Two New Xanthones Isolated from the Stem Bark of *Garcinia lancilimba*

Nian-Yun YANG,[a] Quan-Bin HAN,[a] Xin-Wei CAO,[b] Chun-Feng QIAO,[a] Jing-Zheng SONG,[a] Shi-Lin CHEN,[b] Da-Jian YANG,[b] Hillary YIU,[a] and Hong-Xi XU*,[a]

[a] *Chinese Medicine Laboratory, Hong Kong Jockey Club Institute of Chinese Medicine; Hong Kong, P. R. China: and* [b] *Department of Applied Biology & Chemical Technology, The Hong Kong Polytechnic University; Hung Hom, Kowloon, Hong Kong, P. R. China.* Received March 3, 2007; accepted March 23, 2007

Two new xanthones, 1,5,6-trihydroxy-6′,6′-dimethyl-2*H*-pyrano(2′,3′:3,4)-2-(3-methylbut-2-enyl)xanthone (1) and 1,6,7-trihydroxy-6′,6′-dimethyl-2*H*-pyrano(2′,3′:3,2)-4-(3-methylbut-2-enyl)xanthone (2), have been isolated from the stem bark of *Garcinia lancilimba* (Guttiferae), together with six known xanthones. Their structures were identified on the basis of extensive spectral evidence including detailed 2D NMR and HR-MS data. Two new compounds showed moderate inhibitory effect on human breast cancer MDA-MB-435S cell line.

Key words *Garcinia lancilimba*; Guttiferae; cytotoxicity; xanthone

The medicinal plants of the genus *Garcinia*, which belongs to the Guttiferae family, are well known as rich natural resources of xanthones, biflavones, and benzophenones.[1)] These phenolic constituents have been reported to possess various biological activities, such as antibacterial activity,[2)] antimalarial activity,[3)] and cytotoxicity.[4)] There are twenty-one species in China. Some of them are native, such as *G. lancilimba* C. Y. WU ex Y. H. LI, *G. yunnanensis* HU, and *G. xishuangbannaensis* Y. H. LI, *etc*. These herbs have been seldom studied for their bioactive components, though they all have a long history of being used as herbal medicine by the local people.

In our serial research on the bioactive compounds of *Garcinia* plants, we have reported the anti-bacterial activity and related chemistry of the methanol extract of *Garcinia kola* HECKEL, which is used as chewing sticks in Nigeria.[5,6)] Recent reports from our lab have focused on the cytotoxicities,[7)] stability,[8)] and quantitative analysis[9)] of the prenylated xanthones of *G. hanburyi* whose major component, gambogic acid, has been developed as an anticancer drug in China. We also reported the separation and identification of several new polyprenylated xanthones, especially the C-2 epimers of gambogic acid and its analogues.[10—12)]

Our investigation on the phytochemistry of the stem barks of *G. lancilimba* has led to the isolation of eight prenylated xanthones including two new compounds. The structures of these compounds were elucidated by extensive spectral analysis including 2D NMR and HR-MS spectra. Two new compounds were assayed for their cytotoxicities against breast cancer MDA-MB-435S cell line with adriamycin as the positive control. This paper describes the isolation, structural elucidation, and bioassay results.

Results and Discussion

The $CHCl_3$ fraction of the stem bark of *G. lancilimba* were subjected to repeated column chromatography to yield two new prenylated xanthones (**1** and **2**) together with 6 known compounds (**3**—**8**) (Fig. 2).

Compound **1**, obtained as a yellow amorphous powder, showed a $[M+Na]^+$ peak at *m/z* 417.1323 in the high resolution electrospray ionization mass spectroscopy (HR-ESI-MS), corresponding to a molecular formula of $C_{23}H_{22}O_6$. The IR spectrum showed the presence of hydroxyl groups (3440 cm^{-1}), a conjugated carbonyl group (1633 cm^{-1}), and benzene rings (1587 cm^{-1}). The ^{1}H-NMR spectrum (Table 1) showed the characteristic signals of two *ortho*-aromatic protons [δ 6.99 (1H, d, J=8.4 Hz) and 7.66 (1H, d, J=8.4 Hz)], one phenolic hydroxyl group [δ 13.52 (1H, s)], one prenyl group [δ 3.32 (2H, d, J=7.4 Hz), 5.25 (1H, t, J=7.4 Hz), 1.66 (3H, s) and 1.81 (3H, s)], and a dimethylchromene ring [δ 1.49 (6H, s), and 5.74, 7.07 (each 1H, d, J=10.0 Hz)]. The ^{13}C and DEPT NMR spectra displayed the signals of 23 carbons including 13 quaternary carbons, 5 methine carbons, one methylene carbon, and four methyl carbons, which could be well assigned to a diprenylated xanthone skeleton. The six substituents on the xanthone skeleton were determined on the basis of the heteronuclear multiple bond connectivity (HMBC) spectral analysis (Fig. 2). In the HMBC spectrum, the hydrogen-bonded hydroxyl proton at δ 13.52 correlated with C-1 (δ 160.8), C-2 (δ 112.0), and C-9a (δ 103.1). A prenyl group was located at C-2, since its typical methylene protons (δ 3.32, 2H) showed a clear HMBC correlation with C-1. The *ortho*-aromatic protons at δ 7.66 and 6.99 were assigned as H-8 and H-7, respectively, according to the coupling between the proton at δ 7.66 and the carbonyl carbon C-9 (δ 181.3). The remaining dimethylchromene ring was assigned at C-4 and the oxygenated C-3, by the couplings between C-3 and H-4′ (δ 7.07) and the methylene protons of the prenyl group. These results established a partial structure of **1** as 1-hydroxy-2-prenyl-6′,6′-dimethyl-2*H*-pyrano-(2′,3′:3,4)xanthone. According to the determined molecular formula $C_{23}H_{22}O_6$, two hydroxyl groups were located at C-5 and C-6, though they did not exhibit clear signals in the ^{1}H-NMR spectrum. Thus, the structure of **1** was identified as 1,5,6-trihydroxy-2-prenyl-6′,6′-dimethyl-2*H*-pyrano-(2′,3′:3,4)xanthone.

