		115.5	6.67 (d, J = 8.60)
6‴	120.0	7.35 (d, J = 8.25)	H-2‴	128.4	7.61 (d, J = 8.60)
OCH_3	55.5	3.58 s			
5-OH		13.05 s			13.00 s
5″-OH		13.20 s			13.13 s

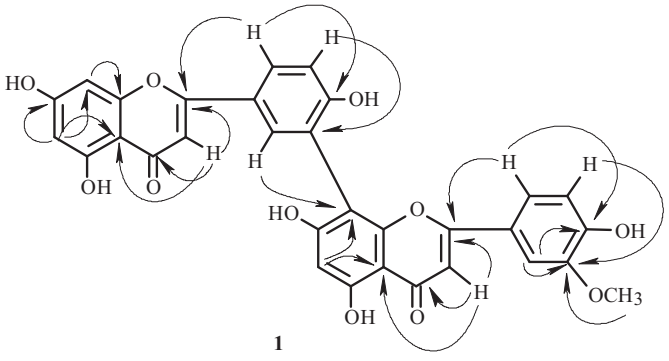

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Fig. 1. HMBC correlations of compound **1**.

Detailed analysis of the ^{1}H NMR data allowed us to deduce that **1** belongs to the amentoflavone series of bioflavonoids linked between C-3′ and C-8″ [4], which was confirmed by ^{13}C NMR and HMBC (Fig. 1) showing the long-range correlations between H-5′ and C-3′ (δ_C 123.9) and between H-2′, H-6″ and C-8″ (δ_C 107.2).

The ^1H and ^{13}C NMR data (Table 1) for compound **1** was very similar to **2**. The main difference between the spectroscopic data of **1** and **2** is due to the substituents on the B′ rings. Studies of its ^1H NMR and HMBC spectra suggested a methoxy at C-3‴ in **1**. The attachment of methoxy was determined from the cross-peaks between H-2‴, H-5‴, and methoxy protons and C-3‴ (δ_C 147.7) in the HMBC spectrum, which was verified by comparing the quasi-molecular ions of **1** (*m/z* 569.1145 $[M + 1]^+$) and **2** (*m/z* 539.0707 $[M + 1]^+$). On the basis of the above results and comparison of the reported data in the literature [5, 6], compound **1** was therefore determined as 3‴-*O*-methylamentoflavone.

Amentoflavone (**2**), obtained also as a yellow amorphous powder, was identified by comparing its spectroscopic data with those reported in [7].

EXPERIMENTAL

General. Column chromatography (CC): silica gel (200–300 mesh) was from Qingdao Marine Chemical Plant, Qingdao, P. R. China; Sephadex LH-20 and ODS was purchased from Amersham Biosciences Inc., USA and YMC Co. Ltd., Japan, respectively.

^1H NMR and ^{13}C NMR, HSQC, and HMBC spectra: Bruker spectrometers operating at 500 MHz; ESI-MS: Agilent 1100 LC/MSD SL; JASCO P-1020 optical rotation apparatus.

Plant Material. The stems of *Lonicera macranthoides* Hand.-Mazz., collected from Longhui, Hunan Province of China in October 2008, were taxonomically identified by Prof. Chang-Qi Yuan. A voucher specimen was deposited in the Nanjing Botanical Garden Mem. Sun Yat-Sen, Nanjing, Jiangsu, China.

Extraction and Isolation. The dried materials (15 kg) were extracted twice with 75% ethanol at room temperature for 30 days and filtered. After removal of the ethanol, the water suspension of the residue was successively extracted with petroleum ether, EtOAc, and *n*-BuOH. The EtOAc extract was subjected to silica gel column chromatography using a gradient of $CHCl_3$–MeOH–H_2O (20:1:0.1→9:1:0.1→ 6:1:0.1→ 3:1:0.1→7:3:0.1) to give seven fractions (I–VII). Fraction II was repeatedly chromatographed on silica gel columns and further purified by RP-C18 (YMC; 12 nm) and Sephadex LH-20 to give compounds **1** (15 mg) and **2** (12 mg).

Compound 1. $C_{31}H_{20}O_{11}$, ESI (+)-MS *m/z* 569 $[M + 1]^+$. For ^1H NMR and ^{13}C NMR (500 and 125 MHz, DMSO-d_6), see Table 1.

Compound 2. $C_{30}H_{18}O_{10}$, ESI (+)-MS *m/z* 539 $[M + 1]^+$. For ^1H NMR and ^{13}C NMR, see Table 1.

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