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ISOPRENYLATED FLAVONES FROM *GARCINIA BRACTEATA* AND THEIR ANTI-TOBACCO MOSAIC VIRUS ACTIVITY

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Abstract – Two new isoprenylated flavones, bracteflavones A and B (**1** and **2**), together with four known isoprenylated flavones (**3-6**) were isolated from the twigs of *Garcinia bracteata*. Their structures were elucidated by spectroscopic methods, including extensive 1D- and 2D- NMR techniques. The anti-tobacco mosaic virus (anti-TMV) activities of compounds **1-6** were evaluated, and the results revealed that **1-6** showed potential anti-TMV activity with inhibition rates in the ranges of 17.6-24.3%, respectively.

The genus *Garcinia* (Clusiaceae) is commonly distributed in tropical and subtropical countries of South East Asia, West and East Africa, and Central and South America. This genus is known to produce xanthenes and benzophenones,¹⁻⁵ and many of these compounds show interesting biological activities including anti-microbial, anti-HIV and anti-oxidant activities.

The *G. bracteata*, a plant of genus *Garcinia*, is distributed in the south of Yunnan and Guangxi Province of China.⁶ In the published literatures, some xanthenes and benzophenones were isolated from this plant,⁷⁻¹¹ and these compounds exhibited various activities. Motivated by a search for new bioactive metabolites from genus *Garcinia*, the chemical constituents of the twigs of *G. bracteata* were reinvestigated by our group. As a result, two new (**1** and **2**) and four known (**3-6**) isoprenylated flavones were isolated from this plants. This paper deals with the isolation and structural characterization of these compounds, and their anti-tobacco mosaic virus (anti-TMV) activity.

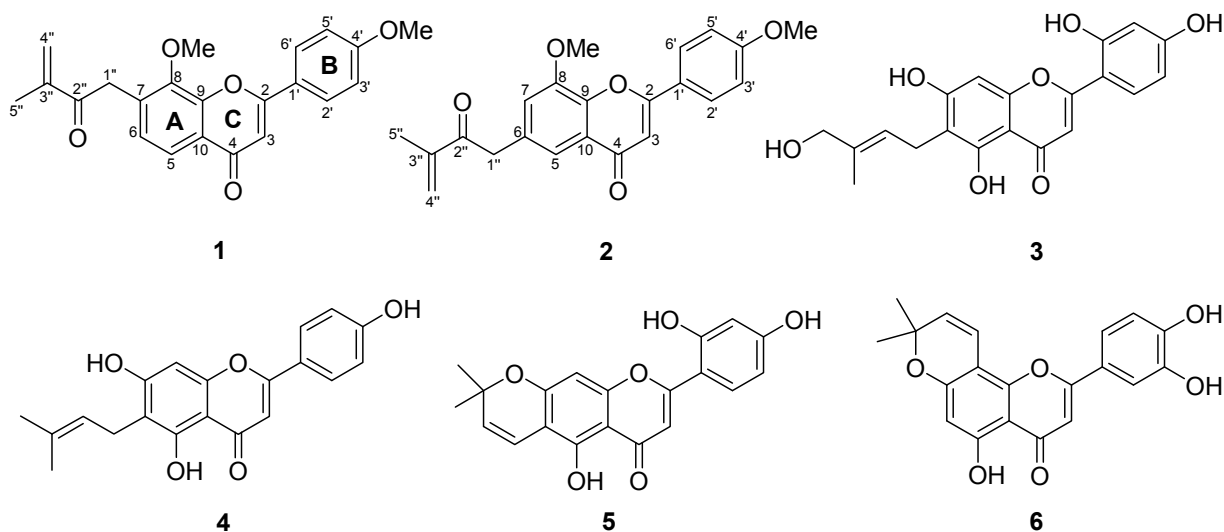


Figure 1. The structures of isoprenylated flavones from *Garcinia bracteata*

The twigs of *G. bracteata* were extracted with 70% aqueous acetone. 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 isoprenylated flavones, bracteflavones A and B (**1** and **2**), together with four known isoprenylated flavones, artocarmin D (**3**),¹² 6-prenylapigenin (**4**),¹³ cycloartocarpesin (**5**),¹⁴ and artochamin C (**6**).¹⁵ The structures of the compounds **1-6** were shown in Figure 1, and the ¹H and ¹³C NMR data of **1** and **2** were listed in Table 1.

