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CYTOTOXIC XANTHONES FROM *GARCINIA HANBURYI*

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Key Word Index—*Garcinia hanburyi*; Guttiferae; gamboge; xanthone; cytotoxicity.

Abstract—Eleven novel cytotoxic xanthones, gambogin, morellin dimethyl acetal, isomoreollin B, moreollic acid, gambogenic acid, gambogenin, isogambogenin, desoxygambogenin, gambogenin dimethyl acetal, gambogellic acid and hanburin were isolated together with four known xanthones, gambogic acid, isomorellin, morellic acid and desoxymorellin, from the dry latex of *Garcinia hanburyi*. The structures were elucidated by a detailed spectroscopic analysis.

INTRODUCTION

Gamboge, which is the resin exuded from various plants in the Guttiferae are used for pigments and as folk medicines. Some bioactive constituents from gamboge have also been reported [1]. In continuation of our investigation on the bioactive phloroglucinol derivatives from Guttiferae species [2–4], potent cytotoxicity was observed in a crude extract of the gamboge resin of *Garcinia hanburyi* Hook. f. In addition to the known gambogic acid (**1**) as a major active constituent [5], we isolated more potent cytotoxic compounds from the resin. In this paper, we describe the isolation and structural elucidation of 11 new and four known xanthones from *G. hanburyi* Hook. f. with cytotoxicity against HeLa and HEL cells.

RESULTS AND DISCUSSION

The methanol extract from the gamboge resin was concentrated and the residue was partitioned between ethyl acetate and water. The cytotoxicity was observed on the organic phase. The xanthone derivatives (**1–15**) were isolated by means of silica gel column chromatography and HPLC. Compounds **1–4** were readily identified as gambogic acid [5], isomorellin [6], morellic acid [7] and desoxymorellin [8] by comparison of their spectral data (^{1}H and ^{13}C) with the published data.

Although the NMR spectra (^{1}H and ^{13}C) of the other isolates (**5–15**) resemble those of gambogic acid (**1**) and morellin analogues (**2–4**), no such compounds have been described before in the literature. These novel compounds were named gambogin (**5**), morellin dimethyl acetal (**6**), isomoreollin **B** (**7**), moreollic acid (**8**), gambogenic acid (**9**), gambogenin (**10**), isogambogenin (**11**), desoxygambogenin (**12**), gambogenin dimethyl acetal (**13**), gambogellic acid (**14**) and hanburin (**15**), respectively.

Gambogin (**5**), obtained as an inseparable diastereomeric mixture, similar to gambogic acid (**1**), was found to have the molecular formula $C_{38}H_{38}O_6$ by high-resolution FAB mass spectrometry $[M + H]^+$ *m/z* 599.3333. The NMR spectra (^{1}H and ^{13}C) of **5** were similar to those of gambogic acid (**1**) except for the lack of signals of the carboxyl group (C-29), the significant upper field shift of H-27 (δ4.43) and another methyl signal. These implied that **5** has two methyl groups at C-28. The structure of gambogin was thus deduced to be **5**.

Compound **6**, $C_{35}H_{42}O_8$ ($[M + H]^+$ *m/z* 591.2193), showed very similar NMR spectra (^{1}H, ^{13}C, ^{1}H–^{1}H COSY and ^{1}H–^{13}C COSY) to morellin (**2**). Differences of the NMR spectra between **6** and **2** are as follows. The aldehyde signals (^{1}H and ^{13}C) of **2** are not observed in those of **6**, H-27 appeared at upper field (δ5.07) in **6**, and new two methoxyl signals (^{1}H: δ3.04, 3.10; ^{13}C: δ53.1, 53.6) and an acetal methine (^{1}H: δ4.48, ^{13}C: δ101.3) signal were observed in **6**. These indicated that compound **6** is the dimethyl acetal of morellin (**2**). The configuration of 27-C and 28-C was deduced as the *z* form because of the finding of a crosspeak between H-26 (δ2.62) and 29-H (δ4.48) in the phase-sensitive NOESY spectrum of **6**.

