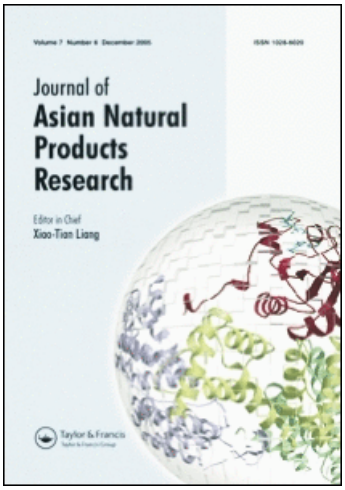

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Five novel prenylated xanthones from *Resina Garciniae*

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Fourteen prenylated xanthone derivatives were isolated from gamboge, the dry latex of *Garcinia hanburyi,* and their structures were elucidated by a detailed spectroscopic analysis. Five of them, isogambogenic acid (**1**), desoxymorellinin (**2**), 10-methoxygambogenic acid (**3**), 10-methoxygambogic acid (**4**) and 10-ethoxy gambogic acid (**5**), are new compounds. All of them showed potent cytotoxicity against HL-60, SMMC-7721 and BGC-83 cells.

Keywords: *Resina Garciniae*; *Guttiferae*; Prenylated xanthone; Isogambogenic acid; Desoxymorellinin; 10-methoxygambogenic acid

1. Introduction

Gamboge, which is the juice secreted from the trunk of *Garcinia* L. Planch, e.g. *Garcinia hanburyi* Hook. and *Garcinia morella* Gesv., is used in traditional Chinese medicine for removing stasis, detoxification, haemostasis, and as an anthelmintic [1]. Chemical studies on gamboge started in the 1960s. A series of polyprenylated xanthonoids have been isolated. A common feature of these compounds is the presence of a bicyclo[2.2.2]octane or tricyclo-4-oxa[4.3.1.0]decan-2-one as part of the xanthonoids [2]. We isolated five new compounds, isogambogenic acid (**1**), desoxymorellinin (**2**), 10-methoxygambogenic acid (**3**), 10-methoxygambogic acid (**4**) and 10-ethoxygambogic acid (**5**), along with 9 known compounds [3] gambogic acid, morellic acid, gambogenic acid, gambogin, desoxygambogenin, desoxymorellin, moreollic acid, gambogellic acid, and hanburin, from gamboge. These new compounds showed significant cytotoxic activity. The UV, IR, MS, and NMR spectral data indicated that all of them were prenylated xanthonoids having a complex caged structural moiety.

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2. Results and discussion

Compound **1** (isogambogenic acid) was obtained as yellow gum. HRESI-MS showed $[M - H]^-$ at *m/z* 629.3107, corresponding to the molecular formula $C_{38}H_{46}O_8$. The UV spectrum exhibited maximum absorption at 359 nm. The IR spectrum showed the presence of hydroxyl (3444 cm^{-1}), carbonyl (1736 cm^{-1}), α,β-unsaturated carboxyl (1688 cm^{-1}), combined carbonyl (1634 cm^{-1}), and phenyl (1601 cm^{-1}) groups. The ^{1}HNMR spectrum (table 2) of **1** showed signals of 8 methyl groups at δ 1.26, 1.29, 1.35, 1.58, 1.67, 1.70, 1.74, and 1.80 (each 3H, s), five alkene proton signals at δ 7.56 (1H, d, $J = 6.9$ Hz, 10-H), 6.63 (1H, m, 27-H), 5.22 (1H, m), 5.14 (1H, m), and 5.06 (1H, m), and the signal of 6-OH at δ 12.79 (1H, s) which disappeared after D_2O exchange. The ^{13}C NMR and DEPT spectral data (table 1) revealed 38 carbon signals including eight CH_3, six CH_2, and seven tertiary carbons. These data were almost the same as those of the known compound gambogenic acid (GGA, table 1) [4]. But a significant downfield shift for H-27 (δ 6.63, $\Delta\delta = 0.8$) and upfield shifts for H-26 (δ 2.53 and 2.63, $\Delta\delta = -0.37$ and -0.66) and CH_3-29 (δ 1.35, $\Delta\delta = -0.35$), comparing with the corresponding signals of gambogenic acid, strongly suggested that the difference of two compounds, similar to the difference between gambogic acid (GBA) and isogambogic acid [4], was in the side-chain bearing the carboxyl group. Thus **1** should also be a geometric isomer of gambogenic acid.