Compound **1** was obtained as a pale yellow gum. It gave an [M+Na]⁺ ion peak at *m/z* 387 in the ESIMS, and was shown to possess the molecular formula C₂₂H₂₀O₅ by HRESIMS (*m/z* 387.1202 [M+Na]⁺).

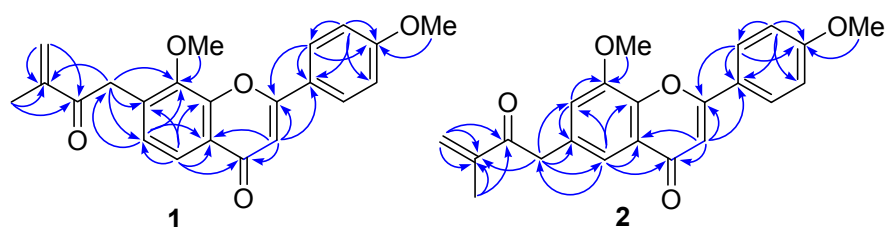


Figure 2. The key HMBC (↷) correlations of **1** and **2**

The UV absorptions at 362, 257, and 210 nm showed an extended chromophore and a substituted benzene ring. Its IR spectral data showed the presence of carbonyl groups (1680 and 1658 cm⁻¹) and phenyl groups (1602, 1561, and 1474 cm⁻¹). The ¹H and ¹³C NMR spectrum of **1** showed 22 carbons and 20 protons, corresponding to a 1,4-disubstituted benzene ring [δ_c 123.0 s, 130.8 d (2C), 115.8 d (2C), and 161.1 s; δ_H 7.73 (d) *J* = 8.8 (2H) and 6.80 (d) *J* = 8.8 (2H)], a 1,2,3,4-tetrasubstituted benzene ring [δ_c 124.1 d, 127.9 d, 133.6 s, 155.2 s, 150.7 s, and 122.1 s; δ_H 7.43 (d) *J* = 8.2 and 6.71 (d) *J* = 8.2], one 2-oxo-3-methylbut-3-enyl group (δ_c 37.9 t, 200.7 s, 144.8 s, 123.4 t, and 18.7 q; δ_H 4.63 s, 5.85, 6.11 s, and 1.90 s),¹⁶ two methoxy groups (δ_c 61.4 q and 56.0 q; δ_H 3.80 s and 3.84 s, 3H each), a pair of olefin proton signal (δ_c 163.6 s and 105.3 d; δ_H 6.42 s, 1H), and a carbonyl carbon (δ_c 181.5). These spectral data indicated that compound **1** should be a flavone derivative bearing a 2-oxo-3-methylbut-3-enyl group and two methoxy groups.¹⁷ The HMBC correlations (Figure 2) of one

methoxy proton signals (δ_{H} 3.80) with C-4' (δ_{C} 162.0) suggested the position of this methoxy group at C-4', this was also supported by the fact of the typical protons signals at [δ_{C} 7.73 (d) $J = 8.8$ (2H) and 6.80 (d) $J = 8.8$ (2H)]. Since the substituents on ring B were evident, the surplus substituents (one 2-oxo-3-methylbut-3-enyl group and one methoxy) should be located at ring A. The HMBC correlations of H-1'' (δ_{C} 4.63) with C-6 (δ_{C} 127.9), C-7 (δ_{C} 133.6), and C-8 (δ_{C} 155.2), and of H-6 (δ_{H} 6.71) with C-1'' (δ_{C} 37.9), suggested the 2-oxo-3-methylbut-3-enyl group should be located at C-7. The other methoxy group located at C-8 was supported by the HMBC correlation of the methoxy proton signal (δ_{H} 3.84) with C-8 (δ_{C} 155.2). Accordingly, the structure of **1** was established, and gives the trivial name of bracteflavones A.