The NMR spectra (^{1}H and ^{13}C) of compounds **7**, $C_{34}H_{40}O_8$ ($[M + H]^+$ *m/z* 577.2815), and **8**, $C_{34}H_{40}O_9$ ($[M + H]^+$ *m/z* 593.2732), were found to be very similar to those of isomoreollin (**7′**) and morreollin (**8′**), reported in 1977 [9] (Table 1). Both **7** and **8** had a methoxyl group (**7**, ^{1}H: δ3.33; ^{13}C: δ56.0. **8**, ^{1}H δ3.32; ^{13}C: δ55.9) instead

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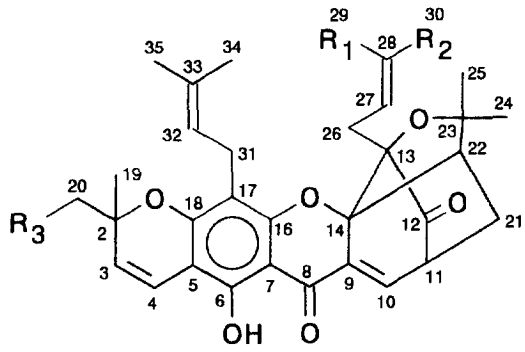
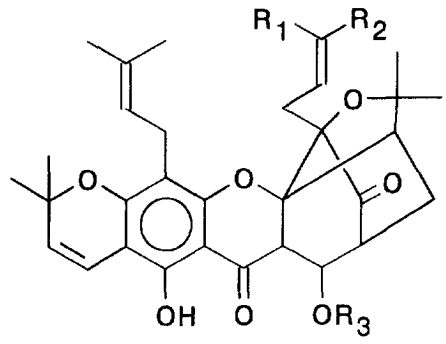
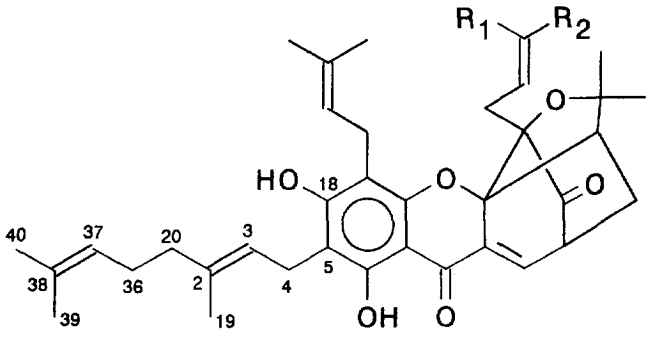


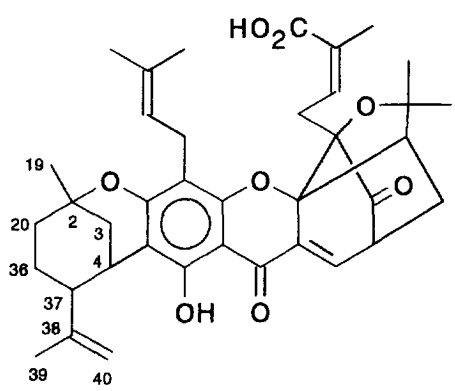
	R_1	R_2	R_3
1	CO_2H	Me	prenyl
2	Me	CHO	H
3	CO_2H	Me	H
4	Me	Me	H
5	Me	Me	prenyl
6	$CH(OMe)_2$	Me	H



	R_1	R_2	R_3
7	Me	CHO	Me
7'	Me	CHO	C_2H_5
8	CO_2H	Me	Me



	R_1	R_2
9	CO_2H	Me
10	CHO	Me
11	Me	CHO
12	Me	Me
13	$CH(OMe)_2$	Me



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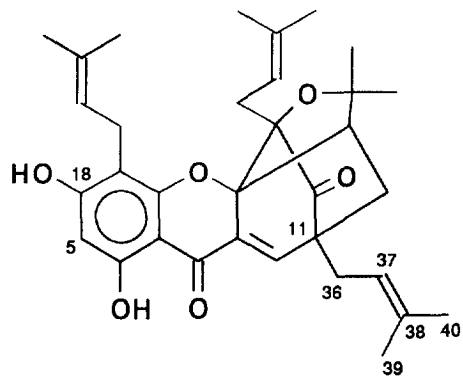

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Table 1. ^{13}C-NMR data of 7, 8, 7′ and 8′ (δ in $CDCl_3$; 68 MHz)

