Cytotoxic Polyprenylated Xanthones from the Resin of *Garcinia hanburyi*

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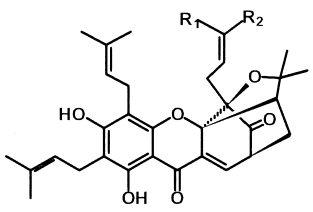
Thirteen xanthones (1—13) were isolated from the resin of *Garcinia hanburyi*. Among them, two new compounds (namely gaudichaudic acid, and isogambogenic acid, 1, 2), and one new natural product (deoxygaudichaudione A, 3) were identified on the basis of extensive spectral evidence including detailed 2D NMR data. Ten of these xanthones were tested for their cytotoxicities against human leukemia K562 (K562/S) and doxorubicin-resistant K562 (K562/R) cell lines, and showed similar inhibitory effects on both cell lines, suggesting that this group of polyprenylated xanthones might not be multidrug resistance (MDR) substrates.

Key words *Garcinia hanburyi*; cytotoxicity; xanthone; multidrug resistance; gaudichaudic acid; isogambogenic acid

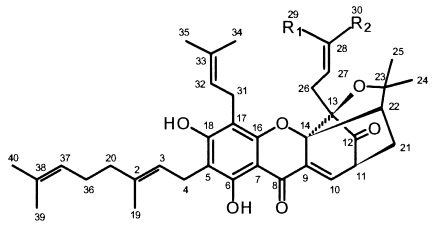
The resin of *Garcinia hanburyi* Hook. f., well-known as a natural fresh orange-yellow pigment, is used internally as a drastic purgative, an emetic and a vermifuge to treat tapeworm. For external use, the resin is applied for the treatment of chronic dermatitis, hemorrhoids, and bedsore. It had been developed as an anti-tumor drug for clinical testing *via* intravenous injection in China in the 1970s. In recent years, many pharmaceutical studies focused on its antitumor activities,[1—7] phytochemistry,[3,8,9] and related chemical stabilities.[10,11] In these previous research on the resin, gambogic acid, isogambogic acid, and relative xanthones were claimed to be the major bioactive constituents. In our phytochemical investigation on the xanthones of the resin, besides gambogic acid and isogambogic acid, eleven xanthones were isolated by repeated preparative high performance liquid chromatography (HPLC). They were identified to be two new compounds (namely gaudichaudic acid, and isogambogenic acid (**1**, **2**)), one new natural product (deoxygaudichaudione A (**3**)),[12] and ten known xanthones: gambogoic acid A (**4**),[13] gambogoic acid B (**5**),[13] isogambogic acid (**6**),[3] gambogenic acid (**7**),[8] desoxygambogenin (**8**),[8] isomorellic acid (**9**),[8] morellic acid (**10**),[8] desoxymorellin (**11**),[8] isomorellinol (**12**),[3] and gambogic acid (**13**) by extensive spectroscopic analysis including 2D NMR technique and comparison of their spectral data with those reported. Ten of these xanthones were tested for their cytotoxicities against human leukemia K562 (K562/S) and doxorubicin-resistant K562 (K562/ADR) cell lines. This paper describes the isolation, structural elucidation, and bioassay results of these xanthones.

Results and Discussion

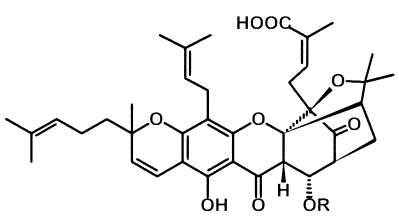
The high resolution electrospray ionization mass spec-



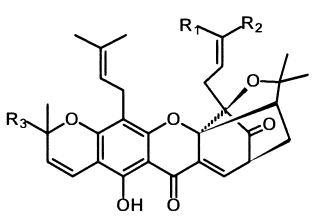
	R_1	R_2
Gaudichaudic acid **1**	COOH	CH_3
Deoxygaudichaudione A **3**	CH_3	CH_3
Gaudichaudione A **14**	CH_3	CHO



	R_1	R_2
Isogambogenic acid **2**	CH_3	COOH
Gambogenic acid **7**	COOH	CH_3
Deoxygambogenin **8**	CH_3	CH_3



	R
Gambogoic acid A **4**	CH_3
Gambogoic acid B **5**	CH_2CH_3



	R_1	R_2	R_3
Isogambogic acid **6**	CH_3	COOH	Prenyl
Isomorellic acid **9**	CH_3	COOH	H
Morellic acid **10**	COOH	CH_3	H
Desoxymorellin **11**	CH_3	CH_3	H
Isomorellinol **12**	CH_3	CH_2OH	H
Gambogic acid **13**	COOH	CH_3	Prenyl