The observation of a significant upfield shift of the C-29 (δ 11.2, $\Delta\delta = -9.5$) due to non-bonding steric interaction (γ effect) with the methylene group at C-26, further represented a *E*-configuration for the double bond between C-27 and C-28. The presence of a correlation between H-26 and methyl group at C-29 in the ROESY spectrum confirm the stereochemistry of this double bond as *E* [4]. Similarly, the presence of the correlation between H-3 and C-20 suggested the double bond between C-2 and C-3 is *E*-configuration too. So, the structure of **1** was elucidated as the geometric isomer of gambogenic acid, named isogambogenic acid (figure 1).

Compound **2** (desoxymorellinin) was isolated as yellow gum. The molecular formula $C_{33}H_{40}O_6$, consistent with 14 degrees of unsaturation, was determined by HRESI-MS which showed a molecular ion peak at *m/z* 555.2784 $[M + Na]^+$. Comparing with the spectral data of **1**, the lack of the signals for an α,β-unsaturated carbonyl group in IR (1688 cm^{-1}) and ^{13}C NMR (δ 171.7) spectra of **2** indicated that the substituent group at C-30 should be a methyl group, similar to the known compound desoxygambogenin. Comparison of the ^{13}C NMR data of **2** with those of desoxygambogenin [3] revealed that two compounds were similar, except for the side-chain located at C-5, in which deoxygambogenin has two isoprenyl segments, while **2** has only one. That was also proved by the correlations between H-4 (δ 3.34 (2H, m) and C-5 (δ 105.8), C-6 (δ 156.0), C-2 (δ 134.7), and C-3 (δ 121.3) in the HMBC spectrum. So the structure of **2** was determined as 20-deisoprenyl-desoxygambogenin, named desoxymorellinin (figure 1).

The NMR spectral data of **3** were very similar to those of gambogenic acid. The absence of C-10 methenyl proton in GGA, along with the appearance of a methoxyl group (δ_H 3.30 (3H, s), δ_C 55.8) in compound **3** suggested a methoxyl group was linked to C-10, that was proved by analysis of HMBC spectrum, in which H-10 (δ 4.37 (1H, dd, $J = 4.7$, 1.3 Hz)) correlated with C-8 and C-12. The α-orientation of the methoxyl group was confirmed by its NOESY spectrum [4], in which obvious NOE signals could be observed between H-9 and H-11, H-10 and H-21, respectively. Thus the structure of compound **3** was established as 10-methoxygambogenic acid (figure 1).

Table 1. The ^{13}CNMR data of compounds **1**–**5** and gambogenic acid (GGA).

