HETEROCYCLES, Vol. 87, No. 5, 2013, pp. 1127 - 1132. © 2013 The Japan Institute of Heterocyclic Chemistry
Received, 21st March, 2013, Accepted, 3rd April, 2013, Published online, 10th April, 2013
DOI: 10.3987/COM-13-12713

NEW XANTHONES FROM *GARCINIA BRACTEATA* AND THEIR CYTOTOXICITIES

Qiufen Hu,[1] Deyun Niu,[1] Xiangli Li,[2] Yunhua Qin,[2] Zongyan Yang,[1] Guoli Zhao,[1] Zhongxiu Yang,[1] Xuemei Gao,[1]* and Zhangyu Chen[1,2,†]

[1] Key Laboratory of Chemistry in Ethnic Medicinal Resources, State Ethnic Affairs Commission & Ministry of Education, Yunnan University of Nationalities, Kunming 650031, P. R. China; [2] Key Laboratory of Tobacco Chemistry of Yunnan Province, Yunnan Academy of Tobacco Science, Kunming 650106, P. R. China. E-mail: gao_xuemei@hotmail.com, huqiufena@yahoo.com.cn

Abstract – Two new xanthones, bracthones A (**1**) and B (**2**), together with four known xanthones (**3**-**6**) were isolated from the stems of *Garcinia bracteata*. Their structures were elucidated by spectroscopic methods, including extensive 1D- and 2D- NMR techniques. Compounds **1-6** were tested for their cytotoxicities against five human tumor cell lines (NB4, A549, SHSY5Y, PC3, and MCF7). Compounds **1** and **2** showed high cytotoxicities against PC3 cell with IC_{50} values of 3.24 and 2.15 μM, respectively.

The species of *Garcinia bracteata* are one of the plants belonging to *Garcinia* genus. This species distributed in the south of Yunnan and Guangxi Province of China.[1] Plants of the genus *Garcinia* (Guttiferae) have been extensively investigated from the phytochemical and biological points of view. Xanthones,[2-5] benzophenones,[4,6,7] depsidones,[8-10] flavonoids,[11,12] biflavonoids,[13] and triterpenes[14] have been reported from *Garcinia* species. Previous phytochemical investigations on *G. bracteata* resulted in the isolation of caged-prenylxanthones and benzophenones.[15-18]

With the aim of multipurpose utilization of *Garcinia* plants and identify bioactive natural products from this genus, the phytochemical investigation on *G. bracteata* was carried out. As a result, two new xanthones (**1**-**2**), together with four known xanthones (**3**-**6**), were isolated from this plant. The structures of new compounds were elucidated on the basis of a comprehensive analysis of the ^{1}H NMR, ^{13}C NMR and 2D NMR spectra. In addition, cytotoxicities of compounds **1-6** were evaluated. The details of the

isolation and structure elucidation and cytotoxicities of the new compounds (**1** and **2**), are reported in this article.

A 70% aq. methanol extract prepared from the stems of *G. bracteata* 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 xanthones, named bracthones A (**1**) and B (**2**), together with four known xanthones, cudraxanthone G (**3**),[19] garcinone A (**4**),[20] 5-O-methylxanthone V1 (**5**),[18] and gerontoxanthone I (**6**).[18] The structures of the compounds **1-6** were as shown in Figure 1, and the ^{1}H and ^{13}C NMR data of **1** and **2** were listed in Table 1.

