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Biphenyls from the Twigs of *Garcinia multiflora* and their Anti-Tobacco Mosaic Virus Activities

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Abstract: For more bioactive compounds, phytochemical investigations of the acetone extract of the twigs *Garcinia multiflora* resulted in the isolation of two new biphenyls, multiflorabiphenyls A and B (**1** and **2**), along with four known biphenyl derivatives (**3**-**6**). Structural elucidations of **1** and **2** were performed by spectral methods such as 1D and 2D NMR spectroscopy, in addition to high resolution mass spectrometry. Compounds **1** and **2** were also evaluated for their anti-tobacco mosaic virus (Anti-TMV) activity. The results showed that compounds **1** and **2** showed high anti-TMV activities with inhibition rates of 25.4% and 28.3%, respectively, which is closed to that of Ningnanmycin (33.5%).

Keywords: Biphenyl; anti-tobacco mosaic virus; *Garcinia multiflora*.

1. Introduction

The genus of Garcinia is medicinally important. Many plants of this genus are commonly used in Traditional Chinese Medicine for their diverse beneficial bioactivities. Previous phytochemical investigations of plants belonging to the genus Garcinia have revealed that it is a rich source of xanthones and benzophenones, in which some have shown antibacterial[1,2], antifungal[3], anti-inflammatory[4], antioxidant[5], apoptosis-inducing[6-11], and cytotoxic effects[12-15].

Garcinia multiflora, which belongs to Garcinia genus, is a dioecious evergreen tree and grows to a height between 3-10 m in southern China. It is used in furniture manufacture and as a dye. Previous phytochemical studies of Garcinia multiflora revealed the presence of xanthones[16], benzophenone derivatives[17,18], and biflavonoids[19,20] as the main components and exhibits a variety of bioactivities, such as anti-inflammatory[17], anti-HIV[20], antioxidant[16,21], and antituberculosis activities[19]. Motivated by a search for new bioactive metabolites from local plants, our group investigated the chemical constituents of the twigs of *Garcinia multiflora* growing in

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Honghe Prefecture, which led to the isolation and characterization of two new (**1** and **2**) and four known (**3**-**6**)biphenyls. This paper deals with the isolation, structural characterization of these compounds, and their anti-tobacco mosaic virus (Anti-TMV) activity.

2. Materials and Methods

2.1. General Experimental Procedures

UV spectra were obtained using a Shimadzu UV-2401A spectrophotometer. A Tenor 27 spectrophotometer was used for scanning IR spectroscopy with KBr pellets. 1D and 2D NMR spectra were recorded on a DRX-500 NMR spectrometer with TMS as internal standard. Chemical shifts (δ) are expressed in ppm with reference to the solvent signals. HRESIMS was performed on a VG Autospec-3000 spectrometer. Semipreparative HPLC was performed on a Shimadzu LC-8A preparative liquid chromatograph with Zorbax PrepHT GF (21.2 mm × 25 cm) or Venusil MP C_{18} (20 mm × 25 cm) columns. Column chromatography (CC) was performed using silica gel (200–300 mesh, Qing-dao Marine Chemical, Inc., Qingdao, People's Republic of China), Lichroprep RP-18 gel (40–63 μm, Merck, Darmstadt, Germany), and MCI gel (75–150 μm, Mitsubishi Chemical Corporation, Tokyo, Japan). Fractions were monitored by TLC, and spots were visualized by heating silica gel plates sprayed with 5% H_2SO_4 in EtOH.

2.2. Plant material

The plant of Garcinia multiflora was collected in Honghe Prefecture, Yunnan Province, People's Republic of China, in September 2013. The identification of the plant material was verified by Dr. Huang Jian-Ping. A voucher specimen (YNNU 2013-09-27) has been deposited in our laboratory.