	GGA	*1*	*2*	*3*	*4*	*5*
2	139.0	138.9	134.7	139.2	81.0	81.2
3	121.4	121.0	121.3	121.6	125.0	124.9
4	39.7	39.4	21.9	39.8	115.8	115.9
5	106.5	106.4	105.8	106.5	102.8	102.8
6	155.9	155.7	156.0	159.4	156.4	156.4
7	100.7	100.4	100.5	102.1	101.8	101.9
8	178.1	178.8	179.4	193.8	193.7	193.9
9	133.7	133.6	134.9	43.4	43.6	43.6
10	133.6	133.2	133.6	74.1	74.0	72.2
11	46.9	46.7	46.7	43.9	43.9	44.4
12	203.3	202.8	203.2	208.3	208.6	208.5
13	83.8	83.4	82.9	82.3	82.1	82.4
14	90.5	90.3	90.0	88.4	88.5	88.4
16	163.6	163.3	162.7	154.0	155.8	155.8
17	107.5	107.4	107.1	108.4	108.8	108.8
18	160.4	160.1	160.0	163.4	161.2	161.3
19	16.2	15.8	16.5	16.2	27.2	27.3
20	26.4	26.1	25.5	26.6	41.9	42.2
21	25.7	25.1	25.5	25.7	20.0	20.0
22	49.0	48.8	48.9	48.0	47.9	48.6
23	84.0	83.3	84.3	86.5	86.4	86.5
24	28.9	28.7	28.6	27.2	27.2	27.8
25	29.9	29.7	29.8	29.8	29.8	29.8
26	29.6	29.4	28.8	27.9	28.0	28.1
27	137.8	136.7	117.5	137.7	140.0	138.6
28	128.2	128.4	134.1	127.3	126.9	127.7
29	20.7	11.2	25.2	20.0	20.5	20.7
30	171.5	171.7	17.6	171.9	172.7	171.4
31	21.14	20.9	21.8	20.6	21.5	21.6
32	121.4	121.7	121.3	121.6	122.6	122.7
33	131.8	131.6	133.6	131.6	131.2	131.4
34	25.7	25.4	25.2	25.8	25.6	25.7
35	18.0	17.7	17.8	18.0	18.1	18.1
36	22.1	21.8		22.0	22.7	22.8
37	123.9	123.6		124.1	123.7	123.8
38	135.1	135.2		135.6	131.8	132.0
39	25.7	25.4		25.7	25.6	25.7
40	17.7	17.4		17.7	17.6	17.7
OMe				55.8	55.8	
OEt						63.8
						15.1

Compound **4** showed the same characteristics as **3**; it has similar NMR data to gambogic acid except for the difference from the 10-methoxyl group (δ_H 3.30 (3H, s), δ_C 55.8) and C-8–C-12. The α-orientation of the methoxyl group was also confirmed by its NOESY spectrum [4], in which obvious NOE correlations could be observed between H-9 and H-11, H-10 and H-21, respectively. Based on the above evidence, the structure of **4** was determined as 10-methoxygambogic acid (figure 1).

The spectral data of **5** were almost identical to those of **4**, except for the substituent group at C-10. The existence of an ethoxyl group at C-10 was proved by ^{1}H NMR and ^{13}C NMR data (δ_H 3.44–3.55 (2H, m), 1.11 (3H, t, $J = 7.1$ Hz), δ_C 63.8, 15.1). The α-orientation of this group was also confirmed by the cross peak between H-9 and H-11, H-10 and H-21 in its NOESY spectrum [4]. So compound **5** was identified as 10-ethoxygambogic acid (figure 1).

Table 2. The ^{1}HNMR data of compounds **1**–**5**.