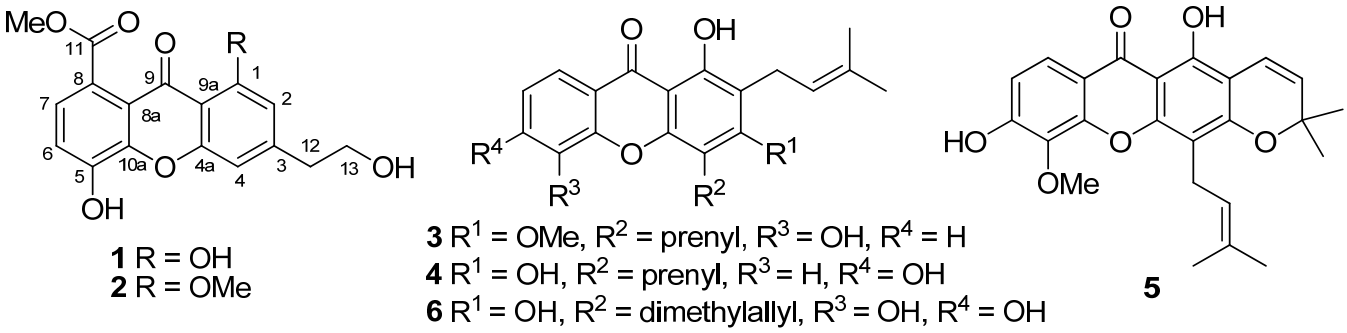

Figure 1. The structures of xanthones from *G. bracteata*

Compound **1** was isolated as a yellow gum. The HRESIMS of **1** gave the pseudomolecular $[M + Na]^{+}$ ion at *m*/*z* 353.0642, corresponding to a molecular formula of $C_{17}H_{14}O_7$. The ^{1}H NMR spectra data (Table 1) showed the presence of two hydroxy groups, two *ortho* coupled aromatic protons, two *meta* coupled aromatic protons, and two methylene protons. These signals could be attributed to a basic xanthone skeleton and an ethanol group. The appearance of the methylene protons (H_2-12) of the ethanol group at δ_H 2.50 together with ^{3}J cross-peaks in the HMBC spectrum with two aromatic methine carbon (C-2, δ_C 110.7; C-4, δ_C 107.9) and a quaternary aromatic carbon (C-3, δ_C 144.1) suggested that the ethanol group was at C-3. The correlation (Figure 2) between one of the *ortho*-coupled aromatic protons (H-7, δ_H 7.61) and C-7 in the HSQC spectrum established the attachment of this proton at C-7. Thus, the other *ortho*-coupled aromatic proton at δ_H 7.45 was attributed to H-6. H-7 also gave HMBC cross-peaks with C-11 (δ_C 168.2) of the ester carbonyl side chain and an aromatic carbon C-8 (δ_C 127.0) in the HMBC spectrum. Thus, the methoxycarbonyl group was placed at C-8. Finally, two hydroxy groups were assigned to C-1 and C-5 on the basis of HMBC correlations between the hydroxy proton (δ_H 12.87) and C-1 (δ_C 162.1), C-2 (δ_C 110.7), and C-9a (δ_C 107.2), as well as those between the other hydroxy proton (δ_H 12.60) and C-5 (δ_C 152.3),

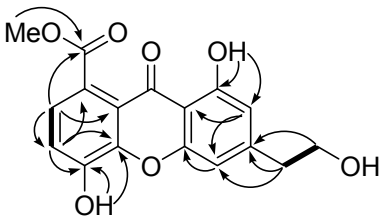

Figure 2. Key HMBC (⌒) and ^{1}H-^{1}H COSY (▬) correlations of **1**

C-6 (δ_C 120.2), and C-10a (δ_C 147.2). Therefore, compound **1** was assigned as 1,5-dihydroxy-3-ethanol-8-methoxycarbonyl-xanthone, and given the trivail name of bracthone A.

Bracthone B (**2**) was also isolated as a yellow gum, and its molecular formula was determined as $C_{18}H_{16}O_7$ through HRESI-MS analysis (pseudomolecular ion $[M + Na]^+$ at *m*/*z* 367.0790). The ^1H spectra data of **2** was very similar to these of **1** (see Table 1), except for the additional methoxy signal at (δ_H 3.80) of compound **2**. HMBC correlations between δ_H 3.80 and C-1 (δ_C 162.9) suggested the methoxy group attached at C-1. The proposed structure was further supported by ^{13}C NMR spectroscopic data with assignments based on the DEPT, HMQC, and HMBC spectra. Thus, compound **2** was determined as 5-hydroxy-3-ethanol-1-methoxy-8-methoxycarbonyl-xanthone.

Compounds **1** and **2** are the first naturally occurring xanthone derivatives possessing an ethanol unit.

Table 1. ^1H NMR and ^{13}C NMR data of compounds **1** - **2** in C_5ND_5 (125 and 500 MHz)

No.	Compound **1**		Compound **2**	
	δ_C (m)	δ_H (m, *J*, Hz)	δ_C (m)	δ_H (m, *J*, Hz)
1	162.1		162.9	
2	110.7	7.10 s	109.0	7.01 s
3	144.1		144.8	
4	107.9	7.23 s	107.1	7.31 s
5	152.3		152.6	
6	120.2	7.45 (d, 9.0)	119.8	7.41 (d, 8.9)
7	125.8	7.61 (d, 9.0)	125.2	7.66 (d, 8.9)
8	127.0		126.4	
9	181.5		182.0	
4a	156.6		156.3	
8a	119.2		118.9	
9a	107.2		105.6	
10a	147.2		147.5	
11	168.2		169.1	
12	35.8	2.50 (t, 7.2)	36.1	2.51 (t, 7.2)
13	63.5	3.72 (t, 7.2)	63.2	3.68 (t, 7.2)
1-OMe	52.6	4.14 s	52.5	4.12 s
11-OMe			56.2	3.80 s
Ar-OH-1		12.87 s		
Ar-OH-5		12.60 s		12.62 s

