A Highly Rearranged Pentaprenylxanthonoid from the Resin of *Garcinia hanburyi*

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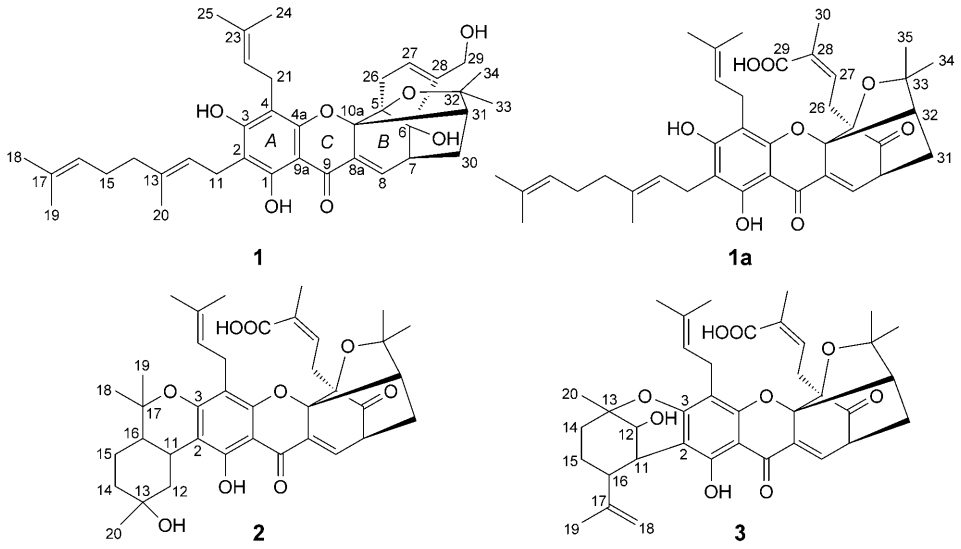
Gamboketanol (**1**), a highly rearranged pentaprenylated xanthonoid, two new caged pentaprenylated xanthonoids, gambogefic acid A (**2**) and gambogellic acid A (**3**), together with two known compounds, were isolated from the acetone extract of the resin of *Garcinia hanburyi*. Their structures were established on the basis of extensive spectroscopic and mass-spectrometric analyses. The cytotoxicity of compounds **1**–**3** against HeLa tumor cell line was evaluated, with all of them being modestly active.

Introduction. – Gamboge, the gum-resin from stems of *Garcinia hanburyi* HOOK. F., is used as a natural pigment or a folk medicine as a potent purgative and against infected wounds [1]. A series of caged polyprenylated xanthones, isolated from the resin, has been reported [2–9], which were considered as the main active ingredients of gamboge, with gambogic acid being the major component [10–13]. In our continuing search for biologically active and structurally unique compounds from medicinal plants, a highly degraded and rearranged pentaprenylxanthonoid, named gamboketanol (**1**), two hitherto unknown caged polyprenylated xanthonoids, namely gambogefic acid A (**2**) and gambogellic acid A (**3**), along with the two known epimers 30-hydroxygambogic acid and 30-hydroxyepigambogic acid [6], were isolated from the acetone extract of the resin of *G. hanburyi*. Their *in vitro* cytotoxic activity against the HeLa tumor cells was investigated. Here, we report the isolation and structure elucidation of compounds **1**–**3**, as well as their cytotoxic activity.

Results and Discussion. – 1. *Structure Elucidation.* Compound **1** was obtained as a yellow gum. Its molecular formula was determined to be $C_{37}H_{46}O_7$ by HR-ESI-MS (*m*/*z* 603.3312 ([*M* + H]$^+$; calc. 603.3322)), 28 mass units less than gambogenic acid (**1a**) [3], a major constituent of *G. hanburyi*. Its ^{1}H- and ^{13}C-NMR spectra, in conjunction with the HSQC spectrum, revealed the presence of seven Me, seven CH_2, and seven CH groups, as well as 16 quaternary C-atoms. Comparison of the NMR data of **1** with those of **1a** revealed the presence of an O-bearing quaternary C-atom (δ(C) 91.7) and a CH_2O group (δ(C) 59.6), but the absence of a saturated CO group, a COOH group, and a Me group. With only 37 C-atoms in the structure instead of the usual 38 C-atoms for a pentaprenylxanthonoid, **1** was likely to be a degraded xanthonoid arising from decarboxylation and rearrangement.