No. H	***1***	***2***	***3***	***4***	***5***
3	5.22 m	5.21 m	5.25 (t, 7.0)	5.45 (d, 10.0)	5.43 (d, 10.2)
4	3.40 m (2H)	3.34 m (2H)	1.96 m (2H)	6.65 (d, 10.0)	6.66 (d, 10.2)
9			3.12 m	3.16 m	3.15 m
10	7.56 (d, 6.9)	7.44 m	4.37 (dd, 4.7, 1.3)	4.34 (d, 4.0)	4.44 (dd, 4.6, 1.1)
11	3.48 (dd, 6.9, 4.3)	3.47 (dd, 6.7, 4.7)	2.85 (br.t, 4.7)	2.82 m	2.79 (t, 5.3)
19	1.80 s (3H)	1.78 s (3H)	1.61 s (3H)	1.35 s (3H)	1.14 s (3H)
20	2.08 m (2H)	1.73 s (3H)	2.03–2.12 m (4H)	1.70 m	1.79 m
				1.72 m	1.59 m
21	2.33 (dd, 13.4, 4.6)	2.32 (dd, 15.4, 4.7)	1.97 m	1.98 m	1.95 m
	1.38 m	1.32 m	1.42 m	1.39 m	1.38 m
22	2.51 (br.d, 9.3)	2.44 (d, 9.4)	2.50 (d, 8.5)	2.48 (d, 8.6)	2.50 (d, 8.4)
24,25-Me	1.70 s (3H)	1.68 s (3H)	1.71 s (3H)	1.36 s (3H)	1.41 s (3H)
	1.29 s (3H)	1.27 s (3H)	1.34 s (3H)	1.14 s (3H)	1.25 s (3H)
26	2.63 m	2.52–2.58 m (2H)	3.31–3.41 m (2H)	3.18–3.24 m (2H)	3.18 m (2H)
	2.53 m				
27	6.63 m	4.44 m	6.61 (t, 6.2)	6.67 (t, 6.2)	6.60 (t, 6.9)
29	1.35 s (3H)	1.37 s (3H)	1.98 s (3H)	1.96 s (3H)	1.96 s (3H)
30		1.03 s (3H)			
31	3.34 m (2H)	3.37 m (2H)	3.15–3.25 m (2H)	3.16–3.21 m (2H)	3.26 m (2H)
32	5.14 m	5.21 m	5.07 (t, 6.2)	5.02 (t, 6.2)	5.01 (t, 6.8)
34,35-Me	1.74 s (3H)	1.81 s (3H)	1.82 s (3H)	1.73 s (3H)	1.73 s (3H)
	1.67 s (3H)	1.75 s (3H)	1.74 s (3H)	1.66 s (3H)	1.62 s (3H)
36	2.05 m		2.03–2.12 m (4H)	2.08 m (2H)	2.04 (dd, 15.8, 5.9)
	1.72 m				
37	5.06 m		5.11 (t, 6.2)	5.10 (t, 6.2)	5.06 (t, 5.9)
39, 40-Me	1.58 s (3H)		1.81 s (3H)	1.62 s (3H)	1.65 s (3H)
	1.26 s (3H)		1.68 s (3H)	1.56 s (3H)	1.55 s (3H)
6-OH	12.79 s	12.92 s		11.92 s	11.93 s
2-OH		6.42 s			
OMe			3.30 s (3H)	3.30 s (3H)	
OEt					3.44–3.55 m (2H)
					1.11 (t, 7.1, 3H)

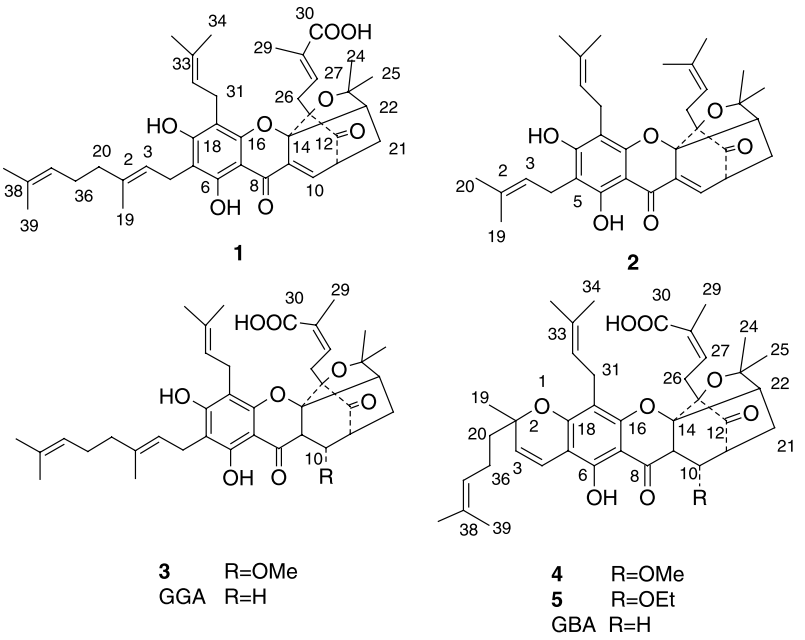

Figure 1. The structures of compounds **1**–**5**, gambogic acid (GBA), and gambogenic acid (GGA).

