Two Unusual Xanthones from the Bark of *Garcinia xanthochymus*

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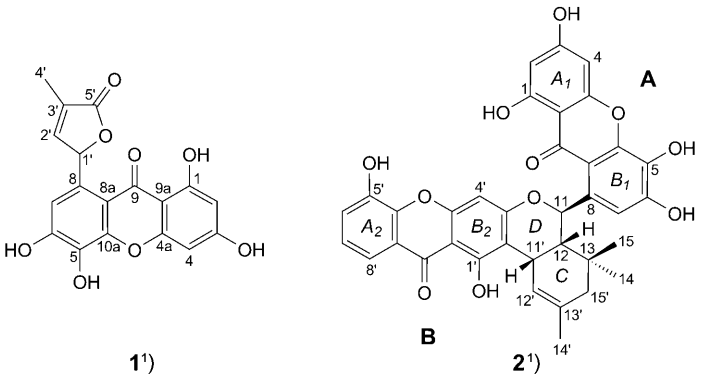
A new xanthone derivative, garcinexanthone F (**1**), which was found to possess an α,β-unsaturated γ-lactone moiety, and a new bisxanthone, bigarcinenone B (**2**), with a terpene bridge providing the xanthone–xanthone linkage, were isolated from the bark of *Garcinia xanthochymus*. Their structures were elucidated by spectroscopic methods, especially 2D-NMR techniques. The antioxidant assay *in vitro* showed that compounds **1** and **2** exhibited significant scavenging activities against 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical with IC_{50} values of 22.32 and 20.14 μM, and against HO$^{\cdot}$ radical with IC_{50} values of 1.16 and 2.85 μM, respectively.

Introduction. – *Garcinia xanthochymus*, belonging to the genus *Garcinia* of the Guttiferae family, is a tree native to the south and southwest of Yunnan Province, P. R. China, which can grow up to 10–20 m, and it is widely used in folk medicine for dispelling worms and removing food toxin [1]. The previous phytochemical studies of the leaves, seeds, fruits, twig bark, and wood revealed the presence of benzophenones [2], flavonoids [3], triterpenes [4], and xanthones [5]. Xanthones are class of polyphenolics that exhibit well-documented pharmacological properties, such as antioxidative, antileukaemic, antitumour, antiulcer, antimicrobial, antihepatotoxic, and CNS (central nervous system) depressant activities [6], mainly due to their O-bearing heterocyclic nature and diversity of functional groups [7]. To determine the bioactive component, we have investigated the bark of *G. xanthochymus*, and isolated and identified two new xanthones **1** and **2**. Different from the previously reported xanthones, garcinexanthone F (**1**) contained an unusual α,β-unsaturated γ-lactone moiety, and bigarcinenone B (**2**) was formally derived from the corresponding diene *via* dimerization by a *Diels–Alder*-type reaction. Here, we describe the isolation and structure elucidation of garcinexanthone F (**1**) and bigarcinenone B (**2**), based on extensive spectroscopic analysis, and their biological activities.

Results and Discussion. – Garcinexanthone F (**1**) was obtained as a yellow powder. Its molecular formula, $C_{18}H_{12}O_8$, was deduced from HR-ESI-MS (positive-ion mode; *m*/*z*