The cytotoxicity of compounds **1**-**6** were tested using a previously reported procedure.[21] All treatments were performed in triplicate. In the MTT assay, the IC_{50} was defined as the concentration of the test compound resulting in a 50% reduction of absorbance compared with untreated cells. The cytotoxic

abilities against NB4, A549, SHSY5Y, PC3, and MCF7 tumor cell lines by MTT-assay (with taxol as the positive control) were shown in Table **2**. Compounds **1** and **2** showed high cytotoxicities against PC3 cell with IC_{50} values of 3.24 and 2.15 μM, respectively. The other compounds also showed moderate cytotoxicities for some tested cell lines with IC_{50} values below 10.

Table 2. cytotoxic activity of compounds **1 - 6**

Compounds	NB4	A549	SHSY5Y	PC3	MCF7
1	7.68	5.47	>10	3.24	>10
2	5.47	>10	4.26	2.15	7.68
3	7.26	>10	5.78	>10	>10
4	8.22	9.65	>10	>10	7.21
5	6.37	>10	5.94	9.21	>10
6	>10	>10	8.65	>10	9.06
Taxol	0.03	0.02	0.2	0.2	0.1

NB4, human leukemia cell; A549, carcinomic human alveolar basal epithelial cell; SHSY5Y, human neuroblastoma cell; PC3, Human prostate cancer cell; MCF7, Human breast adenocarcinoma cell.

EXPERIMENTAL

General. UV spectra were obtained using a Shimadzu UV-2401A spectrophotometer. IR spectra were obtained in KBr disc on a Bio-Rad Wininfmred 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 DRX-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 stems of *G. bracteata* were collected in Xishuangbanna Prefecture, Yunnan Province, People's Republic of China, in September 2010. The identification of the plant material was verified by Prof. Ren P. Y (Xishuangbanna Botanical Garden). A voucher specimen (YNNI-2010-86) has been deposited in our laboratory.

Extraction and Isolation. The air-dried and powdered stems of *G. bracteata* (4.5 kg) were extracted four times with 70% MeOH (4 × 10 L) at room temperature and filtered. The crude extract (103 g) was applied 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 A (9:1, 18.5 g) by silica gel column chromatography, eluted with petroleum ether-EtOAc (9:1, 8:2, 7:3, 6:4, 1:1), yielded mixtures D1–D5. Fraction D2 (8:2, 3.8 g) was subjected to preparative HPLC (68% MeOH, flow rate 12 mL/min) to give **3** (15.2 mg), **4** (14.6 mg), and **5** (16.6 mg). The further separation of fraction B (8:2, 2.6

g) by silica gel column chromatography, and preparative HPLC (60% MeOH, flow rate 12 mL/min) to give **1** (10.5 mg), **2** (11.2 mg), and **6** (16.4 mg).

Cytotoxicity Assay. The cytotoxicity tests for the isolates were performed by against NB4, A549, SHSY5Y, PC3, and MCF7 tumor cell lines by MTT-assay (with doxorubicin as the positive control).[21]

Bracthone A (1). Obtained as a yellow gum; UV (MeOH) λ_{max} (log ε) 210 (4.22), 240 (3.22), 305 (3.87) nm; IR (KBr) ν_{max} 3428, 3080, 2916, 2873, 1728, 1653, 1597, 1542, 1463, 1379, 1122, 1069, 873, 722 cm^{-1}; ESIMS *m/z* (positive ion mode) 353 $[M+Na]^+$; HRESIMS (positive ion mode) *m/z* 353.0642 $[M+Na]^+$ (calcd $C_{17}H_{14}NaO_7$ for 353.0637).

Bracthone B (**2**): Obtained as a yellow gum; UV (MeOH) λ_{max} (log ε) 210 (4.31), 243 (3.31), 309 (3.94) nm; IR (KBr) ν_{max} 3426, 3083, 2910, 2876, 1726, 1650, 1595, 1546, 1460, 1381, 1118, 1076, 885, 718 cm^{-1}; ESIMS *m/z* (positive ion mode) 367 $[M+Na]^+$; HRESIMS (positive ion mode) *m/z* 367.0790 $[M+Na]^+$ (calcd $C_{18}H_{16}NaO_7$ for 367.0794).