According to the above data and reported compounds, the presence of 9,10-double bond in these compounds could be proved by the UV absorption near 360 nm, and the 1,2-pyran could be confirmed by the UV absorption near 290 nm [4].

The median inhibition concentrations (IC_{50}, mmol/L) of five new compounds against cultured HL-60, SMMC-7721 and BGC-83 cells are given in table 3. Compared with gambogic acid (GBA), these compounds showed lower cytotoxicities against three kinds of cultured cells.

3. Experimental

3.1 General experimental procedures

IR spectra were obtained by a Nicolet Impact 410 IR spectrophotometer KBr disk; UV spectra were recorded on a Shimadzu UV-260 spectrophotometer; Optical rotations were measured with a Perkin–Elmer 241MC polarimeter; MS data were gained by Agilent 1100

Table 3. Cytotoxicity of compounds **1**–**5** against three kinds of cultured cancer cell lines (IC_{50}, mmol/L).

	Compound					
Cell line	***1***	***2***	***3***	***4***	***5***	*GBA*
HL-60	1.544×10^{-4}	1.725×10^{-6}	1.096×10^{-6}	1.322×10^{-6}	3.996×10^{-6}	8.610×10^{-7}
SMMC-7721	5.942×10^{-3}	1.131×10^{-4}	5.277×10^{-5}	3.887×10^{-6}	7.962×10^{-5}	1.054×10^{-6}
BGC-83	4.327×10^{-5}	2.269×10^{-5}	3.035×10^{-6}	2.641×10^{-6}	3.649×10^{-5}	1.200×10^{-6}

HPLC-ESI-MS; ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz, $CDCI_3$ and TMS as internal standard) were recorded on a Brucker DRX-500 NMR spectrometer. The ODS (15–35, 40–60 μm) for column chromatography was the product of Dikma Corp.

3.2 Plant material

The gamboge resin of *G. hanburyi* was bought in Nanjing, Jiangsu province, China, in 1999. The voucher specimen was identified by the authors and deposited at Department of Phytochemistry, China Pharmaceutical University.

3.3 Isolation of the xanthone derivatives

The dried gum resin (30 g) was extracted according to the method proposed by Chen [5]. Gambogic acid was obtained after filtering, and the mother liquid was acidified with 0.1 M HCl, then extracted with EtOAc. The solvent was evaporated under reduced pressure to obtain a gummy residue (6.8 g), which was chromatographed on a column of silica gel (400 g) with a gradient elution using petroleum with increasing proportions of EtOAc to give nine fractions. Fraction III was purified over a column of ODS to give compound **1** (7 mg), Fraction V was subjected to ODS column chromatography eluted with MeOH/H_2O (6:4) to yield compound **2** (10 mg). Repeated chromatography of fraction VI on a column of ODS eluted gradiently by the component solvent of MeOH and water to obtain compounds **3** (27 mg) and **5** (8 mg). Compound **4** (12 mg) was purified with a ODS column chromatography from fraction VIII.

3.3.1 Isogambogenic acid (1). Yellow gum; UV λ_{max}^{MeOH} nm: 292 (sh), 359. IR ν_{max}^{KBr} (cm^{-1}): 3444, 2972, 2927, 1736, 1688, 1634, 1601, 1582, 1456, 1444, 1372, 1175, 1116. ^{13}C NMR (in $CDCl_3$, 125 MHz): see table 1. ^{1}H NMR (in $CDCl_3$, 500 MHz): see table 2. HRESI-MS *m/z* 629.3107 $[M - H]^-$ (calcd for $C_{38}H_{45}O_8$, 629.3114).