379.0432 ($[M+\mathrm{Na}]^+$, $C_{18}H_{12}NaO_8^+$; calc. 379.0429), indicating 13 degrees of unsaturation. The UV spectrum of **1** displayed characteristic xanthone absorptions at 245, 285, and 327 nm. Its ^{1}H- and ^{13}C-NMR spectra, in conjunction with HSQC spectrum, revealed the presence of one Me group, five CH groups, and twelve quaternary C-atoms, including two CO groups with signals at δ(C) 182.3 and 174.7. The NMR data of **1** did not show much similarity to those of the prenylated xanthones previously isolated from *Garcinia* plant [8]. The ^{1}H-NMR spectrum of **1** exhibited two characteristic signals of a 1,2,3,5-tetrasubstitued benzene ring (δ(H) 6.23 (br. *s*, 1 H) and 6.47 (br. *s*, 1 H)), of a chelated OH H-atom (δ(H) 13.0 (*s*, HO–C(1))), of an aromatic H-atom (δ(H) 6.95 (*s*, 1 H)), of an O-bearing CH group (δ(H) 7.12 (*s*, 1 H)), which correlated with the C-atom signal at δ(C) 79.9 in HSQC spectrum, of an olefinic H-atom (δ(H) 7.52 (br. *s*, 1 H)), and of one Me group (δ(H) 1.87 (*s*, 3 H)). The HMBCs between a chelated OH group (δ(H) 13.0) and C(1), C(2), and C(9a), and between an aromatic H-atom (δ(H) 6.23) and C(4) and C(9a) indicated the locations of the two OH groups at C(1) and C(3) (*Fig. 1*). In HMBC spectrum, the correlations H–C(1′)/C(2′)[1]) (δ(C) 151.5) and C(3′) (δ(C) 127.7), and H–C(2′)/C(1′) (δ(C) 79.9), C(3′), and C(5′) (δ(C) 174.7), and Me(4′)/C(2′), C(3′), and C(5′) suggested the presence of an α,β-unsaturated γ-lactone moiety, which was supported by EI-MS fragment-ion peaks at *m*/*z* 97 ($C_5H_5O_2^+$) and 69 ($C_4H_5O^+$). The H–C(1′) signal appeared at δ(H) 7.12, which was a more deshielded value than that usually observed for this functionality, due to the effect

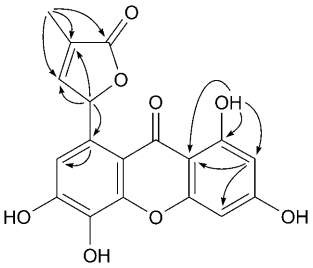


Fig. 1. *Selected HMBCs of compound* **1**

[1]) Arbitrary atom numbering. For systematic names, see *Exper. Part.*

of the C(9)=O group. Clearly, an α,β-unsaturated γ-lactone moiety was located at C(8) which was further supported by the HMBCs from H–C(1′) to C(7) and C(8). Thus, the structure of **1** was deduced. Prenylated xanthones constitute one of the most abundant classes of naturally occurring xanthones. In these isopentenylated derivatives, the C_5 unit is generally present as either 3-methylbut-2-enyl or 1,1-dimethylallyl group. The C_5 units can also occur as fused 2,2-dimethylchromene or 2,3-dihydro-2,3,3-trimethylfuran ring, or as the corresponding hydrated forms [9]. As far as we know, compound **1** represents the first example of xanthone with an α,β-unsaturated γ-lactone moiety.

Compound **2**, which was obtained as a yellow amorphous powder, exhibited an $[M+Na]^+$ ion peak at *m/z* 659.1515 in the HR-ESI-MS (positive-ion mode), corresponding to the molecular formula $C_{36}H_{28}O_{11}$. The ^{1}H- and ^{13}C-NMR data (*Table 1*) suggested that **2** possessed two xanthone moieties.

Table 1. *^{1}H- and ^{13}C-NMR and HMBC Data of Compound* **2** *in (D_6)DMSO*[1])