C	7	8	7′	8′
2	78.6	78.5	78.6	78.6
3	126.5	126.4	126.6	126.6
4	115.2	115.3	115.6	115.4
5	103.3	103.1	103.4	103.3
6	156.4	156.4	156.8	156.7
7	101.8	101.9	102.1	102.0
8	193.3	193.7	193.6	193.7
9	43.5	43.5	43.8	43.8
10	74.1	73.9	72.6	72.6
11	43.7	43.9	44.2	44.2
12	208.2	208.4	208.3	208.3
13	82.1	82.5	82.1	82.1
14	88.3	88.3	88.5	88.5
16	155.5	155.6	155.0	155.8
17	109.2	109.2	109.3	109.2
18	160.9	161.0	161.1	161.1
19	27.6	27.2	27.8	27.4
20	28.2	28.2	28.3	28.3
21	20.0	19.9	20.1	20.1
22	48.5	48.0	49.2	49.1
23	85.9	86.4	86.2	86.2
24	28.6	28.6	28.6	28.6
25	29.8	29.8	29.9	29.8
26	27.3	27.9	27.4	25.7
27	148.9	138.1	149.0	142.9
28	139.9	127.9	140.1	137.2
29	9.3	171.3	8.1	191.4
30	195.2	20.7	195.2	16.5
31	21.6	21.5	21.7	21.6
32	122.3	122.5	122.8	122.7
33	131.5	131.3	131.6	131.5
34	18.1	18.1	18.1	18.1
35	25.7	25.7	25.7	25.4
$OC\underline{H}_3$	56.0	55.9	—	—
$OC\underline{H}_2CH_3$	—	—	63.9	63.9
$OCH_2\underline{C}H_3$	—	—	15.2	15.2

of the ethoxyl group present in 7′ and 8′ at C-10. Compound 7 was almost the same as 7′, except for the substitution at C-10, but compound 8 had several differences from 8′. The NMR spectra of 8 showed a carboxyl group (δ171.2) and the absence of an aldehyde group. These indicated that 8 has a 29-carboxyl carbon. As the stereochemistry of 7′ and 8′ had not been reported, we elucidated the stereochemistry of these compounds by phase-sensitive NOESY spectroscopy of 7. The significant NOE were observed between H-9 and H-11 and between H-10 and H-21, as shown in Fig. 1. The structures of isomoreollin B and moreollic acid were thus assigned to be 7 and 8, respectively.

The high-resolution FAB mass spectrometry of gambogenic acid (9), $[M + H]^+$ m/z 631.3248, indicated the molecular formula $C_{38}H_{46}O_8$. The comparison of the NMR spectra (^{1}H, ^{1}H–^{1}H COSY and ^{1}H–^{13}C COSY) of 1 and 9 showed that two characteristic proton signals of H-3 and H-4 of gambogic acid (1) were absent in 9, whereas the other signals, the distinctive signals of the isoprene unit including four olefinic multiplet (δ5.03, 5.05, 5.17 and 5.83) and a broad signal of the hydroxyl group (δ6.41), were present. These revealed that 9 has a geranyl and a hydroxyl group instead of the ether ring in 1. In order to confirm this, gambogenic acid 9 was treated with the neutral oxidant, dichlorodicyano-*p*-benzoquinone (DDQ) in benzene at reflux temperature to yield gambogic acid (1) (53.2%). The configuration at the C-2 double bond of 9 was determined to be *E* form by the chemical shift of the 19-methyl carbon (δ16.1). The 19-C signal of the *Z* form compounds should appear more downfield, because of the decreasing γ-effect. Consequently, the structure of gambogenic acid can be revealed as 9.

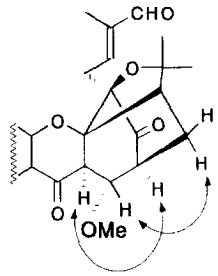

Fig. 1. Correlation in the NOESY spectrum of isomoreollin (7).

