A Pair of Novel Cytotoxic Polyprenylated Xanthone Epimers from Gamboges

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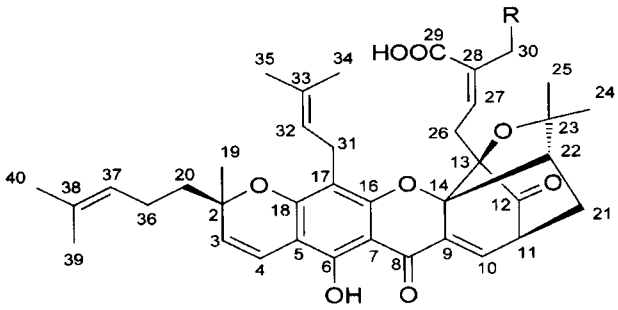
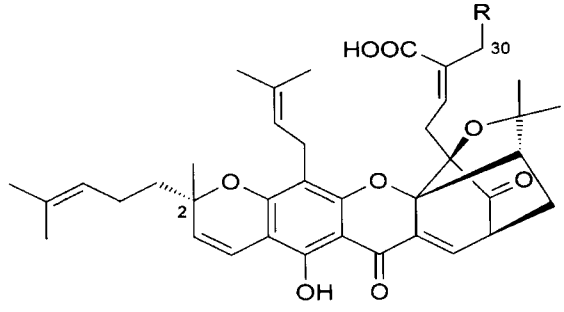
Two new polyprenylated xanthone epimers were isolated from gamboges of *Garcinia hanburyi*, and identified by detailed spectroscopic analysis as 30-hydroxygambogic acid (**2a**) and its (2*S*)-epimer 30-hydroxyepigambogic acid (**2b**). Both compounds exhibited significant cytotoxicities against the human leukemia K562/S and the corresponding doxorubicin-resistant K562/R cell lines (*Table 2*).

Introduction. – Gamboges, the resin from various *Garcinia* species, including *G. morella* and *G. hanburyi*, is rich in antitumor gambogic acid[1]) [1–7]. This compound had always been isolated as an inseparable C(2)-epimeric mixture **1a**/**1b**, whose structure could not be determined completely, until the (*R*)-epimer (**1a**) was obtained by crystallization of its pyridine salt and identified by single-crystal X-ray diffraction [8][9]. In our previous report, the (2*S*)-epimer **1b**, also called epigambogic acid, was separated from the (2*R*)-epimer for the first time [10]. Both epimers show similarly strong cytotoxicities against human leukemia K562/S and doxorubicin-resistant K562/R cell lines. They have been suggested as potential chemotherapeutic drugs that are not substrates of the multidrug-resistance (MDR) transporter. More interestingly, these



1a R = H
2a R = OH



1b R = H
2b R = OH

[1]) *CAS* No. 2752-65-0.

two epimers exhibit different inhibitory effects towards the cytochrome P450 (CYP) enzyme CYP2C9, the (2*S*)-epimer **1b** being a 20-fold stronger inhibitor than the corresponding (2*R*)-epimer.

Our continued search for related (epimeric) gambogic acid derivatives by means of HPLC/ESI-MS analysis now led to the isolation of the novel C(2)-epimeric compounds **2a,b**. Their structures were elucidated by detailed analyses of spectroscopic data, including HR-MS and 2D-NMR. In this paper, we report their isolation, structure elucidation, configuration, and cytotoxic properties towards two types of K562 cell lines.

Results and Discussion. – 1. *Structure Elucidation.* The epimeric compounds **2a** and **2b**, initially obtained as a 2:3 mixture after preparative reverse-phase HPLC separation on a C_{18} column, were further separated on a C_8 column. Both compounds exhibited the $[M+H]^+$ peak at *m/z* 645 in HPLC/ESI-MS analyses, indicating a molecular formula of $C_{38}H_{44}O_9$. Accordingly, they contained an additional O-atom compared to gambogic acid (**1a**). The ^{1}H-, ^{13}C-, and 2D-NMR spectra of **2a,b** were almost identical, suggesting epimeric compounds. On the basis of careful spectroscopic analyses and detailed comparison with the spectroscopic data previously reported for gambogic acid epimers, the new compounds were identified as 30-hydroxygambogic acid (**2a**) and 30-hydroxyepigambogic acid (**2b**).