Bracteflavones B (**2**) was also obtained as yellow gum. It HRESIMS at m/z 387.1215 $[M+Na]^+$ revealed that compounds **1** and **2** had the same molecular formula. The ^1H and ^{13}C NMR spectra of **2** (Table 1) were very similar to those of **1**. The obvious chemical shift differences came from the variations of the NMR data for ring A of the two compounds. This indicated the substituents position on the ring A should be varied. The 2-oxo-3-methylbut-3-enyl group located at C-6 was supported by the HMBC correlations of H-1'' (δ_{C} 4.65) with C-5 (δ_{C} 124.5), C-6 (δ_{C} 132.2), and C-7 (δ_{C} 118.8), and of H-5 (δ_{H} 7.04) and H-7 (δ_{H} 6.66) with C-1'' (δ_{C} 38.1).

The other precise substituents positions, two methoxy group located at C-8 and C-4', respectively, were also conducted by further analysis of its HMBC correlations. The structure of **2** is therefore determined.

Since certain of the flavonoids exhibit potential anti-TMV activity,¹⁸⁻²¹ compounds **1-6** were tested for their anti-TMV activity. The anti-TMV activity were tested using the half-leaf method.²² Ningnanmycin (a commercial product for plant disease in China), was used as a positive control. Their antiviral inhibition

Table 1. ^1H NMR and ^{13}C NMR data of compounds **1** and **2**

No.	Compound 1		Compound 2	
	δ_{C} (m.)	δ_{H} (m, J, Hz)	δ_{C} (m.)	δ_{H} (m, J, Hz)
2	163.6 s		164.6 s	
3	105.3 d	6.42 s	105.8 d	6.43 s
4	181.5 s		182.6 s	
5	124.1 d	7.43 (d) 8.2	124.5 d	7.04 (d) 2.1
6	127.9 d	6.71 (d) 8.2	132.2 s	
7	133.6 s		118.8 d	6.66 (d) 2.1
8	155.2 s		154.6 s	
9	150.7 s		149.2 s	
10	122.1 s		125.1 s	
1'	123.0 s		123.6 s	
2',6'	130.8 d	7.73 (d) 8.8	130.9 d	7.67 (d) 8.8
3',5'	115.8 d	6.80 (d) 8.8	115.7 d	6.81 (d) 8.8
4'	161.1 s		161.3 s	
1''	37.9 t	4.63 s	38.1 t	4.65 s
2''	200.7 s		201.5 s	
3''	144.8 s		144.3 s	
4''	123.4 t	5.85, 6.11 s	122.8 t	5.85, 6.15 s
5''	18.7 q	1.90 s	18.6 q	1.89 s
-OMe-8	61.4 q	3.80 s	56.0 q	3.80 s
-OMe-4'	56.0 q	3.84 s	56.2 q	3.83 s

Table 2. TMV Infection Inhibition Activities of **1-6**

Compounds	Inhibition rate (%)	IC ₅₀ (μm)
1	22.2 \pm 3.0	65.2
2	18.6 \pm 2.8	83.5
3	24.0 \pm 2.5	58.1
4	22.4 \pm 2.6	60.3
5	24.3 \pm 2.8	55.9
6	17.6 \pm 2.7	86.7
ningnanmycin	32.2 \pm 3.2	40.5

All results are expressed as mean \pm SD; n = 3 for all groups.

rates at the concentration of 20 μM were listed in Table 2. Compounds **1-6** showed potential anti-TMV activity with inhibition rates in the ranges of 17.6-24.3%, respectively.

EXPERIMENTAL

General. UV spectra were obtained using a Shimadzu UV-2401A spectrophotometer. IR spectra were obtained in KBr disc on a Bio-Rad Win infrared spectrophotometer. ESI-MS were measured on a VG Auto Spec-3000 MS spectrometer. ^1H , ^{13}C and 2D NMR spectra were recorded on Bruker 500 instrument with TMS as internal standard. Column chromatography was performed on silica gel (200-300 mesh), or on silica gel H (10 ~ 40 μm , Qingdao Marine Chemical Inc., China). Second separate was used an Agilent 1100 HPLC equipped with ZORBAX- C_{18} (21.2 mm \times 250 mm, 7.0 μm) column and DAD detector.

Plant material. The twigs of *Garcinia bracteata* C. Y. Wu ex Y. H. Li were collected in Pu'er Prefecture, Yunnan Province, People's Republic of China, in September 2012. The identification of the plant material was verified by Prof. Ren P. Y (Xishuangbanna Botanical Garden). A voucher specimen (YNNI-2012-88) has been deposited in our laboratory.