The NMR spectra (^{1}H and ^{13}C) of compounds (10–13) resemble those of gambogenic acid (9), respectively. Gambogenin (10), $C_{38}H_{46}O_7$ ($[M + H]^+$ m/z 615.3365), and isogambogenin (11), ($[M + H]^+$ m/z 615.3358), had an aldehyde group (10, ^{1}H: δ9.71; ^{13}C: δ189.4. 11, ^{1}H: δ9.24; ^{13}C: δ194.5) at C-28 instead of the carboxyl group of the gambogenic acid 9. The C-29 (methyl) signal of 11 appeared at more upper field (δ8.7) than the C-30 signal (δ16.3) of 10. This implied that 10 and 11 have the *E* and the *Z* configuration at the C-27 double bond, respectively.

In the ^{13}C-NMR and DEPT spectra of desoxygambogenin (12), nine methyl signals and no carboxyl group were observed. The H-27 signal is field (δ4.43) than that of 9. This suggests that 12 has another methyl carbon at C-28, instead of the carboxyl carbon (C-29) of 9. The high-resolution FAB mass spectrometry established the molecular formula $C_{40}H_{52}O_8$ ($[M + H]^+$ m/z 601.3474) to support the structure 12.

Compound 13, with the molecular formula $C_{40}H_{52}O_8$ ($[M + H]^+$ m/z 661.3724), showed similar NMR spectra (^{1}H, ^{13}C, ^{1}H–^{1}H COSY and ^{1}H–^{13}C COSY) as 10 except for a dimethyl acetal function instead of aldehyde H, the upper field shift of the H-27 signal (δ5.05). In the NOESY spectrum of 13, H-29 (δ4.51) showed a correlation with H-26 (δ2.61), as with morellin dimethyl acetal (6). Therefore 13 is the dimethyl acetal of gambogenin (10).

Gambogellic acid (14), ($[M + H]^+$ m/z 629.3083) has the same molecular formula $C_{38}H_{44}O_8$ as gambogic acid (1). The ^{1}H NMR spectrum of 14 showed characteristic proton signals of an *exo*-methylene function (^{1}H: δ4.21, 4.51; ^{13}C: δ108.6) instead of the signals due to H-3 and H-4 and prenyl group at C-20 of 1. The NMR signals (^{1}H

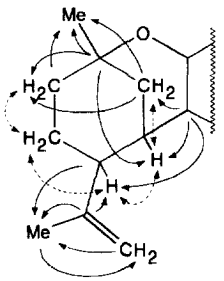

Fig. 2. Correlations (^{13}C–^{1}H) in the COLOC spectrum and (^{1}H–^{1}H) in the ^{1}H–^{1}H COSY spectrum of gambogellic acid (**14**).

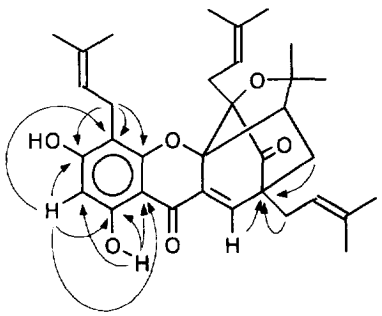

Fig. 3. Correlation (^{13}C–^{1}H) in the HMBC spectrum of hanburin (**15**).

and ^{13}C) of **14** (from 5- to 18-position and from 21- to 35-position) were assigned by a detail analysis of the two-dimensional NMR (^{1}H–^{1}H, ^{1}H–^{13}C COSY, HMBC, COLOC) spectra of **14** and **1**. The remaining groups, two methyls, four methylenes, two methines and quaternary carbons, an *exo*-methylene and a terpene moiety, were also confirmed by the two-dimensional NMR experiments. The ^{1}H–^{1}H and long-range ^{1}H–^{13}C correlations of the monoterpene moiety of **14** are indicated by arrows in Fig. 2. These results clarified the plane structure of gambogellic acid (**14**). The stereostructure of **13** could not be determined.

Hanburin (**15**) has the molecular formula $C_{33}H_{40}O_6$, based on high-resolution FAB mass spectrometry $[M+H]^+$ *m/z* 533.2904. The NMR spectra (^{1}H and ^{1}H–^{1}H COSY) of **15** showed signals similar to desoxymorellin **4** except for the appearance of a phenolic H and a lack of signals derived from the ether ring and one of the isoprene units in **4**. Furthermore, the coupling pattern of H-10 appeared to be *s* (δ7.23) in **15**, instead of *dd* (δ7.44, J = 7.0 Hz) of **4**. The long-range ^{1}H–^{13}C correlations (HMBC) of compound **15** were observed between the aromatic signal (H-5) and C-6, C-7, C-17, C-18, between a hydrogen-bonded hydroxyl signal (OH-6) and C-5, C-6, C-7, between a methylene signal (H-31) of one of isoprene groups and C-16, C-17, C-18 and between H-10, H-21, H-36 and C-11 (Fig. 3). These indicates the substituents on the aromatic ring and the cyclohexane ring. Hence, hanburin **15** is formulated as shown in Fig. 3.

