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

Yuehong Yang,[1] Limei Li,[2] Jie Lou,[2] Yang Cheng,[1] Yede Wang,[2] Duntao Shu,[1] Lei Shi,[1] Xue-Mei Gao,[2] Ping Ning,[1,*] and Qiufen Hu[2,*]

[1] Faculty of Environmental Science and Engineering, Kunming University of Science and Technology, Kunming 650500, China; [2] Key Laboratory of Chemistry in Ethnic Medicinal Resources, Yunnan University of Nationalities, Kunming 650500, P. R. China.
E-mail: ningping58@sina.com, huqiufena@aliyun.com

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 xanthones 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 xanthones 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.

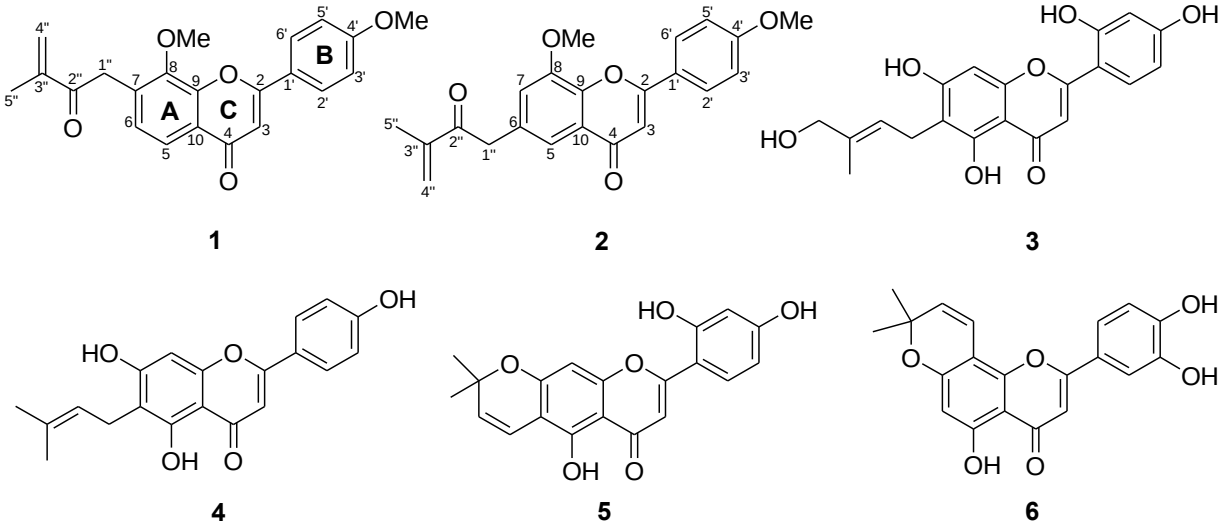

Figure 1. The structures of isoprenylated flavones from *Garcinia bracteata*

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 ^{1}H and ^{13}C NMR data of **1** and **2** were listed in Table 1.

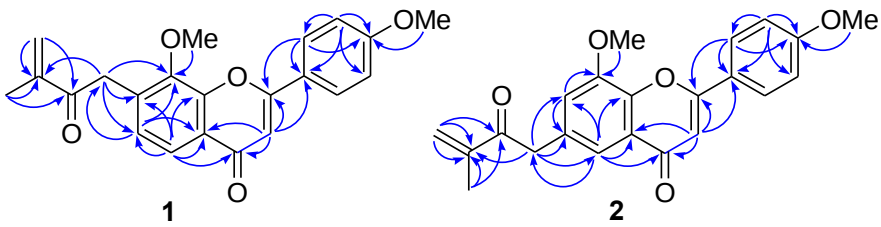

Figure 2. The key HMBC (↷) correlations of **1** and **2**

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 $C_{22}H_{20}O_5$ by HRESIMS (*m/z* 387.1202 $[M+Na]^+$). 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 ^{1}H and ^{13}C 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).

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

The other precise subsituents 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 2. TMV Infection Inhibition Activities of **1**-**6**

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

All results are expressed as mean ± 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. ^{1}H, ^{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 × 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} × dilution ratio)/E0.1%, 260 nm (1cm)]. The purified virus was kept at -20 °C and was diluted to 32 μg/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 × 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**): $C_{22}H_{20}O_5$, 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 $[M+Na]^+$ (calcd $C_{22}H_{20}O_5$ for 387.1208).

Bracteflavones B (**1**): $C_{22}H_{20}O_5$, 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 $[M+Na]^+$ (calcd $C_{22}H_{20}O_5$ for 387.1208).