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troscopy (HR-ESI-MS) of **1** presented a $[M+H]^+$ ion peak at *m/z* 563.2652 (Calcd 563.2644 for $C_{33}H_{39}O_8$), in equivalent with the molecular formula $C_{33}H_{38}O_8$. In the ^{1}H-NMR spectrum, there were three characteristic prenyl signals (δ 5.10, 5.19, 5.83, each 1H, t, J=7.0 Hz). The absence of a prenyl substitution was suggested by comparison with that of gambogic acid (GA). One of these three prenyls was located at C-5, similar to that of a known compound, gaudichaudione A (**14**) isolated from *G. gaudichaudii*,[14] according to the HMBC correlations among this prenyl's olefinic proton (δ 5.19), OH-6 (δ 12.81) and OH-18 (δ 6.48) with C-5 (δ 107.8). Comparison between the NMR data of **1** with those of the known gaudichaudione A indicated that these two compounds were very similar except the prenyl substitution at C-13. This prenyl's olefinic proton (δ 5.83) showed correlations with a carboxy carbon (δ 170.1) instead of an aldehyde carbon in the HMBC spectrum of **1**. It was then deduced that the aldehyde group in gaudichaudione A was replaced by a carboxy group in **1**. Furthermore, the olefinic bond of this prenyl group was determined to be *cis*-configuration by the NOE between the methyl protons (δ 1.72, 3H, s) and the olefinic proton. Therefore, compound **1** was elucidated to be as shown in Fig. 1, and named gaudichaudic acid. All the remaining ^{1}H- and ^{13}C-NMR data were clearly assigned based on the extensive 2D NMR spectra, which supported the deduced structure of **1**. Similarly, compound **3** was established to be a new natural product deoxygaudichaudione A, whose ^{1}H- and ^{13}C-NMR data were assigned unambiguously by analysis of its 2D NMR spectra.

Compound **2** was determined to have the same molecular formula $C_{38}H_{46}O_8$ as that of the known gambogenic acid (**7**) by its HR-MS (*m/z* 631.3282, Calcd 631.3270 for $C_{38}H_{47}O_8$, $[M+H]^+$). Detailed analysis of its HMBC spectra indicated that **2** had the same skeleton as that of **7**. However, there was not any NOE between the olefinic proton (δ 6.63) of the prenyl at C-13 of **2** and the methyl protons (δ 1.30, 3H, s) in the ROESY spectrum of **2**. This observation suggested **2** to be the *trans*-isomer of **7**. This deduction was confirmed by