2.3. Extraction and Isolation

The air-dried and powdered twigs of G. multiflora (2.2 kg) were extracted four times with 70% acetone (4 × 5 L) at room temperature and filtered. The filtrate was concentrated and successively partitioned with CH_2Cl_2 and EtOAc. The EtOAc fraction (35 g) was submitted to silica gel (200–300 mesh) column chromatography, eluting with a $CHCl_3$-$(CH_3)_2CO$ gradient system (20:1, 9:1, 4:1, 7:3, 3:2, 1:1), to give six fractions A–F. The further separation of fraction B (9:1, 6.40 g) by silica gel column chromatography, eluted with petroleum ether-EtOAc (9:1, 4:1, 7:3, 3:2, 1:1), yielded mixtures C1–C5. Fraction C2 (4:1, 640 mg) was subjected to preparative HPLC (60% MeOH, flow rate 12 mL/min) to give **1** (6.25 mg), **4** (7.32 mg), and **6** (10.8 mg). The further separation of fraction C3 (7:3, 1.7 g) by silica gel column chromatography, and preparative HPLC (55% MeOH, flow rate 12 mL/min) to give **2** (8.25 mg), **3** (7.22 mg), and **5** (6.95 mg).

Multiflorabiphenyl A (1), pale yellow gum; UV (MeOH) λ_{max} (log ε): 210 (4.18), 240 (3.68), 272 (3. 43) nm; IR (KBr) ν_{max} 3440, 2935, 2873 1610, 1544, 1476, 1415, 1362, 1248, 1158, 1036, 961, 874 cm^{-1}; ^1H and ^{13}C NMR data (CD_3OD, 500 and 125 MHz), see Table 1. Positive ESIMS *m/z* 337 $[M+Na]^+$; Positive HRESIMS *m/z* 337.1410 $[M+Na]^+$ (calcd for $C_{19}H_{22}NaO_4$, 337.1416).

Multiflorabiphenyl B (2), pale yellow gum; UV (MeOH) λ_{max} (log ε) 210 (4.12), 345 (3.62), 278 (3.54) nm; IR (KBr): ν_{max} 3436, 2936, 2820, 1685, 1604, 1537, 1468, 1357, 1253, 1164, 1040, 957, 869 cm^{-1}; ^1H and ^{13}C NMR data (CD_3OD, 500 and 125 MHz), see Table 1; ESIMS *m/z* 337; HRESIMS *m/z* 337.1058 $[M+Na]^+$ (calcd for $C_{18}H_{18}NaO_5$, 337.1052).

3. Results and Discussion

3.1. Structure elucidation

The twigs of Garcinia multiflora were extracted with 70% aqueous $(CH_3)_2CO$. The extract was subjected repeatedly to column chromatography on Silica gel, Sephadex LH-20, RP-18 and Preparative HPLC to afford compounds **1**-**6**, including two new biphenyl derivatives,

multiflorabiphenyls A and B (**1** and **2**), together with four known biphenyl derivatives, bractebiphenyl B (**3**) [22], doitungbiphenyl B (**4**) [23], schomburgbiphenyl (**5**) [24], and tababiphenyl C (**6**) [25]. The structures of the compounds **1-6** were shown in Figure. 1, and the ^{1}H and ^{13}C NMR data of 1 and 2 were listed in Table. 1.