3.3.2 Desoxymorellinin (2). Yellow gum, $[\alpha]_D^{30}$ −108 (*c* 0.08, MeOH), UV λ_{max}^{MeOH} nm: 358. IR ν_{max}^{KBr} (cm^{-1}): 3417, 2974, 2927, 1738, 1633, 1606, 1440, 1383, 1174, 1136. ^{13}C NMR (in $CDCl_3$, 125 MHz): see table 1. ^{1}H NMR (in $CDCl_3$, 500 MHz): see table 2. HRESI-MS *m/z* 555.2784 $[M + Na]^+$ (calcd for $C_{33}H_{40}O_6Na$, 555.2723).

3.3.3 10-methoxygambogenic acid (3). Yellow gum, $[\alpha]_D^{30}$ −142 (*c* 0.17, MeOH), UV λ_{max}^{MeOH} nm: 296. IR ν_{max}^{KBr} (cm^{-1}): 3452, 2969, 2929, 1742, 1684, 1644, 1628, 1585, 1452, 1444, 1378, 1180, 1111. ^{13}C NMR (in $CDCl_3$, 125 MHz): see table 1. ^{1}H NMR (in $CDCl_3$, 500 MHz): see table 2. HRESI-MS *m/z* 663.3517 $[M + H]^+$ (calcd for $C_{39}H_{51}O_9$, 663.3532).

3.3.4 10-methoxygambogic acid (4). Yellow gum, $[\alpha]_D^{30}$ −140 (*c* 1.0, MeOH), UV λ_{max}^{MeOH} nm: 278, 318. IR ν_{max}^{KBr} (cm^{-1}): 3435, 2972, 2927, 1742, 1688, 1641, 1628, 1584, 1453, 1440, 1376, 1175, 1112. ^{13}C NMR (in $CDCl_3$, 125 MHz): see table 1. ^{1}H NMR (in $CDCl_3$,

500 MHz): see table 2. HRESI-MS *m/z* 683.2928 $[M + Na]^+$ (calcd for $C_{39}H_{48}O_9Na$, 683.3196).

3.3.5 10-ethoxygambogic acid (5). Yellow gum, UV λ_{max}^{MeOH} nm: 279, 318. IR ν_{max}^{KBr} (cm^{-1}): 2972, 2926, 1743, 1687, 1644, 1628, 1584, 1455, 1437, 1375, 1251, 1178, 1109. ^{13}C NMR (in $CDCl_3$, 125 MHz): see table 1. ^1H NMR (in $CDCl_3$, 500 MHz): see table 2. HRESI-MS *m/z* 697.3374 $[M + Na]^+$ (calcd for $C_{40}H_{50}O_9Na$, 697.3352).

3.4 Cell growth inhibition

By colorimetric MTT assay, the logarithmic cells were dispersed with 0.02% EDTA to prepare cell suspension, and partitioned into wells of 96-well plates at 100 μl/well for 4 h culture in a 5% CO_2 incubator under 37°C. The cell culture wells were then exposed to different concentrations GA (100 μl/well). After 20, 44 and 68 h culture, 5 mg/ml MTT solution (20 μl/well) was added. After culture for 4 h, the supernatant was discarded and DMSO was added (100 μl/well). The suspension was placed on a micro-vibrator for 5 min and the absorbance (*A*) was measured at λ 570 nm by an enzyme immunoassay instrument (DJ-3200, Huadong Electron Tube Co.). Cell inhibitory ratio was calculated by the following formula:

$$\text{Inhibitory ratio}\,(\%) = \frac{\text{Average absorbance of treated group}}{(1 - \text{Average absorbance of control group})} \times 100\%$$

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