In the ^{13}C-NMR spectra of **2** (*Table 1*), four clear resonances of oxygenated quaternary C-atoms were observed at δ(C) *ca.* 80–90 due to C(2), C(13), C(14), and C(23). These signals are characteristic for the polyprenylated skeleton of gambogic acid. In the ^{1}H-NMR spectra of **2**, there were characteristic signals at δ(H) 7.56 (*d*) and two coupled resonances at 5.44 (*d*, $J=10$ Hz) and 6.62 (*d*, $J=10$ Hz), assignable to H–C(10), H–C(3), and H–C(4), respectively. Three additional signals at δ(H) 6.39 (*t*), 5.04 (*t*), and 5.10 (*t*), all with *J* values of 7.6 Hz, were due to H–C(27), H–C(37), and H–C(32), respectively, which indicated great structural similarity to **1**.

A detailed comparison of NMR data finally revealed the unique structural differences of **2** from **1**. In the ^{1}H- and ^{13}C-NMR spectra of **2**, there were signals for one additional oxygenated CH_2 group [δ(C) 64.7; δ(H) 4.09, 4.01 (2*d*, $J=13.2$ Hz each) for **2a**, and 4.13, 4.04 (2*d*, $J=13.2$ Hz each) for **2b**] instead of a Me resonance found in the spectra of **1**. We, therefore, concluded that one of the Me groups of **1** was oxygenated in **2**.

The additional oxygenated CH_2 group was located at C(30), based on HMBC correlations of the CH_2 H-atoms with the carboxy C(29) atom at δ(C) 169.9 and two olefinic C-atoms (C(27) and C(28)). Furthermore, H–C(27) exhibited clear HMBC couplings with a non-oxygenated CH_2 C-atom (C(26)), an oxygenated quaternary C-atom (C(13)), and the above-mentioned resonances for C(28), C(29), and C(30), respectively, which confirmed this deduction. All the ^{1}H- and ^{13}C-NMR (DEPT) signals could be fully assigned (*Table 1*), based on a detailed analysis of COSY, HMQC, HMBC, and ROESY spectra. In addition, from key NOEs between CH_2(30) and H–C(27), the (*Z*)-configuration of the pertinent C=C bond was inferred, which is the same as in **1**. Thus, compounds **2** were identified as epimeric 30-hydroxy derivatives of gambogic acid.

The configuration at C(2) of **2a,b** was determined by comparing the key ^{1}H- and ^{13}C-NMR spectroscopic patterns with those of the reported C(2)-epimers of **1** [10]. In

Table 1. *^{1}H- and ^{13}C-NMR Data of Compounds* **2a** *and* **2b**. At 400 (^{1}H) and 100 MHz (^{13}C), resp., in C_5D_5N, δ in ppm, J in Hz.