ACKNOWLEDGMENT

This research was supported by the National Natural Science Foundation of China (No. 21002085), the Excellent Scientific and Technological Team of Yunnan High School (2010CI08), the Yunnan University of Nationalities Green Chemistry and Functional Materials Research for Provincial Innovation Team (2011HC008), the National Undergraduates Innovating Experimentation Project (2011HX18), and start-up funds of Yunnan University of Nationalities.

REFERENCES

1. X. W. Li, J. Li, and P. F. Stevens, *Flora of China*. Chinese Science Press: Beijing, 2007, Vol. 13, p. 1267.
2. Y. J. Xu, Y. H. Lai, Z. Imiyabir, and S. H. Goh, *J. Nat. Prod.*, 2001, **64**, 1191.
3. X. M. Gao, T. Yu, M. Z. Cui, J. X. Pu, X. Du, Q. B. Han, Q. F. Hu, T. C. Liu, K. Q. Luo, and H. X. Xu, *Bioorg. Med. Chem. Lett.*, 2012, **22**, 2350.
4. J. H. Wu, Y. T. Tung, C. F. Chyu, S. C. Chien, S. Y. Wang, S. T. Chang, and Y. H. Kuo, *J. Wood. Sci.,* 2008, **54**, 383.
5. Z. H. Mbwambo, M. C. Kapingu, M. J. Moshi, F. Machumi, S. Apers, P. Cos, D. Ferreira, J. P. J. Marais, D. V. Berghe, L. Maes, A. Vlietinck, and L. Pieters, *J. Nat. Prod.,* 2006, **69**, 369.
6. 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.
7. L. J. Zhang, C. T. Chiou, J. J. Cheng, H. C. Huang, L. M. Yang Kuo, C. C. Liao, K. F. Bastow, K. H. Lee, and Y. H. Kuo, *J. Nat. Prod.,* 2010, **73**, 557.

8. X. M. Gao, T. Yu, F. S. F. Lai, Y. Zhou, X. Liu, C. F. Qiao, J. Z. Song, S. L. Chen, K. Q. Luo, and H. X. Xu, *Bioorg. Med. Chem.,* 2010, **18**, 4957.
9. C. Ito, M. Itoigawa, Y. Mishina, H. Tomiyasu, M. Litaudon, J. P. Cosson, T. Mukainaka, H. Tokuda, H. Nishino, and H. Furukawa, *J. Nat. Prod.*, 2001, **64**, 147.
10. V. Rukachaisirikul, W. Naklue, S. Phongpaichit, N. H. Towatana, and K. Maneenoon, *Tetrahedron*, 2006, **62**, 8578.
11. S. Deachathai, W. Mahabusarakam, S. Phongpaichit, and W. C. Taylor, *Phytochemistry*, 2005, **66**, 2368.
12. M. Ilyas, M. Kamil, M. Parveen, and M. S. Khan, *Phytochemistry*, 1994, **36**, 807.
13. Q. B. Han, S. F. Lee, C. F. Qiao, Z. D. He, J. Z. Song, H. D. Sun, and H. X. Xu, *Chem. Pharm. Bull.,* 2005, **53**, 1034.
14. V. Rukachaisirikul, S. Saelim, P. Karnsomchoke, and S. Phongpaichit, *J. Nat. Prod.*, 2005, **68**, 1222.
15. Z. Na, H. B. Hu, and Q. F. Fan, *Chin. Chem. Lett.,* 2010, **21**, 443.
16. Z. Na, H. B. Hu, and Q. F. Fan, *Helv. Chim. Acta,* 2010, **5**, 958.
17. O. Thoison, J. Fahy, V. Dumontet, A. Chiaroni, C. Riche, M. V. Tri, and T. Sèveneta, *J. Nat. Prod.,* 2000, **63**, 441.
18. O. Thoison, D. D. Cuong, A. Gramain, A. Chiaroni, N. V. Hung, and T. Sèveneta, *Tetrahedron,* 2005, **61**, 8529.
19. M. J. Balunas, B. Su, R. W. Brueggemeier, and A. D. Kinghorn, *J. Nat. Prod.,* 2008, **71**, 1161.
20. A. K. Sena, K. K. Sarkara, P. C. Mazumdera, N. Banerjia, R. Uusvuoria, and T. A. Hasea, *Phytochemistry,* 1982, **21**, 1747.
21. T. Mosmann, *J. Immunol. Methods,* 1983, **65**, 55.