Compound **2** was obtained as a yellow amorphous powder. The negative HR-ESI-MS showed the $[M-H]^-$ at *m/z* 393.1314 in correspondence with $C_{23}H_{22}O_6$, same as that of **1**. General analysis of its UV, IR, ^{1}H, ^{13}C and DEPT NMR spectra suggested that **2** was a close analogue of **1**, which was also a tri-hydroxylated xanthone with one prenyl group and one dimethylchromene ring. Similarly HMBC correlations were observed between the hydrogen-bonded hydroxyl proton at δ 13.52 correlated with C-1 (δ 156.1), C-2 (104.8), and C-9a (δ 103.5). Different from **1**, there was no *ortho*-

* To whom correspondence should be addressed. e-mail: xuhongxi@hkjcicm.org

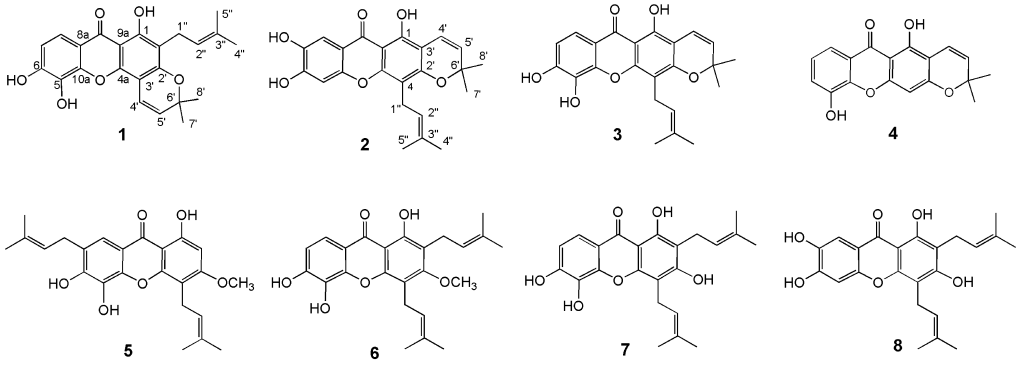

Fig. 1. Chemical Structures of **1**—**8**

Table 1. ^{1}H- and ^{13}C-NMR Data of **1** and **2** at 400 MHz (for ^{1}H-NMR) and 100 MHz (for ^{13}C-NMR) in $CDCl_3$, δ in ppm, J in Hz

	1		**2**	
	^{1}H	^{13}C	^{1}H	^{13}C
1		160.8		156.1
2		112.0		104.8
3		158.7		158.0
4		101.9		107.9
4a		150.9		155.2
5		133.0	7.01 s	103.6
6		152.4		152.7
7	6.99 d (8.4)	113.6		144.0
8	7.66 d (8.4)	117.8	7.55 s	109.2
8a		114.8		113.7
9		181.3		180.9
9a		103.1		103.5
10a		147.0		154.2
4′	7.07 d (10.0)	116.3	6.69 d (10.0)	116.2
5′	5.74 d (10.0)	127.7	5.73 d (10.0)	128.4
6′		78.8		78.7
7′	1.49 s	28.3	1.48 s	28.4
8′	1.49 s	28.3	1.48 s	28.4
1″	3.32 d (7.4)	21.7	3.46 d (7.4)	22.0
2″	5.25 t (7.4)	123.1	5.24 t (7.4)	123.2
3″		131.5		131.7
4″	1.81 s	18.0	1.89 s	18.1
5″	1.66 s	25.9	1.66 s	25.9
1-OH	13.52 s		13.52 s	

coupled aromatic proton on the xanthone skeleton of **2**. A singlet signal at δ 7.55 was assigned as H-8 by its HMBC correlation with C-9. The other aromatic proton (δ 7.01, s) was not assigned as H-7 but as H-5, according to its coupling with C-8a. The dimethylchromene ring was linked to C-2 and C-3 by the HMBC correlation of its olefinic proton with C-1, C-2, and C-3 (Fig. 1). The prenyl group was located at C-4 since the methylene protons coupled with C-3 and the olefinic proton correlated with C-4. Therefore, compound **2** was established as 1,6,7-trihydroxy-6′,6′-dimethyl-2*H*-pyrano(2′,3′:3,2)-4-(3-methylbut-2-enyl)xanthone.