The NMR data of rings *A* and *C*, and the substituents on ring *A* of **1** were very similar to those of **1a**, indicating that they are endowed with the same partial structure in this region. The HMBC interactions H–C(11)/C(2) and H–C(21)/C(4) confirmed the connections of a geranyl group and of a prenyl group to C(2) and C(4), respectively, as in **1a**. The HMBCs of H–C(7) with C(5), C(6), C(8), and C(8a), and of H–C(8) with C(6), C(7), and C(10a) indicated the presence of ring *B*, which showed the only difference from **1a** by an O-bearing quaternary C-atom (δ(C) 91.7, C(6)) in **1** *vs.* the presence of a non-conjugated ketone in **1a**. The HMBC interactions of H–C(30) with C(6), C(7), C(8), C(10a), C(31), and C(32), of H–C(31) with C(7), C(10a), C(32), and C(34), and of Me(33) and Me(34) with C(31) and C(32) supported the presence of the caged structure, which represents the structural feature of the gambogenic acids. The aforementioned features suggested that the degradation and rearrangement took place in the prenyl group at C(5) and the ketone C-atom C(6) in **1a**. The HMBCs of H–C(29) with C(27) and C(28), of H–C(27) with C(5), C(26), C(28), and C(29), and of H–C(26) with C(5), C(6), and C(10a) indicated the connection of the degraded prenyl group to C(5). The HMBCs of H–C(29) with C(5), and of H–C(27) with C(6) evidenced the linkage of C(6) and C(28), which was further supported by the molecular weight and unsaturated degrees of **1**.

The *Scheme* outlines the postulated biosynthesis pathway of **1**, which represents a further support for its structure. Thus, formula **1** was established for gamboketanol.

Compound **2** had the molecular formula of $C_{38}H_{46}O_9$ as deduced from the HR-ESI-MS (*m*/*z* 647.3228 ([*M* + H]$^+$; calc. 647.3220)), indicating a pentaprenylxanthonoid. The ^{1}H- and ^{13}C-NMR data (*Tables 1* and *2*) of **2** were compared with those of gambogefic acid [9]. The only difference between them was the presence of a OH group at C(13) in **2** instead of a C(12)=C(13) bond in gambogefic acid. The ^{1}H,^{1}H-COSY experiment indicated a contiguous spin system comprising H–C(12)/H–C(11)/H–C(16)/H–C(15)/H–C(14), together with the HMBCs of H–C(12), H–C(14), and

Scheme. *Postulated Biosynthetic Pathway Leading to* **1**

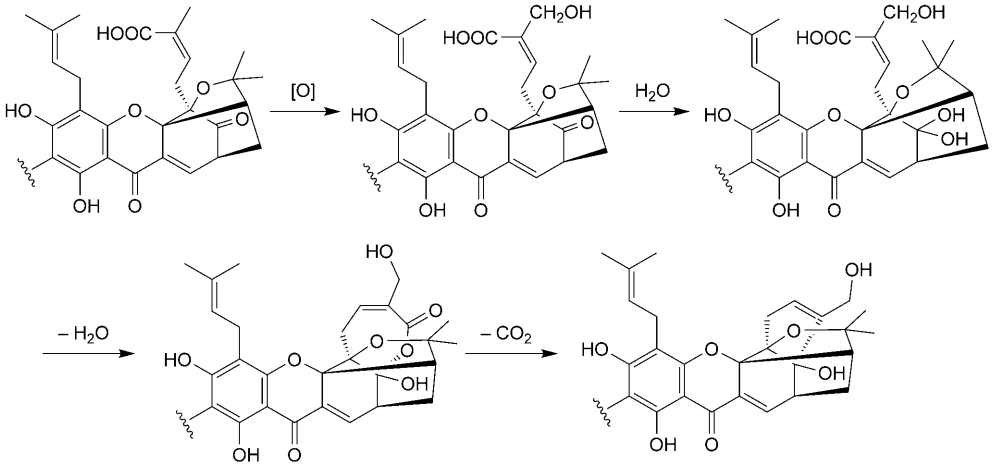


Table 1. *^{1}H-NMR Data of Compounds* **1**–**3**. In $CDCl_3$; δ in ppm, J in Hz.