	A				B		
	^{1}H-NMR	^{13}C-NMR	HMBC (H → C)		^{1}H-NMR	^{13}C-NMR	HMBC (H → C)
C(1)		163.2 (*s*)		C(1′)		160.0 (*s*)	
H–C(2)	6.12 (br. *s*)	98.1 (*d*)	1, 3, 4, 9a	C(2′)		108.3 (*s*)	
C(3)		165.3 (*s*)		C(3′)		161.8 (*s*)	
H–C(4)	6.39 (br. *s*)	93.7 (*d*)	2, 3, 4a, 9a	H–C(4′)	6.38 (*s*)	93.4 (*d*)	2′, 3′, 4a′, 9a′
C(4a)		156.4 (*s*)		C(4a′)		154.9 (*s*)	
C(10a)		145.0 (*s*)		C(10a′)		146.2 (*s*)	
C(5)		132.2 (*s*)		C(5′)		146.5 (*s*)	
C(6)		150.8 (*s*)		H–C(6′)	7.30 (*d*, *J* = 7.5)	120.8 (*d*)	8′, 10a′
H–C(7)	7.06 (*s*)	114.6 (*d*)	6, 8, 8a, 11	H–C(7′)	7.24 (*t*, *J* = 7.5)	124.1 (*d*)	5′, 6′, 8a′
C(8)		133.2 (*s*)		H–C(8′)	7.58 (*d*, *J* = 7.5)	114.6 (*d*)	6′, 9′, 10a′
C(8a)		110.4 (*s*)		C(8a′)		120.7 (*s*)	
C(9)		182.1 (*s*)		C(9′)		180.6 (*s*)	
C(9a)		102.1 (*s*)		C(9a′)		102.4 (*s*)	
H–C(11)	7.10 (br. *s*)	73.0 (*d*)	7, 8, 12, 11′	H–C(11′)	3.85 (br. *s*)	30.7 (*d*)	
H–C(12)	2.05–2.10 (*m*)	45.2 (*d*)	11, 11′, 12′	H–C(12′)	5.44 (br. *s*)	121.3 (*d*)	
C(13)		32.2 (*s*)		C(13′)		133.0 (*s*)	
Me(14)	0.30 (*s*)	30.2 (*q*)	12, 13, 15, 15′	Me(14′)	1.65 (*s*)	23.2 (*q*)	12′, 13′, 15′
Me(15)	1.05 (*s*)	29.3 (*q*)	12, 13, 14, 15′	CH_2(15′)	1.45 (*d*, *J* = 18), 1.85 (*d*, *J* = 18)	40.1 (*t*)	
HO–C(1)	13.2 (*s*)		1, 2, 9a	HO–C(1′)	13.6 (*s*)		1′, 2′, 9a′

The ^{1}H- and ^{13}C-NMR data revealed that the part **A** of **2** resembled the same part of the known compound griffipavixanthone [10]. The ^{1}H-NMR spectrum of the part **A** of **2** exhibited signals of one chelated OH group at δ(H) 13.2 (*s*, 1 H), of one set of *meta*-aromatic H-atoms at δ(H) 6.12 (br. *s*, 1 H) and 6.39 (br. *s*, 1 H), and an aromatic H-atom a *singlet* at δ(H) 7.06 (*s*, 1 H). The H-atoms with signals at δ(H) 6.12 (br. *s*, 1 H) and 6.39 (*s*, 1 H) were attributed to H–C(2)[1]) and H–C(4) in the 1,2,3,5-tetrasubstitued benzene ring A_1, respectively. This was confirmed by comparing the NMR data of ring A_1 with those of griffipavixanthone and the correlations observed in

the HMBC spectrum (*Table 1*). The HMBCs of an aromatic H-atom *singlet* at δ(H) 7.06 with C(6) (δ(C) 150.8), C(8) (δ(C) 133.2), and C(8a) (δ(C) 110.4) suggested that this H-atom may be assigned to H–C(7). Hence, the part **A** of **2** can be deduced as shown in the *Formulae*.