Table 2. Cytotoxicity against HeLa and HEL cells (*MICs*, $\mu g/ml^{-1}$)

Compound	HeLa	HEL
1	12.5	12.5
2	25.0	25.0
3	$\geqslant$ 50.0	$\geqslant$ 50.0
4	0.39	0.39
5	6.25	3.13
6	1.56	0.78
7	6.25	6.25
8	3.13	3.13
9	12.5	3.13
10	12.5	6.25
11	3.13	0.78
12	0.78	0.78
13	6.25	3.13
14	1.56	0.78
15	12.5	12.5

The minimum inhibitory concentrations (*MICs*, $\mu g\,ml^{-1}$) of each of the isolates against cultured HeLa and HEL cells are given in Table 2. Desoxymorellin (**4**) showed highest activity. Most of the isolated compounds have higher cytotoxicities, except isomorellin (**2**), morellic acid (**3**) and hanburin (**15**), than gambogic acid (**1**) which was reported as a cytotoxic compound [5]; However there are no significant differences in the activities of these compounds against two kinds of cultured cells – HeLa and HEL cells, respectively.

EXPERIMENTAL

Mps uncorr.; MS: direct inlet, 70 eV; FABMS: 2,2′-dithiodiethanol as a matrix; ^{1}H and ^{13}C NMR: 270 and 68 MHz, respectively, $CDCl_3$ TMS as int. standard; HPLC was performed on an ODS column (Inertsil PREP-ODS, eluted with MeOH–H_2O) and on the gel permeation column (Asahipak GS310, eluted with MeOH or EtOAc).

Plant material. The gamboge resin of *G. hanburyi* was collected in Guiyang city, Guizhou province, China in 1993.

Cytotoxicity test. Each compound was dissolved in methanol to make 10000 $\mu g\,ml^{-1}$ soln, and then the soln was diluted $\times$ 100 with Eagle's minimum essential medium (MEM) to remove the influence of methanol to cell cultures. For the cytotoxicity test, all compounds were diluted further with MEM in duplicate 96 well plastic microplates (Falcon Co. Ltd) to required concns. HeLa and human embryonic lung fibloblast (HEL #17, the 16th passage level) cell suspension (5.0×10^5 cells ml^{-1}) with growing medium (MEM containing 0.11% of $NaHCO_3$, 5.0% of fetal bovine serum) were added to all wells (100 $\mu l\,well^{-1}$). After gentle mixing, the plates were incubated 24 hr in a 36° CO_2 incubator. The cytotoxicity of the compounds were determined by microscopic observation.

Isolation of the xanthone derivatives. The dried gum resin (18 g) was extracted with MeOH (400 ml) at room temp. for 1 week. The solvent was removed under reduced pressure and the residue partitioned between EtOAc and H_2O. The organic layer was evapd and the gummy residue was chromatographed over silica gel (400 g) using hexane–EtOAc with increasing the polarity. Each fractions was purified by the repeated HPLC. The yields of the known xanthone derivatives **1**, **2**, **3** and **4** were 5.5 g, 70.2 mg, 574 mg and 7.8 mg respectively.