	2a			**2b**		
	^{1}H	^{13}C	HMBC	^{1}H	^{13}C	HMBC
C(2)		81.4	3, 4, 19, 20, 36		81.3	4, 19, 20, 36
H–C(3)	5.36 (*d*, *J* = 10.0)	124.7	4, 19, 20	5.44 (*d*, *J* = 10.0)	125.0	4, 19, 20
H–C(4)	6.57 (*d*, *J* = 10.0)	115.7	3	6.62 (*d*, *J* = 10.0)	115.8	3
C(5)		102.8	3, 4		103.0	3, 4
C(6)		157.4	4		157.5	4
C(7)		100.5			100.5	
C(8)		179.0	10		179.1	10
C(9)		133.2	10, 11		133.1	10, 11
H–C(10)	7.53 (*d*, *J* = 6.8)	135.8	11, 21	7.56 (*d*, *J* = 6.8)	135.8	11, 21
H–C(11)	3.48 (*m*)	46.8	10, 21, 22	3.49 (*m*)	46.9	10, 21, 22
C(12)		203.2	10, 11, 26		203.1	10, 11, 26
C(13)		83.7	11, 21, 26, 27		83.6	11, 21, 26, 27
C(14)		90.9	10, 26		90.9	10, 26
C(16)		157.3	31		157.4	31
C(17)		107.7	31, 32		108.0	31, 32
C(18)		161.6	4, 31		161.5	4, 31
Me(19)	1.36 (*s*)	27.7	3, 20	1.35 (*s*)	27.6	3, 20
CH_2(20)	1.57, 1.74 (2*m*)	41.9	3, 19, 36, 37	1.57, 1.74 (2*m*)	41.7	3, 19, 36, 37
CH_2(21)	2.34, 1.40 (2*m*)	25.1	10, 11, 22	2.34, 1.40 (2*m*)	25.1	10, 11, 22
H–C(22)	2.52 (*d*, *J* = 9.2)	48.9	11, 21, 24, 25	2.53 (*d*, *J* = 9.2)	48.9	11, 21, 24, 25
C(23)		84.1	21, 22, 24, 25		84.1	21, 24, 25
Me(24)	1.24 (*s*)	28.8	22, 25	1.29 (*s*)	28.8	22, 25
Me(25)	1.67 (*s*)	29.9	22, 24	1.70 (*s*)	29.9	22, 24
CH_2(26)	3.00 (*d*, *J* = 6.8)	29.1	27	2.98 (*d*, *J* = 6.8)	29.1	27
H–C(27)	6.39 (*t*, *J* = 7.6)	140.5	26, 30	6.39 (*t*, *J* = 7.6)	140.5	26, 30
C(28)		131.0	26, 27, 30		131.2	26, 27, 30
C(29)		169.9	27, 30		169.9	27, 30
CH_2(30)	4.09, 4.01 (2*d*, *J* = 13.2 each)	64.7	27	4.13, 4.04 (2*d*, *J* = 13.2 each)	64.7	27
CH_2(31)	3.27, 3.14 (2*m*)	21.6	32	3.30, 3.16 (2*m*)	21.6	32
H–C(32)	5.01 (*t*, *J* = 7.6)	122.0	31, 34, 35	5.04 (*t*, *J* = 7.6)	122.0	31, 34, 35
C(33)		131.8	31, 32, 34, 35		131.7	31, 32, 34, 35
Me(34)	1.70 (*s*)	18.1	32, 25	1.74 (*s*)	18.2	32, 25
Me(35)	1.61 (*s*)	25.6	32, 34	1.64 (*s*)	25.7	32, 34
CH_2(36)	2.00 (*m*)	22.7	20, 37	2.08 (*m*)	22.5	20, 37
H–C(37)	5.01 (*t*, *J* = 7.6)	123.7	20, 36, 39, 40	5.10 (*t*, *J* = 7.6)	123.7	20, 36, 39, 40
C(38)		131.8	36, 37, 39, 40		132.3	36, 37, 39, 40
Me(39)	1.52 (*s*)	17.6	37, 40	1.59 (*s*)	17.6	37, 40
Me(40)	1.62 (*s*)	25.6	37, 39	1.67 (*s*)	25.7	37, 39
6-OH	12.74 (*s*)			12.77 (*s*)		

the ^{1}H-NMR spectrum of (2*R*)-configured gambogic acid proper (**1a**), previously identified by X-ray diffraction, the signals of H–C(37) and H–C(32) were completely overlapping at δ(H) 5.02. In the spectrum of (2*S*)-configured epigambogic acid (**1b**), however, two completely separated signals had been observed at δ(H) 5.07 and 5.00 (2*t*,

$J = 7.0$ Hz each) for H–C(37) and H–C(32), respectively. Furthermore, the ^{13}C-NMR signal for C(38), which was completely isochronic with that of C(33) in **1a**, had been found to be significantly shifted downfield in the (2*S*)-epimer **1b**. These differences, regarded as the key NMR spectroscopic characteristics to distinguish the two epimers, were also observed in the corresponding spectra of **2a** and **2b**. Small downfield shifts of H–C(37) from δ(H) 5.01 in **2a** to 5.10 in **2b**, and, similarly, of C(38) from δ(C) 131.8 to 132.3, were clearly observed. Accordingly, **2a** was assigned the (2*R*)-configuration, and **2b**, thus, corresponded to the (2*S*)-epimer.