Position	**1**	**2**	**3**
1-OH	13.01 (*s*)	13.92 (*s*)	12.67 (*s*)
3-OH	6.50 (*s*)		
7	3.16 (*dd*, *J* = 3.2, 6.8)	3.45[a])	3.46 – 3.50 (*m*)
8	7.52 (*d*, *J* = 7.2)	7.57 (*d*, *J* = 6.6)	7.45 (*d*, *J* = 6.9)
11	3.37 (*d*, *J* = 7.2)	3.56 – 3.62 (*m*)	3.60 – 3.65 (*m*)
12	5.23[a])	1.65 – 1.71, 1.80 – 1.86 (2*m*)	3.84 (*d*, *J* = 2.1)
14	2.06[a])	1.52 – 1.55, 2.02 – 2.10 (2*m*)	1.60 – 1.63, 1.97 – 2.05 (2*m*)
15	2.08[a])	1.50 – 1.53, 1.64 – 1.68 (2*m*)	1.28 – 1.36 (*m*)
16	5.05 (*t*, *J* = 6.4)	1.53[a])	2.29[a])
18	1.67 (*s*)	1.54 (*s*)	4.28, 4.62 (*s*)
19	1.59 (*s*)	1.12 (*s*)	1.90 (*s*)
20	1.80 (*s*)	1.35 (*s*)	1.48 (*s*)
21	3.32 – 3.45 (*m*)	2.96 – 3.05, 3.34 – 3.38 (2*m*)	3.22 – 3.32 (*m*)
22	5.21[a])	4.95 – 4.99 (*m*)	5.16 (*t*, *J* = 6.6)
24	1.77 (*s*)	1.72 (*s*)	1.75 (*s*)
25	1.70 (*s*)	1.63 (*s*)	1.67 (*s*)
26	2.42[a])	2.52 – 2.60, 3.46 – 3.50 (2*m*)	2.77 – 2.85, 3.21 – 3.29 (2*m*)
27	5.47 (br. *s*)	6.23 – 6.28 (*m*)	5.54 – 5.60 (*m*)
29	4.12 (*dd*, *J* = 12.8, 26.4)		
30	1.29 – 1.35, 2.41 – 2.47 (2*m*)	1.72 (*s*)	1.61 (*s*)
31	2.19 (*d*, *J* = 10.0)	1.35 – 1.40, 2.25 – 2.31 (2*m*)	1.38 – 1.44, 2.28 – 2.34 (2*m*)
32		2.49 (*d*, *J* = 9.3)	2.54 (*d*, *J* = 9.6)
33	1.57 (*s*)		
34	1.77 (*s*)	1.28 (*s*)	1.28 (*s*)
35		1.69 (*s*)	1.69 (*s*)

[a]) Overlapping.

Table 2. *^{13}C-NMR Data of Compounds* **1**–**3**. In $CDCl_3$; δ in ppm.