Except for ^{1}H- and ^{13}C-NMR signal of part **A** mentioned above, signals of three H-atoms of *ABM* system at δ(H) 7.30 (*d*, $J = 7.5$), 7.24 (*t*, $J = 7.5$), and 7.58 (*d*, $J = 7.5$), together with those of three aromatic C-atoms (δ(C) 146.5, 146.2, and 120.7) and three aromatic CH C-atoms (δ(C) 120.8, 124.1, 114.6) were attributed to 1,2,3-trisubstitued benzene ring A_2. The HMBCs between H–C(8′), and C(6′), C(9′), and C(10a′) indicated that the OH group of ring A_2 was located at C(5′). The remaining aromatic H-atom (δ(H) 6.38 (*s*, 1 H)) and chelated OH group (δ(H) 13.6 (*s*, 1 H)) were assigned to ring B_2. In the HMBC spectrum, the correlations of the chelated OH group (δ(H) 13.6 (*s*, 1 H)) with three quaternary aromatic C-atoms, C(1′) (δ(C) 160.0), C(2′) (δ(C) 108.3), and C(9a′) (δ(C) 102.4), and of an aromatic H-atom (δ(H) 6.38 (*s*, 1 H)) with the C-atoms C(2′) (δ(C) 108.3), C(3′) (δ(C) 161.8), C(4a′) (δ(C) 154.9), and C(9a′) (δ(C) 102.4) indicated that *para*-position of the OH group was unsubstituted, and C(3′) was substituted with O-function. Thus, the partial structure B_2 of **2** was confirmed.

The remaining structure was determined by ^{1}H- and ^{13}C-NMR (DEPT) to consist of three CH groups (one O-bearing), one CH_2 group, three Me group, one quaternary C-atom, and one C=C bond with signals at δ(C) 121.3 (CH) and 133.0 (C). From the ^{1}H,^{1}H-COSY and HSQC spectra, the correlation of H–C(11) (δ(H) 7.10 (br. *s*, 1 H)) with H–C(12) (δ(H) 2.05 – 2.10 (*m*, 1 H)), and of H–C(12) with H–C(11′) (δ(H) 3.85 (br. *s*, 1 H)) led to the partial structure –CH(O)–CH–CH–. Based on these data and the HMBC correlations Me(14′)/C(12′), C(13′), C(15′); Me(14) and Me(15)/C(12), C(13), C(15′); H–C(12)/C(11), C(11′), C(12′); and H–C(11)/C(11′), C(12), the connectivity from C(11) to C(11′) was established. From these results, the remaining structure was established as depicted as ring *C*. The connectivity of ring *C* and ring B_1 was revealed by the HMBCs of H–C(11) to C(7) and C(8), and H–C(7) to C(11). Thus, the connection was established between C(8) and C(11). The aforementioned groups accounted for 22 out of 23 degrees of unsaturation in compound **2**, indicating the presence of an additional ring. Since C(3′) of ring B_2 and C(11) of ring *C* were O-bearing quaternary and CH C-atoms, respectively, an O-bridge was established between C(3′) and C(11). The remaining connection between ring B_2 and ring *C* should from C(2′) through C(11′) to form six-membered ring *D*.

Based on the spectral analysis documented above, the planar structure of **2** was obtained as depicted. The relative configuration of **2** was established on the basis of the ROESY spectrum (*Fig. 2*). The ROESY correlations of H–C(11) with H_α–C(15′) and Me(14) suggested location on the same face and α-orientation. H–C(11′) was found to correlate with H–C(12) and Me(15), indicating their β-orientation. Therefore, bigarcinenone B was deduced to be as shown for **2**. However, the absolute configuration of **2** was not determined.

Naturally occuring bisxanthones from guttiferae plants were rare. Dimeric xanthones, which have been reported so far, were all characterized by a direct C–C bond [11], or an ether linkage [12], or a terpene bridge [13] between two xanthone moieties. To the best of our knowledge, bigarcinenone B (**2**) is the first example in which the two xanthone moieties are connected by 6/6-membered rings from a double