Table 1. ^{1}H- and ^{13}C-NMR Data of **1**—**5** 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**		**3**		**4**		**5**	
	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C
2		134.4		139.0		135.2		81.1		81.1
3	5.19 t (7.0)	121.5	5.23 t (6.8)	121.3	5.22 t (7.0)	121.8	5.45 d (10.0)	124.8	5.43 d (10.0)	124.8
4	3.29 d (2H, 7.0)	21.2	3.38 m 2H	21.2	3.39 d (2H, 7.0)	21.5	6.68 d (10.0)	115.8	6.66 d (10.0)	115.8
5		107.8		107.6		107.4		102.6		102.6
6		160.4		160.4		160.3		156.3		156.3
7		100.7		100.7		100.8		101.7		101.7
8		179.2		179.1		179.7		193.7		193.8
9		135.1		133.4		133.8	3.18 s	47.9	3.23 s	48.5
10	7.53 d (6.8)	135.2	7.56 d (6.8)	135.6	7.45 d (6.8)	133.9	4.36 d (5.0)	74.0	4.44 d (4.4)	72.0
11	3.49 dd (6.8, 4.4)	46.9	3.49 m	46.9	3.49 dd (6.8, 4.4)	47.0	2.85 m	43.9	2.80 t (5.2)	44.2
12		203.3		203.2		203.6		208.6		208.6
13		84.0		83.6		84.6		82.2		82.4
14		90.5		90.5		90.3		88.4		88.3
16		155.9		156.0		156.3		155.7		155.6
17		106.2		106.7		106.2		108.7		108.6
18		163.5		163.6		163.0		161.2		161.2
19	1.78 s 3H	18.0	1.80 s 3H	16.2	1.82 s 3H	18.0	1.41 s 3H	27.7	1.41 s 3H	27.7
20	1.72 s 3H	25.7	2.06 m 2H	39.7	1.76 s 3H	25.8	1.63, 1.79 m	42.0	1.63, 1.79 m	42.0
21	2.32, 1.37 m	25.3	2.33, 1.42 m	25.3	2.33, 1.31 m	25.6	1.39 m 2H	19.9	1.39 m 2H	20.0
22	2.50 d (13.2)	48.9	2.52 d (9.2)	49.0	2.46 d (13.2)	49.1	2.50 d (8.0)	43.5	2.50 d (8.0)	43.5
23		83.9		83.7		83.2		86.3		86.4
24	1.29 s 3H	29.0	1.26 s 3H	28.9	1.28 s 3H	29.1	1.15 s 3H	27.2	1.15 s 3H	27.2
25	1.65 s 3H	29.7	1.70 s 3H	29.9	1.68 s 3H	30.1	1.36 s 3H	29.7	1.36 s 3H	29.7
26	3.14, 2.87 m	29.5	2.65, 2.56 m	28.9	2.55 d (7.0) 2H	29.7	3.15 m 2H	28.0	3.15 m 2H	28.0
27	5.83 t (7.0)	137.7	6.63 t (7.0)	136.9	4.42 t (7.0)	117.8	6.67 m	139.8	6.61 m	139.8
28		128.6		128.8		134.4		127.0		127.6
29		170.1	1.30 s 3H	11.4	1.01 s 3H	16.8		172.4		172.3
30	1.72 s 3H	20.8		172.4	1.37 s 3H	25.8	1.95 s 3H	20.5	1.96 s 3H	20.6
31	3.32 d (7.0)	22.1	3.35 m 2H	22.1	3.36 d (2H, 7.0)	22.1	3.23, 3.27 m	21.5	3.26 m 2H	21.5
32	5.10 t (7.0)	121.8	5.06 m	122.0	5.22 t (7.0)	121.6	5.04 m	122.5	5.03 m	122.5
33		135.1		133.8		135.0		131.2		131.3
34	1.74 s 3H	18.0	1.67 s 3H	18.0	1.79 s 3H	18.1	1.73 s 3H	18.0	1.73 s 3H	18.1
35	1.70 s 3H	25.8	1.62 s 3H	25.7	1.73 s 3H	25.9	1.62 s 3H	25.6	1.62 s 3H	25.6
36			2.11 m 2H	26.4			2.06 m 2H	22.7	2.06 m 2H	22.7
37			5.14 m	123.9			5.10 m	123.7	5.09 m	123.7
38				131.8				131.9		131.9
39			1.59 s 3H	17.7			1.55 s 3H	17.6	1.55 s 3H	17.6
40			1.70 s 3H	25.7			1.65 s 3H	25.6	1.65 s 3H	25.6
6-OH	12.81 s		12.79 s		12.95 s		12.76 s		12.75 s	
18-OH	6.48 s				6.47 s					
OCH_3							3.31 s 3H	55.8		
$OC\underline{H}_2CH_3$									3.45, 3.56 m	63.7
$OCH_2C\underline{H}_3$									1.11 t (7.0) 3H	15.0

Table 2. The Inhibitory Effects of **1**—**4** and **7**—**12** against K562/ADR and K562/S Cell Lines (IC_{50}, μg/ml±S.E.M.)

	K562/ADR	K562/S	RF
1	0.61±0.05	0.41±0.03	1.5
2	2.86±0.16	2.10±0.14	1.4
3	3.04±0.18	1.74±0.22	1.7
4	1.65±0.12	1.38±0.04	1.2
7	3.01±0.45	2.41±0.13	1.2
8	2.43±0.13	0.91±0.04	2.7
9	1.86±0.13	0.91±0.07	2.0
10	2.29±0.14	1.48±0.09	1.5
11	1.53±0.05	0.64±0.03	2.4
12	0.62±0.03	0.57±0.04	1.1
ADM	1.79±0.17	0.11±0.01	16.8

comparison of the ^{1}H-NMR data of other pairs of *E*,*Z*-isomers, *e.g.* gambogic acid (**13**) and isogambogic acid (**6**), and isomorellic acid (**9**) and morellic acid (**10**). In the *trans*-isomers, the olefinic proton H-27 was in the downfield beyond δ 6.50, while in the *cis*-isomers it significantly upfield shifted to δ 6.00. Compound **2** displayed this signal at δ 6.63, while that of **7** was at δ 5.85. Therefore, compound **2** was deduced to be the *trans*-isomer of **7**, and named isogambogenic acid.

Compounds **4** and **5** had been revealed to be the derivative of the addition of a molecular methanol (or ethanol) to the olefinic bond between C-9 and C-10 of gambogic acid (**13**),[11] and named gambogoic acid A and gambogoic acid B, respectively. Different from other reported analogues from *Garcinia* plants like isomorreolin B and morreolic acid,[8] however, H-9 of **4** and **5** was assigned as β-orientated by the clearly displayed NOEs among H-9, H-10, and H-21 in the NOESY spectra. On the basis of 2D NMR techniques, their NMR data were completely assigned for the first time (Table 1).