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REFERENCES

1. X. M. Gao, T. Yu, F. Lai, C. F. Qiao, Y. Zhou, X. Liu, J. Z. Song, K. Q. Luo, and H. X. Xu, *Tetrahedron Lett.*, 2010, **51**, 2442.
2. X. Liu, T. Yu, X. M. Gao, Y. Zhou, C. F. Qiao, Y. Peng, S. L. Chen, K. Q. Luo, and H. X. Xu, *J. Nat. Prod.*, 2010, **73**, 1355.
3. T. Ritthiwigrom, S. Laphookhieo, and S. G. Pyne, *Int. J. Sci. Technol.*, 2013, **7**, 212.
4. Y. P. Wu, W. Zhao, Z. Y. Xia, G. H. Kong, X. P. Lu, Q. F. Hu, and X. M. Gao, *Phytochem. Lett.*, 2013, **6**, 629.
5. Y. P. Wu, W. Zhao, Z. Y. Xia, G. H. Kong, X. P. Lu, Q. F. Hu, and X. M. Gao, *Molecules*, 2013, **18**, 9663.
6. X. W. Li, J. Li, and P. F. Stevens, *Floral of China*, Vol. 13, Science Press, Beijing, 2007, p. 43.
7. Z. Na, H. B. Hu, and Q. F. Fan, *Helv. Chim. Acta*, 2010, **93**, 958.

8. S. L. Niu, Z. L. Li, F. Ji, G. Y. Liu, N. Zhao, X. Q. Liu, Y. K. Jing, and H. M. Hua, *Phytochemistry*, 2012, **77**, 280.
9. O. Thoison, J. Fahy, V. Dumontet, A. Chiaroni, C. Riche, M. V. Tri, and T. Sèveneta, *J. Nat. Prod.*, 2000, **63**, 441.
10. O. Thoison, D. D. Cuong, A. Gramain, A. Chiaroni, N. V. Hung, and T. Sèveneta, *Tetrahedron*, 2005, **61**, 8529.
11. Q. F. Hu, D. Y. Niu, X. L. Li, Y. H. Qin, Z. Y. Yang, G. L. Zhao, Z. X. Yang, X. M. Gao, and Z. Y. Chen, *Heterocycles*, 2013, **87**, 1127.
12. N. T. Nguyen, M. H. K. Nguyen, H. X. Nguyen, N. K. N. Bui, and M. T. T. Nguyen, *J. Nat. Prod.*, 2012, **75**, 1951.
13. B. M. Abegaz, B. T. Ngadjui, E. Dongo, and H. Tamboue, *Phytochemistry*, 1998, **49**, 1147.
14. Z. P. Zheng, K. W. Cheng, J. T. K. To, H. Li, and M. Wang, *Mol. Nutr. Food Res.*, 2008, **52**, 1530.
15. Y. H. Wang, A. J. Hou, L. Chen, D. F. Chen, H. D. Sun, Q. S. Zhao, K. F. Bastow, Y. Nakanish, X. H. Wang, and K. H. Lee, *J. Nat. Prod.*, 2004, **67**, 757.
16. J. Kwon, N. T. Hiep, D. W. Kim, B. Y. Hwang, H. J. Lee, W. Mar, and D. Lee, *J. Nat. Prod.*, 2014, **77**, 1893.
17. D. Q. Yu and J. S. Yang, Handbook of Analytical Chemistry (7th vol.), *Nuclear Magnetic Resonance Spectroscopy*, 2nd ed, Chemical Industry Press, Beijing, 1999, p. 816.
18. Z. Y. Chen, J. L. Tan, G. Y. Yang, M. M. Miao, Z. Y. Chen, and T. F. Li, *Phytochem. Lett.*, 2012, **5**, 233.
19. J. X. Chen, H. Q. Leng, Y. X. Duan, W. Zhao, G. Y. Yang, Y. D. Guo, Y. K. Chen, and Q. F. Hu, *Phytochem. Lett.*, 2013, **6**, 144.
20. W. Zhao, X. Y. Zeng, T. Zhang, L. Wang, G. Y. Yang, Y. K. Chen, Q. F. Hu, and M. M. Miao, *Phytochem. Lett.*, 2013, **6**, 179.
21. B. N. Jing, Z. Q. Ma, J. T. Feng, H. Y. Liang, C. Li, and X. J. Zhang, *Phytopathol.*, 2012, **160**, 181.
22. Q. F. Hu, B. Zhou, J. M. Huang, X. M. Gao, L. D. Shu, G. Y. Yang, and C. T. Che, *J. Nat. Prod.*, 2013, **76**, 292.