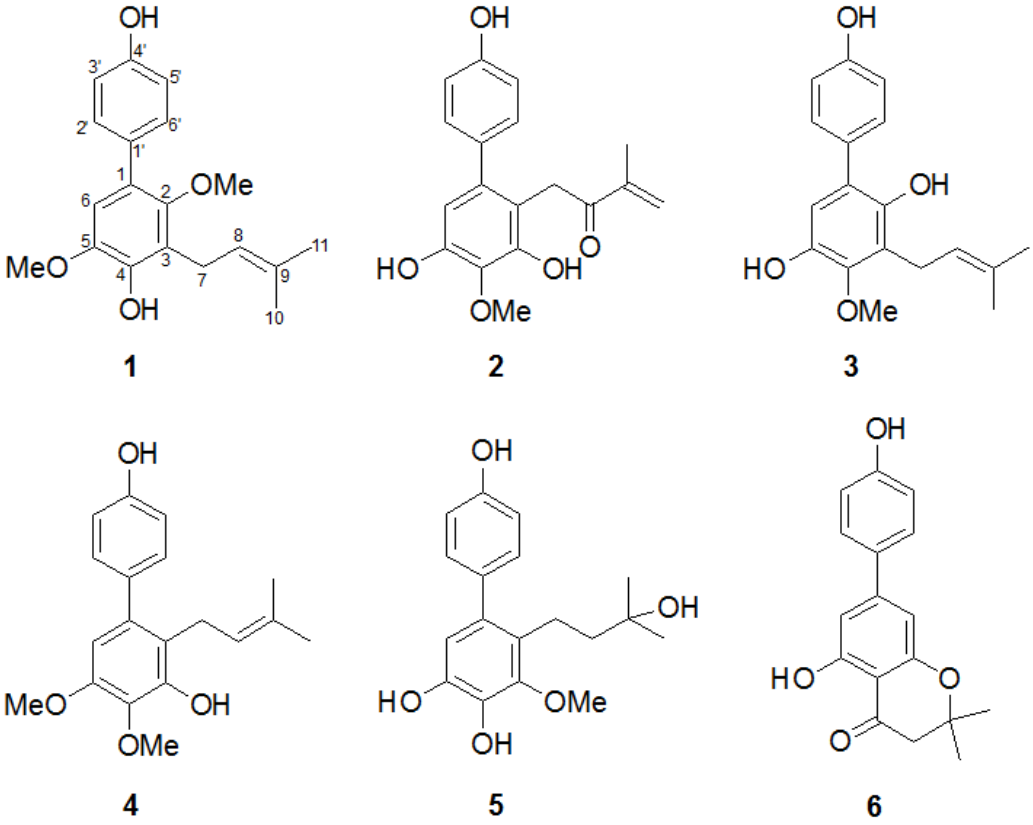

Figure 1. The structures of biphenyls from *Garcinia multiflora*.

Multiflorabiphenyl A (**1**) was obtained as a pale yellow gum, and exhibited an ion peak at *m/z* 337.1410 $[M+Na]^{+}$ in the HRESIMS spectrum, corresponding to the molecular formula $C_{19}H_{22}O_4$, which indicated 9 degrees of unsaturation. The inspection of its ^{1}H and ^{13}C NMR data (Table 1) suggested the presence of four methyls (two oxygenated), one methylenes, six olefinic methines, and eight olefinic quaternary carbons. The UV absorption bands at λmax 210, 240 and 272 nm indicated the presence of benzene chromophore. The absorption bands in its IR spectrum suggested the presence of hydroxy groups (3440 cm^{-1}) and aromatic group (1610, 1544, 1476 cm^{-1}). ^{1}H NMR and ^{13}C NMR spectra of **1** (Table 1) display a 1,2,3,4,5-pentasubstituted aromatic ring (δ_C 131.8 s, 147.9 s, 127.5 s, 146.9 s, 137.5 s, 113.4 d; δ_H 6.56 s), a 1,4-disubstituted aromatic ring (δ_C 132.9 s, 130.3 d (2C), 116.2 d (2C), 157.2 s; δ_H 7.23(d, J = 8.6 Hz), 6.87(d, J = 8.6 Hz)), a prenyl group (δ_C 27.4 t, 123.7 d, 132.5 s, 17.5 q, 25.6 q; δ_H 3.07(d, J = 6.8 Hz), 5.31(d, J = 6.8 Hz), 1.54 s, 1.73 s) [22], and two methoxy groups (δ_C 60.0 and 61.9 q, δ_H 3.81 and 3.85 s). The HMBC correlations of H-6 (δ_H 6.56 s) with C-1′ (δ_C 132.9 s), of H-2′,6′ (δ_H 7.23) with C-1 (δ_C 131.8) indicated **1** should be processed a biphenyl skeleton [22,24]. The prenyl group was placed on C-3 because the methylene protons H-7 (δ_H 3.07) showed HMBC correlations (Figure 2) with C-2 (δ_C C 147.9), C-3 (δ_C 127.5), and C-4 (δ_C 146.9); whereas, two methoxy groups were placed at C-2 and C-5, because of the methoxy protons (δ_H 3.81 and 3.85) showed HMBC correlations with C-2 (δ_C 147.9) and C-5 (δ_C 137.5), respectively. In addition, two phenolic groups should be located at C-4 and C-4′ to support the 1,2,3,4,5-pentasubstituted aromatic ring and 1,4-disubstituted aromatic ring in **1**. The structure of **1** was therefore assigned.