Position	**1**	**2**	**3**	Position	**1**	**2**	**3**
1	160.1 (*s*)	158.6 (*s*)	162.8 (*s*)	17	131.9 (*s*)	73.3 (*s*)	146.4 (*s*)
2	106.9 (*s*)	105.7 (*s*)	100.2 (*s*)	18	25.7 (*q*)	29.0 (*q*)	109.6 (*t*)
3	163.1 (*s*)	164.3 (*s*)	163.4 (*s*)	19	17.7 (*q*)	27.9 (*q*)	23.0 (*q*)
4	106.3 (*s*)	107.2 (*s*)	106.4 (*s*)	20	16.2 (*q*)	28.4 (*q*)	23.7 (*q*)
4a	157.0 (*s*)	155.7 (*s*)	155.9 (*s*)	21	22.2 (*t*)	21.7 (*t*)	21.9 (*t*)
5	84.3 (*s*)	84.2 (*s*)	84.6 (*s*)	22	122.0 (*d*)	122.2 (*d*)	122.3 (*d*)
6	91.7 (*s*)	203.7 (*s*)	204.2 (*s*)	23	133.6 (*s*)	131.2 (*s*)	131.5 (*s*)
7	37.4 (*d*)	46.6 (*d*)	47.0 (*d*)	24	18.0 (*q*)	18.2 (*q*)	18.2 (*q*)
8	142.6 (*d*)	135.5 (*d*)	134.3 (*d*)	25	25.8 (*q*)	25.7 (*q*)	25.7 (*q*)
8a	131.0 (*s*)	133.4 (*s*)	134.3 (*s*)	26	35.1 (*t*)	27.9 (*t*)	29.4 (*t*)
9	180.5 (*s*)	179.0 (*s*)	179.0 (*s*)	27	128.9 (*d*)	137.9 (*d*)	136.2 (*d*)
9a	100.5 (*s*)	100.2 (*s*)	100.1 (*s*)	28	143.5 (*s*)	126.9 (*s*)	128.2 (*s*)
10a	91.9 (*s*)	91.1 (*s*)	90.1 (*s*)	29	59.6 (*t*)	168.0 (*s*)	168.6 (*s*)
11	21.2 (*t*)	26.2 (*d*)	36.6 (*d*)	30	26.1 (*t*)	21.4 (*q*)	21.0 (*q*)
12	121.4 (*d*)	38.4 (*t*)	71.3 (*d*)	31	48.6 (*d*)	24.8 (*t*)	25.1 (*t*)
13	139.1 (*s*)	77.0 (*s*)	79.5 (*s*)	32	84.1 (*s*)	49.1 (*d*)	48.8 (*d*)
14	39.7 (*t*)	39.8 (*t*)	38.2 (*t*)	33	28.1 (*q*)	83.8 (*s*)	83.6 (*s*)
15	26.3 (*t*)	19.4 (*t*)	22.0 (*t*)	34	30.2 (*q*)	28.7 (*q*)	28.8 (*q*)
16	123.7 (*d*)	52.4 (*d*)	48.2 (*d*)	35		29.8 (*q*)	29.9 (*q*)

H–C(20) with C(13), confirming the attachment of the OH group to C(13). The ROESY correlations H–C(11)/H–C(16) suggested the *cis*-relative configuration of these two H-atoms. The structure of **2** was thus elucidated as gambogefic acid A.

Compound **3** was assigned the molecular formula $C_{38}H_{44}O_9$ by HR-ESI-MS (*m*/*z* 645.3055 ($[M + H]^+$; calc. 645.3064)), 16 mass units more than that of gambogellic acid [3]. Careful comparison of the ^{1}H- and ^{13}C-NMR data of **3** with those of gambogellic acid revealed that they only differ from each other by an OH-bearing CH group in **3** (δ(H) 3.84 (*d*, *J* = 2.1, H–C(12)); δ(C) 71.3, C(12)) *vs.* a CH_2 group in gambogellic acid. The HMBCs of H–C(12) with C(2), C(11), and C(13), in conjunction with the fragment of CH(12)–CH(11)–CH(16)–CH_2(15)–CH_2(14) established by the ^{1}H,^{1}H-COSY spectrum, supported the structure assignment (*Tables 1* and *2*). The relative configuration of the isopropenyl-substituted six-membered ring of **3** was elucidated by means of ROESY spectrum and a computer-generated 3D structure, which was obtained by Chem 3D Ultra V 9.0, with MM2 forcefield calculations for energy minimization (*Fig.*). Key ROESY correlations of H–C(11) with H–C(12), H–C(16), and Me(19) and of H–C(12) with Me(20) were observed. Thus, the relative configuration of **3** was elucidated as shown in the *Figure*, and the structure was established as gambogellic acid A.