2. *Biological Studies.* The (2*R*)-epimer **2a** exhibited considerable cytotoxic activities against human leukemia K562/S and doxorubicin-resistant K562/R cell lines, with IC_{50} values of 1.27 and 2.89 μg/ml, respectively (*Table 2*), doxorubicin being used as positive control ($IC_{50} = 0.11$ and 1.79 μg/ml, resp.). The (2*S*)-epimer **2b** was slightly less active than **2a** towards these two cell lines, giving rise to IC_{50} values of 3.61 and 4.49 μg/ml, respectively. Just as gambogic acid (**1a**) and epigambogic acid (**1b**), compounds **2a** and **2b** might not be substrates of the MDR transporter [10]. Based on the data given in *Table 2*, epimerization at C(2) of **2** has a relatively small effect on their cytotoxicity. Also note that, compared to the parent compounds **1**, the 30-hydroxylated congeners **2** showed somewhat reduced activities (*Table 2*).

Table 2. *Cytotoxicities of Gambogic Acid Derivatives against Two Types of Human Leukemia K562 Cell Lines*

Compound	IC_{50} [μM]	
	K562/R	K562/S
2a	2.89 ± 0.35	1.27 ± 0.15
2b	4.49 ± 0.31	3.61 ± 0.17
Doxorubicin[a])	1.79 ± 0.17	0.11 ± 0.01
Gambogic acid (**1a**)[b])	1.32	0.89
Epigambogic acid (**1b**)[b])	1.11	0.86
Doxorubicin[a])[b])	10.78	0.66

[a]) Positive control. [b]) Published data [10], tested at different exposure times.

Experimental Part

General. 1D- and 2D-NMR Spectra: *Brucker AM-400* and *DRX-500* spectrometers; δ in ppm, J in Hz, in C_5D_5N soln., with Me_4Si as internal standard. MS: *VG Autospec-3000* spectrometer; in *m/z* (rel. %). LC/MS Analysis: *Agilent 1100*, combined with a *Micromass Q-TOF-2* spectrometer.

Plant Material. The resin of *Garcinia hanburyi* was purchased in Guangzhou, P. R. China. A voucher specimen (CMS-0283) was deposited at the Herbarium of the Hong Kong Jockey Club Institute of Chinese Medicine, Hong Kong, China.

Extraction and Isolation. The resin (1 g) was dissolved in acetone (10 ml), and purified by prep. HPLC (*Alltima* C_{18}, 10 μm, 22 × 250 mm; 0.1% aq. H_3PO_4/MeOH 10:90; flow rate 1 ml/min, UV detection at 360 nm) to afford a mixture of **2a**,**b** (40 mg; t_R 8.5 min). Further purification by prep. HPLC (*Alltima* C_8, 5μm, 9.2 × 250 mm; 0.1% aq. AcOH/50% aq. 1,4-dioxane/MeCN 25:10:65) yielded **2a** (6 mg) and **2b** (8 mg).

30-Hydroxygambogic Acid (**2a**). Yellow, amorphous powder, barely sublimable. $[\alpha]_D^{28} = -500.6$ ($c = 0.314$, $CHCl_3$). ^{1}H- and ^{13}C-NMR: see *Table 1*. ESI-MS (pos.): 645 ($[M+H]^+$). HR-ESI-MS (pos.): 645.3059 ($C_{38}H_{45}O_9$; calc. 645.3063).

30-Hydroxyepigambogic Acid (**2b**). Yellow, amorphous powder, barely sublimable. $[\alpha]_D^{28} = -405.6$ ($c = 0.288$, $CHCl_3$). ^1H- and ^{13}C-NMR: see *Table 1*. ESI-MS: 645 ($[M+H]^+$). HR-ESI-MS: 645.3054 ($C_{38}H_{45}O_9^+$; calc. 645.3063).

Cytotoxicity Assay. Both epimers of **2** were tested for their cytotoxicities against human leukemia K562/S and doxorubicin-resistant K562/R cell lines, using the SRB method, as previously described [11][12], with doxorubicin as pos. control. IC_{50} Values were calculated from sigmoidal plots of the optical density (OD) data.

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