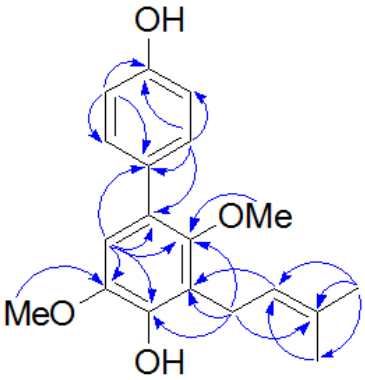

Figure 2. Selected HMBC (↷) correlations of compound **1**.

Compound **2** was also obtained as a pale yellow gum, and assigned the molecular formula $C_{18}H_{18}O_5$ by HRESIMS at *m/z* 337.1058 $[M+Na]^+$, which indicated 10 degrees of unsaturation. The ^1H NMR and ^{13}C NMR spectra of **2** (Table 1) display a 1,2,3,4,5-pentasubstituted aromatic ring, a 1,4-disubstituted aromatic ring, a 2-oxo-3-methylbut-3-enyl group (C-7 ~ C-11, H-7, H-10, and H-11) [26], and one methoxy group. The HMBC correlations of H-6 with C-1′, of H-2′,6′ with C-1 indicated **2** should be processed a biphenyl skeleton [22,24]. The HMBC correlations of H-7 with C-1, C-2, and C-3 indicated the 2-oxo-3-methylbut-3-enyl group located at C-2; the methoxy group located at C-4 was supported by the HMBC correlations of methoxy protons with C-4. In addition, three phenolic groups should be located at C-3, C-5 and C-4′ to support the 1,2,3,4,5-pentasubstituted aromatic ring and 1,4-disubstituted aromatic ring in **2**. The structure of multiflorabiphenyl B (**2**) is therefore determined.

Table 1. ^1H and ^{13}C NMR data for compounds **1 and 2** (500 and 125 MHz, in CD_3OD).

No.	**1**		**2**	
	δ_C	δ_H (m, *J*, Hz)	δ_C	δ_H (m, *J*, Hz)
1	131.8 s		132.2 s	
2	147.9 s		122.3 s	
3	127.5 s		147.9 s	
4	146.9 s		138.3 s	
5	137.5 s		145.2 s	
6	113.4 d	6.56 s	114.9 d	6.58 s
7	27.4 t	3.07 d (6.8)	36.9 t	4.58 s
8	123.7 d	5.31 t (6.98)	200.9 s	
9	132.5 s		144.2 s	
10	17.5 q	1.54 s	123.9 t	5.85, 6.17 s
11	25.6 q	1.73 s	18.8 q	2.03 s
1′	132.9 s		133.9 s	
2′,6′	130.3 d	7.23 d (8.6)	131.7 d	7.24 d (8.6)
3′,5′	116.2 d	6.87 d (8.6)	116.2 d	6.86 d (8.6)
4′	157.2 s		156.9 s	
2-OMe	61.0 q	3.81 s		
4-OMe	61.9 q	3.85 s	61.0 q	3.83 s

Compounds **1** and **2** were tested for their anti-TMV activities. The anti-TMV activities were tested using the half-leaf method [27]. Ningnanmycin (with inhibition rates of 33.5%, a commercial product for plant disease in China) was used as a positive control. On the basis of the results, compound **1** and **2** showed high anti-TMV activities with inhibition rates of 25.4% and 28.3%, respectively, which is closed to that of Ningnanmycin (33.5%).

3.2. Anti-TMV Assay

The Anti-TMV activities were tested according to literature [28]. In Anti-TMV activity test, the antiviral inhibition rates of the compounds at the concentration of 20 μM were tested by the half-leaf method.

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Supporting Information

Supporting Information accompanies this paper on http://www.acgpubs.org/RNP

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