The structures of the two known compounds, 30-hydroxygambogic acid and 30-hydroxyepigambogic acid, were identified by comparison of their spectroscopic data with literature values [6].

2. *Biological Studies.* Compounds **1**–**3** were evaluated for their cytotoxic activity against the HeLa human cervical cell lines *in vitro* by means of the MTT (= 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2-tetrazolium bromide) method according to the protocols described in [14] with adriamycin as the positive control (IC_{50} = 0.07 μM

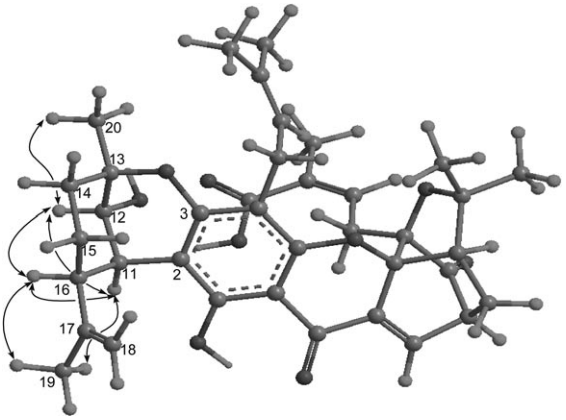


Figure. *Key ROESY* (H ↔ H) *correlations of compound* **3**

against HeLa cells). The IC_{50} values in the HeLa cell system were 3.82, 2.11, and 1.73 μM for **1**, **2**, and **3**, respectively.

Experimental Part

General. Column chromatography (CC): silica gel (SiO_2; 200–300 mesh; *Qing-dao Hai-yang Chemical Co., Ltd.*) and *Sephadex LH-20* gel (*Amersham Biosciences*). TLC: precoated silica-gel plates (*Yan-tai Zi Fu Chemical Group Co.*). Reversed-phase (RP) HPLC: *Agilent 1100* series liquid chromatograph using a *VWD G1314A* detector at 230 nm, and a semi-prep. *Exlipse XDB-C_{18}* (5 μm, 9.4 × 250 mm) column (*Agilent*) was employed for the purification. Optical rotation: in $CDCl_3$; *Perkin-Elmer 341* polarimeter. UV Spectra: *Shimadzu UV-2450* spectrophotometer; λ_{max} (log ε) in nm. IR Spectra: *Perkin-Elmer 577* spectrometer; KBr pellets; in cm^{-1}. NMR Spectra: *Bruker DRX-400* instrument; at 400 (^1H) or 100 MHz (^{13}C); in $CDCl_3$; δ in ppm, *J* in Hz. ESI- and HR-ESI-MS: *Finnigan LCQ-Deca* and *Waters Micromass Q-TOF-Ultima* mass spectrometers, in *m*/*z*.

Plant Material. The gamboge resin of *G. hanburyi* was purchased in Shanghai, China, in September 2007, and identified by Prof. *D.-A. G.* A voucher specimen (SC0091010) was deposited with the Herbarium of Shanghai Research Center for TCM Modernization, Shanghai Institute of Materia Medica, Chinese Academy of Sciences.

Extraction and Isolation. The dried gum resin of *G. hanburyi* (800 g) was powdered and extracted with acetone (3 × 4 l) at r.t. for 2 d. The filtered soln. was concentrated *in vacuo* to give a brown residue (550 g), which was chromatographed on a SiO_2 column eluted successively with a petroleum ether (PE)/acetone gradient (100 : 0 to 0 : 100) to obtain seven fractions. *Fr. 6* (70 g) was subjected to CC (SiO_2; $CHCl_3$/MeOH 100 : 0 to 4 : 1), *Sephadex LH-20* (PE/$CHCl_3$/MeOH 2 : 1 : 1), and prep. HPLC (MeOH/0.1‰CF_3COOH 86 : 14) to afford compounds **1** (22 mg), **2** (8 mg), and **3** (11 mg). *Fr. 7* (10 g) was repeatedly subjected to *Sephadex LH-20* CC (PE/$CHCl_3$/MeOH 2 : 1 : 1) to obtain 30-hydroxygambogic acid (32 mg) and 30-hydroxyepigambogic acid (23 mg).