Anti-TMV Assays. TMV (U1 strain) was obtained from the Key Laboratory of Tobacco Chemistry of Yunnan Province, Yunnan Academy of Tobacco Science, P. R. China. The virus was multiplied in *Nicotiana tabacum* cv. K326 and purified as described. The concentration of TMV was determined as 20 mg/mL with a UV spectrophotometer [virus concentration = $(A_{260} \times \text{dilution ratio})/E_{0.1\%}^{1\text{cm}}$, 260 nm (1cm)]. The purified virus was kept at -20 °C and was diluted to 32 $\mu\text{g/mL}$ with 0.01 M PBS before use.

Nicotiana glutinosa plants were cultivated in an insect-free greenhouse. *N. glutinosa* was used as a local lesion host. The experiments were conducted when the plants grew to the 5–6-leaf stage. The tested compounds were dissolved in DMSO and diluted with distilled H_2O to the required concentrations. A solution of equal concentration of DMSO was used as a negative control. The commercial antiviral agent ningnanmycin was used as a positive control.

For the half-leaf method, the virus was inhibited by mixing with the solution of compound. After 30 min, the mixture was inoculated on the left side of the leaves of *N. glutinosa*, whereas the right side of the leaves was inoculated with the mixture of DMSO solution and the virus as control. The local lesion numbers were recorded 3 or 4 days after inoculation. Three repetitions were conducted for each compound. The inhibition rates were calculated according to the formula:

$$\text{inhibition rate (\%)} = [(C - T)/C] \times 100\%$$

where C is the average number of local lesions of the control and T is the average number of local lesions of the treatment.

Extraction and isolation. The air-dried and powdered twigs of *G. bracteata* (2.5 kg) were extracted four times with 70% acetone (4 \times 3.0 L) at room temperature and filtered. The filtrate was concentrated and

successively partitioned with CH_2Cl_2 and EtOAc. The EtOAc fraction (68 g) was submitted to silica gel (200–300 mesh) column chromatography, eluting with a CHCl_3 -acetone gradient system (20:1, 9:1, 8:2, 7:3, 6:4, 5:5), to give six fractions A–F. The further separation of fraction B (9:1, 20 g) by silica gel column chromatography, eluted with petroleum ether-EtOAc (9:1, 8:2, 7:3, 6:4, 1:1), yielded mixtures B1–B5. Fraction B2 (8:2, 1.6 g) was subjected to preparative HPLC (65% MeOH, flow rate 12 mL/min) to give **1** (13.2 mg) and **2** (10.6 mg). The further separation of fraction B3 (7:3, 1.2 g) by silica gel column chromatography, and preparative HPLC (58% MeOH, flow rate 12 mL/min) to give **3** (14.0 mg), **4** (16.7 mg), **5** (18.5 mg), and **6** (15.8 mg).

Bracteflavones A (1): $\text{C}_{22}\text{H}_{20}\text{O}_5$, Pale yellow gum; UV (MeOH) λ_{max} (log ϵ) 362 (3.60), 257 (3.72), 210 (4.28) nm; IR (KBr): ν_{max} 2947, 2832, 1680, 1658, 1602, 1561, 1474, 1338, 1285, 1142, 1071, 845, 762 cm^{-1} ; ^1H and ^{13}C NMR data (in CDCl_3 , 500 and 125 MHz), see Table 1; ESIMS m/z 387; HRESIMS m/z 387.1202 $[\text{M}+\text{Na}]^+$ (calcd $\text{C}_{22}\text{H}_{20}\text{O}_5$ for 387.1208).

Bracteflavones B (1): $\text{C}_{22}\text{H}_{20}\text{O}_5$, Pale yellow gum; UV (MeOH) λ_{max} (log ϵ) 365 (3.68), 262 (3.75), 210 (4.32) nm; IR (KBr): ν_{max} 2958, 2826, 1685, 1654, 1608, 1576, 1461, 1327, 1276, 1157, 1064, 869, 753 cm^{-1} ; ^1H and ^{13}C NMR data (in CDCl_3 , 500 and 125 MHz), see Table 1; ESIMS m/z 387; HRESIMS m/z 387.1215 $[\text{M}+\text{Na}]^+$ (calcd $\text{C}_{22}\text{H}_{20}\text{O}_5$ for 387.1208).

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