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NEW XANTHONES FROM *GARCINIA BRACTEATA* AND THEIR CYTOTOXICITIES

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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.

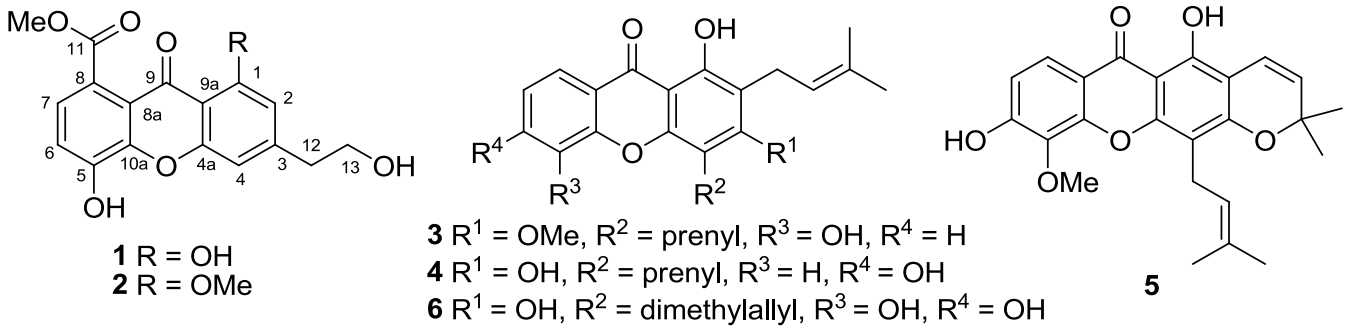

Figure 1. The structures of xanthones from *G. bracteata*

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),

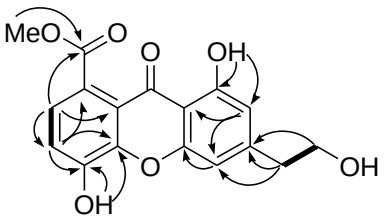

Figure 2. Key HMBC (⌒) and ^{1}H-^{1}H COSY (—) correlations of **1**

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).

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