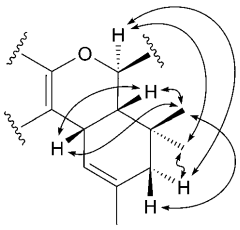
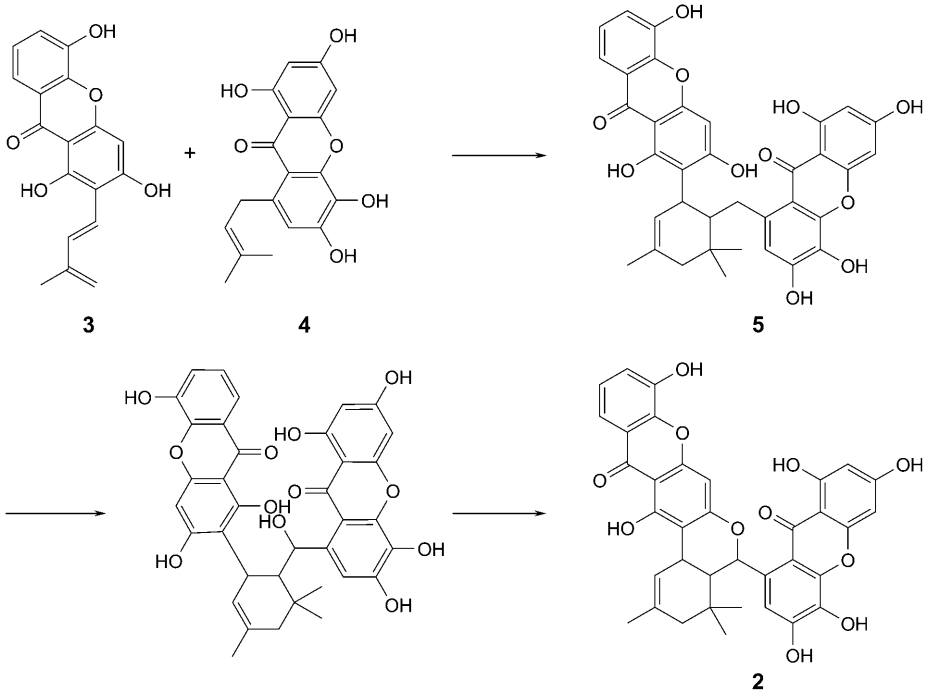


Fig. 2. *Key ROESY correlations of compound* **2**

cyclization involving two prenyl groups. A plausible biosynthetic pathway for **2** was proposed in the *Scheme:* an initial *Diels–Alder* reaction of the prenyl group of two xanthones **3** and **4** to provide a cyclohexene derivative **5**, followed by another cyclization to form a fused six-membered ring.

Scheme. *Plausible Biogenetic Route for Compound* **2**



Compounds **1** and **2** were evaluated for their antioxidant activities by 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical-scavenging method [14] and luminol–H_2O_2–Co^{II}–EDTA luminescence system [15]. The results (*Table 2*) showed that compounds **1** and **2** exhibited significant scavenging activities against DPPH radical with IC_{50} values of 22.32 and 20.14 μM, and against HO$^{\bullet}$ radical with IC_{50} values of 1.16 and 2.85 μM, respectively.

Table 2. *Antioxidant Activities of Compounds* **1** *and* **2**

Compounds	Antioxidant activities (IC_{50})[a]	
	DPPH[b])	HO·
1	22.32 ± 0.18	1.16 ± 0.03
2	20.14 ± 0.08	2.85 ± 0.14
Ascorbic acid	13.16 ± 0.03	0.76 ± 0.03
Gallic acid	5.86 ± 0.03	0.67 ± 0.03

[a]) Values expressed in μM. [b]) DPPH: 1,1-diphenyl-2-picrylhydrazyl radical.

Experimental Part

General. TLC: Pre-coated silica gel GF_{254} plates (*Qingdao Haiyang Chemical Co., Ltd.*, P. R. China). Column Chromatography (CC): silica gel (SiO_2, 200–300 mesh; *Qingdao Haiyang Chemical Co.*, *Ltd.*, P. R. China) and C_{18} reversed-phase (RP) silica gel (*YMC CO., Ltd.*, Japan). UV Spectra: *SP-2102UVPC* spectrometer; λ_{max} (log ε) in nm. ^{1}H- and ^{13}C-NMR spectra: *Bruker-AM-400* instrument; δ in ppm rel. to Me_4Si as internal standard (= 0 ppm), *J* in Hz. EI-MS: *Finnigan-MAT-95* mass spectrometer (70 eV); in *m*/*z* (rel. %). ESI-MS and HR-ESI-MS: *Finnigan LCQ-Deca* and *Waters*/*Micromass Q-Tof-Ultima* mass spectrometers, resp., in *m*/*z* (rel. int.).