Ten of these xanthones (**1**—**4**, **7**—**12**) were tested for their cytotoxicities against human leukemia K562 (K562/S) and doxorubicin-resistant K562 (K562/ADR) cell lines, with doxorubicin (ADM) as the positive control. All the tested xanthones showed considerable cytotoxicities against both human leukemia K562 cell lines. Their resistant fold (RF, also called the ratio of doxorubicin IC_{50}) between K562/ADR and K562/S are much lower than that of doxorubicin, suggesting these xanthones to be non-substrates of MDR transporter.

Experimental

General ESI-MS spectra were recorded on a VGAuto Spec-3000 spectrometer. 1D- and 2D-NMR spectra were obtained on Brucker AM-400 and DRX-50 instruments with TMS as an internal standard. Analytical and preparative HPLC was carried out on an Agilent 1100 system using three Altima C_{18} (5 μm, 4.6×250 mm, and 9.2×250 mm, and 10 μm, 22×250 mm) columns.

Plant Material The resin of *Garcinia hanburyi* was collected from Shanxi province, China in 2004. It was identified by Dr. Chun-Feng. Qiao. The identification was confirmed by chromatographic comparison with reference standard herb which was provided by National Institute of the Control of Pharmaceutical and Biological Products (NICPBP) of China. A voucher specimen (CMS-0284) is deposited in the Herbarium of Hong Kong Jockey Club Institute of Chinese Medicine, Hong Kong, China.

Extraction and Isolation The dried gum resin (20 g) was powdered and dissolved in acetone (20 ml) and then filtered with a 0.25μm filter. The filtrate was loaded on HPLC system through the column (10 μm, 22×250 mm) using $CNCH_3/H_2O$ (85 : 15) as mobile phase. The detection UV wavelength was set at 360 nm. Ten fractions were collected according to their retention times. Fractions 3—8 were further loaded on HPLC using a smaller column (5 μm, 9.2×250 mm), eluting with different aqueous acetonitrile solution, respectively. As a result, compounds **4**—**6** and **13** were isolated from fr. 7 and 8, compounds **2** and **7** from fr. 6, **8**—**12** from fr. 3—5. Compound **1** was obtained using the analytical column (5 μm, 4.6×250 mm) from fr. 2 in the similar chromatographic condition.

Gaudichaudic Acid (**1**): A bright yellow amorphous powder. $[\alpha]_D^{28}$ −535° (c=0.065, $CHCl_3$). ESI-MS *m*/*z*: 563 $[M+H]^+$; HR-ESI-MS *m*/*z*: 563.2652 $[M+H]^+$, (Calcd 563.2644 for $C_{33}H_{39}O_8$).

Isogambogenic Acid (**2**): A bright yellow amorphous powder. $[\alpha]_D^{20}$ −488° (c=0.290, $CHCl_3$). ESI-MS *m*/*z*: 631 $[M+H]^+$; HR-ESI-MS *m*/*z*: 631.3282 $[M+H]^+$, (Calcd 631.3270 for $C_{38}H_{47}O_8$).

Cytotoxicity Assay Both drug-sensitive K562 (K562/S) cell line and doxorubicin-resistant cell subline (K562/R) were purchased from Tianjin Institute of Hematopathy. K562/ADR and K562/S cells were cultured in PRMI1640 (Gibco) medium, supplemented with 10 fetal bovine serums at 37 °C in a humidified incubator with 5% CO_2. SRB assay was performed as described in literatures[15,16]: Cells were seeded into 96-well plates at 4000 viable cells per well. Blank wells and control wells were set up. The tested compound with different concentrations was loaded in a final volume of 200 μl per well. After 44 h incubation, the plates were centrifuged at the speed of 2000 rpm for 10 min. Afterwards, the medium was removed. The cells were fixed by addition of 100 μl of cold 16% trichloroacetic acid (TCA, 4 °C) in each well. The plate was incubated at 4 °C for 1 h before being gently washed five times with tap water to remove TCA. The plates were air dried. Then, 40 μl of 0.4% w/v SRB dissolved in 1% acetic acid in water was added to each well for 30 min. At the end of the staining period, unbound SRB was removed by washing four times with 1% acetic acid. The plate was air dried again, and 100 ml of 10 mM aqueous Tris base [tris(hydroxymethyl)aminomethane] (pH 10.0) was added into each well to solubilize the cell-bound dye. The plate was shaken for 15 min on a gyratory shaker followed by a reading of the optical density (OD) at 570 nm in a microplate spectrophotometer. The mean value of each concentration (n=3 wells) was obtained. Absorbance of untreated controls was taken as 100%. Survival rate was calculated as follows: Cell survival rate (%)=$(T-B)/(U-B)\times100\%$, T (treated) is the absorbance of compound in treated cell, U (untreated) is the absorbance of untreated cells, and B (blank) is the absorbance when neither cells nor compound was added. The IC_{50} values of the compound to K562 cells were calculated by using sigmoidal plot.

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