Compounds **1** and **2** were tested for their cytotoxicities against human breast cancer MDA-MB-435S cells using the previously described method with adriamycin as the positive control (IC_{50}=0.24±0.01 μg/ml).[13] They showed moderate effects with IC_{50}=5.88±0.49, and 6.05±0.21 μg/ml, respectively.

The six known compounds were determined to be xanthone V_1 (**3**),[14] 6-deoxy-jacareubin (**4**),[15] parvifolixanthone B (**5**),[16] dulxanthone B (**6**),[17] xanthone V_{1a} (**7**),[14] cudratricusxanthone E (**8**),[18] by comparison of their spectral data (^{1}H- and ^{13}C-NMR, MS) with those reported.

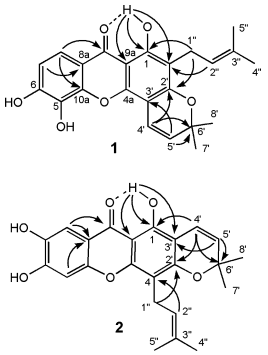

Fig. 2. Key HMBC Correlations of **1** and **2**

Experimental

General UV spectra were obtained on a Perkin-Elmer Lambda L14 spectrometer, IR spectra were obtained on a Perkin-Elmer 577 spectrometer with KBr disk. NMR spectra were recorded on a Bruker AV-400 spectrometer with TMS as internal standard. MS were measured on an Esqurie 2000 instrument. HR-ESI-MS were measured on an Esqurie 4000 instrument. Analytical and preparative HPLC was carried out on an Agilent 1100 system using three Altima C_{18} columns (5 μm, 4.6×250 mm, and 9.2×250 mm, and 10 μm, 22×250 mm).

Plant Material The stem bark of *Garcinia lancilimba* C. Y. Wu ex Y. H. Li, was collected from Yunnan province, China in 2006. It was identified by Dr. Chun-Feng. Qiao. A voucher specimen (CMS-0470) is deposited in the Herbarium of Hong Kong Jockey Club Institute of Chinese Medicine, Hong Kong, China.

Extraction and Isolation The air-dried stem bark of *G. lancilimba* (6.0 kg) was extracted with acetone. The acetone extract (265 g) was dissolved in hot water, and successively extracted with $CHCl_3$, EtOAc, and *n*-butanol. The $CHCl_3$ portion (83 g) was chromatographed over silica gel eluting with $CHCl_3$–CH_3OH (in gradient, 100 : 0 to 10 : 1, v/v) to yield fractions 1—4 on the basis of TLC analysis. Fraction 2 was separated by semi-preparative HPLC on Alltima C18 columns, eluting with 0.1% acetic acid/CH_3CN (40 : 60), to give **1** (15 mg), **2** (5 mg), **3** (35 mg), **4** (38 mg), **5** (18 mg). Compounds **6**—**8** (9, 3, and 2 mg, respectively) were isolated from fraction 3 by the same method.

1,5,6-Trihydroxy-2-prenyl-6′,6′-dimethyl-2*H*-pyrano(2′,3′:3,4)xanthone (**1**): Yellow amorphous powder, UV λ_{max} nm (log ε): 258 (4.53), 334 (4.13); IR ν_{max} cm^{-1}: 3440 (OH), 2973, 2926, 1633, 1587, 1435, 1328, 1288, 1122; ^{1}H- and ^{13}C-NMR [$(CD_3)_2CO$] (Table 1); ESI-MS *m/z*: 393 $[M-H]^-$ (100); HR-ESI-MS *m/z*: 417.1323 $[M+Na]^+$ (Calcd for $C_{23}H_{22}O_6Na$ 417.1314).

1,6,7-Trihydroxy-6′,6′-dimethyl-2*H*-pyrano(2′,3′:3,2)-4-(3-methylbut-2-enyl)xanthone (**2**): Yellow amorphous powder, UV λ_{max} nm (log ε): 296 (5.48), 341 (4.95); IR ν_{max} cm^{-1}: 3500 (OH), 2972, 2924, 1640, 1604, 1588, 1479, 1378, 1296, 1190; ^{1}H- and ^{13}C-NMR [$(CD_3)_2CO$] (Table 1); ESI-MS

m/z: 393 $[M-H]^-$ (100); HR-ESI-MS *m/z*: 393.1314 $[M-H]^-$ (Calcd for $C_{23}H_{21}O_6$ 393.1333).

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