Plant Material. The bark of *Garcinia xanthochymus* was collected from Xishuangbanna Prefecture, Yunnan Province, P. R. China, and identified by *Xishuangbanna Prefecture National Medicine Research Institute*. The voucher specimen (06061201) was deposited with the Herbarium of College of Pharmacy, South Central University for Nationalities, P. R. China.

Extraction and Isolation. The powdered bark of *G. xanthochymus* (6.5 kg) was extracted with 95% EtOH and then successively partitioned with petroleum ether (PE) (3 × 3.0 l), AcOEt (3 × 3.0 l), and BuOH (3.0 l). The combined extract of AcOEt (590 g) was subjected to CC (SiO_2; PE/Me_2CO 9 : 1, 8 : 2, 7 : 3, 1 : 1, 3 : 7, 0 : 1 (*v*/*v*)) to give 13 fractions, *Frs. 1–13*. *Fr. 9* (10.8 g) was subjected to CC (SiO_2; toluene/Me_2CO 9 : 1 → 3 : 7 gradient system; and *RP-18*; MeOH/H_2O 3 : 7 → 7 : 3 gradient system) to afford **1** (8.5 mg) and **2** (4.2 mg).

Garcinexanthone F (= *1-(2,5-Dihydro-4-methyl-5-oxodihydrofuran-2-yl)-3,4,6,8-tetrahydroxy-9*H-*xanthen-9-one*; **1**). Yellow amorphous powder. UV (MeOH): 245 (3.95), 285 (sh) (3.92), 327 (3.94). ^{1}H-NMR (400 MHz, (D_6)acetone): 1.87 (*s*, Me(4′)); 6.23 (br. *s*, H–C(2)); 6.47 (br. *s*, H–C(4)); 6.95 (br. *s*, H–C(7)); 7.12 (br. *s*, H–C(1′)); 7.52 (br. *s*, H–C(2′)); 13.0 (*s*, HO–C(1)). ^{13}C-NMR (100 MHz, (D_6)acetone): 10.1 (C(4′)); 79.9 (C(1′)); 94.2 (C(4)); 98.7 (C(2)); 102.9 (C(9a)); 109.5 (C(7)); 111.1 (C(8a)); 127.7 (C(3′)); 128.7 (C(8)); 133.1 (C(5)); 147.1 (C(10a)); 151.5 (C(2′)); 151.6 (C(6)); 157.6 (C(4a)); 164.2 (C(3)); 166.2 (C(1)); 174.7 (C(5′)); 182.3 (C(9)). EI-MS: 97 (6), 69 (100), 51 (36). HR-ESI-MS: 379.0432 ($[M + Na]^+$, $C_{18}H_{12}NaO_8^+$; calc. 379.0429).

Bigarcinenone B (= rel-(*4a*R,*5*S,*14b*R)*-3,4a,5,14b-Tetrahydro-9,14-dihydroxy-2,4,4-trimethyl-5-(3,4,6,8-tetrahydroxy-9-oxo-9*H*-xanthen-1-yl)-4*H*,*13*H*-isochromeno[4,3-*b*]xanthen-13-one*; **2**). Yellow amorphous powder. UV (MeOH): 260 (4.21), 343 (4.20). ^{1}H- and ^{13}C-NMR: see *Table 1*. EI-MS: 295 (100), 91 (56), 69 (60), 57 (52). HR-ESI-MS: 659.1515 ($[M + Na]^+$, $C_{36}H_{28}NaO_{11}^+$; calc. 659.1529).

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