Gamboketanol (=*(11a*R**)-8-[(2*E*)-3,7-Dimethylocta-2,6-dien-1-yl]-3a,4,12,13-tetrahydro-3a,7,9-trihydroxy-3-(hydroxymethyl)-13,13-dimethyl-10-(3-methylbut-2-en-1-yl)-4,12-methanocyclopenta[c]furo[2,3-d]xanthen-6(1H)-one*; **1**). Yellow gum. $[\alpha]_D^{26} = -4$ ($c = 0.106$, $CHCl_3$). UV (MeOH): 204 (4.41), 340 (4.07). IR (KBr): 3415, 2966, 2920, 2854, 1637, 1603, 1442, 1340, 1132, 850. ^1H- and ^{13}C-NMR ($CDCl_3$): see *Tables 1* and *2*, resp. ESI-MS (pos.): 603.3 ($[M + H]^+$). ESI-MS (neg.): 601.3 ($[M - H]^-$). HR-ESI-MS: 603.3312 ($[M + H]^+$, $C_{37}H_{47}O_7^+$; calc. 603.3322).

Gambogefic Acid A (=*(2Z)-4-[(5*R**,16a*S**)-3a,4,5,7,9,10,11,12,12a,13-Decahydro-8,10-dihydroxy-3,3,10,13,13-pentamethyl-15-(3-methylbut-2-en-1-yl)-7,17-dioxo-3*H,*8b*H-*1,5-methanofuro[3,4-g]isochro-*

*meno[4,3-*b*]xanthen-1-yl]-2-methylbut-2-enoic Acid*; **2**). Yellow gum. $[\alpha]_D^{26} = -611$ ($c = 0.095$, $CHCl_3$). UV (MeOH): 215 (4.58), 361 (4.20). IR (KBr): 3448, 2952, 2930, 2854, 1736, 1633, 1589, 1456, 1431, 1377, 1329, 1172, 1149, 1049, 856. ^{1}H- and ^{13}C-NMR ($CDCl_3$): see *Tables 1* and *2*, resp. ESI-MS (pos.): 647.2 ($[M + H]^+$). ESI-MS (neg.): 645.2 ($[M - H]^-$). HR-ESI-MS: 647.3228 ($[M + H]^+$, $C_{38}H_{47}O_9^+$; calc. 647.3220).

Gambogellic Acid A (=*(2*Z*)-2-Methyl-4-[3a,4,5,7,10,11,12,13-Octahydro-(5*R**,16a*S**)-8,17-dihydroxy-3,3,13-trimethyl-15-(3-methylbut-2-en-1-yl)-7,18-dioxo-10-(prop-1-en-2-yl)-3*H,9H*-1,5 : 9,13-dimethanofuro[3,4-*g*]oxocino[3,2-*b*]xanthen-1-yl]but-2-enoic Acid*; **3**). Yellow gum. $[\alpha]_D^{26} = -344$ ($c = 0.090$, $CHCl_3$). UV (MeOH): 216 (4.59), 361 (4.21). IR (KBr): 3433, 2952, 2928, 2854, 1736, 1633, 1593, 1431, 1379, 1327, 1172, 1142, 877, 754. ^{1}H- and ^{13}C-NMR ($CDCl_3$): see *Tables 1* and *2*, resp. ESI-MS (pos.): 645.2 ($[M + H]^+$). ESI-MS (neg.): 643.2 ($[M - H]^-$). HR-ESI-MS: 645.3055 ($[M + H]^+$, $C_{38}H_{45}O_9^+$; calc. 645.3064).

The research work was financially supported by the *National Science & Technology Major Project 'Key New Drug Creation and Manufacturing Program', China* (Nos. 2009ZX09308-005, 2009ZX09311-001, 2009ZX09502-020, and 2009ZX09304-002) and the *National Science Foundation of Shanghai*, P. R. China (No. 10ZR1436400).

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Received November 24, 2009