Gambogin (**5**). A mixture of the diastereomeric isomers; yellow gum (7.8 mg); $[\alpha]_D^{27.0}$ − 360° ($CHCl_3$; *c* 0.1); HRFABMS $[M + H]^+$ *m/z* 599.3333; calcd for $C_{38}H_{46}O_6$ $[M + H]^+$ 599.3373. EIMS *m/z* (rel. int.): 598 $[M]^+$ (15), 570 (40), 555 (14), 515 (100), 487 (79), 473 (17), 469 (16), 215 (32); IR ν_{max}^{KBr} cm^{-1}: 3450, 2970, 2925, 2850, 1732, 1635, 1592, 1438, 1329, 1170, 1132; UV λ_{max}^{EtOH} nm (log ε): 362 (4.10), 292 (4.21), 283 (4.18); ^1H NMR: δ1.03 (3H, *s*), 1.29 (3H, *s*), 1.34 (1H, *dd*, *J* = 9.3, 13.6 Hz), 1.37 (3H, *s*), 1.40/1.41 (3H, *s*), 1.57 (3H, *s*), 1.66 (6H, *s*), 1.64–1.74 (2H, *m*), 1.71 (3H, *s*), 1.76 (3H, *s*), 2.07 (2H, *m*), 2.31 (1H, *dd*, *J* = 4.7, 13.6 Hz), 2.48 (1H, *d*, *J* = 9.3 Hz), 2.57 (2H, *d*, *J* = 7.4 Hz), 3.32 (2H, *m*), 3.49 (1H, *dd*, *J* = 4.7, 7.0 Hz), 4.43 (1H, *m*), 5.07 (1H, *m*), 5.21 (1H, *m*), 5.45/5.46 (1H, *d*, *J* = 10.0 Hz), 6.68 (1H, *d*, *J* = 10.0 Hz), 7.44 (1H, *d*, *J* = 7.0 Hz), 12.89 (1H, *s*); ^{13}C NMR; δ16.71/16.74, 17.6/17.7, 18.2, 21.6, 22.7/22.8, 25.5, 25.6, 25.7, 25.8, 27.3/27.4, 28.8, 29.1, 30.11/30.14, 41.8/41.9, 46.9, 49.2, 81.0/81.1, 83.2, 84.6/84.7, 90.5, 100.5, 102.6, 107.8/107.9, 116.0/116.1, 117.9, 122.2/122.3, 123.7/123.8, 124.7/124.8, 131.7, 132.0, 133.7, 133.8, 135.0, 157.5, 157.9, 160.8/160.9, 179.5, 203.56/203.59.

Morellin dimethyl acetal (**6**). Yellow gum (2.3 mg); $[\alpha]_D^{24.0}$ − 405° ($CHCl_3$; *c* 0.1); HRFABMS $[M + H]^+$ *m/z* 591.2913; calcd for $C_{35}H_{42}O_8$ $[M + H]^+$ 591.2958. EIMS *m/z* (rel. int.): 590 $[M]^+$ (18), 530 (54), 515 (46), 243 (84), 236 (45), 125 (100); UV λ_{max}^{EtOH} nm (log ε): 361 (3.88), 290 (3.97), 280 (4.00); ^1H NMR: δ1.29 (3H, *s*), 1.36 (1H, *dd*, *J* = 9.5, 13.4 Hz), 1.42 (3H, *s*), 1.45 (3H, *d*, *J* = 1.4 Hz), 1.47 (3H, *s*), 1.67 (3H, *s*), 1.71 (3H, *s*), 1.77 (3H, *s*), 2.33 (1H, *dd*, *J* = 4.4, 13.4 Hz), 2.51 (1H, *d*, *J* = 9.5 Hz), 2.62 (2H, *d*, *J* = 7.3 Hz), 3.04 (3H, *s*), 3.10 (3H, *s*), 3.30 (2H, *d*, *J* = 7.2 Hz), 3.49 (1H, *dd*, *J* = 4.4, 6.8 Hz), 4.48 (1H, *s*), 5.07 (1H, *m*), 5.17 (1H, *m*), 5.51 (1H, *d*, *J* = 9.9 Hz), 6.64 (1H, *d*, *J* = 9.9 Hz), 7.50 (1H, *d*, *J* = 6.8 Hz), 12.85 (1H, *s*); ^{13}C NMR; δ18.2, 18.3, 21.7, 25.4, 25.8, 27.5, 28.4, 28.8, 29.0, 30.0, 46.9, 49.2, 53.1, 53.6, 78.6, 83.4, 83.8, 90.7, 100.6, 101.3, 103.0, 108.0, 115.4, 122.1, 122.7, 126.0, 131.7, 133.6, 134.8, 135.1, 157.6, 157.7, 161.0, 179.2, 203.4.

Isomoreollin B (**7**). Powder (5.1 mg), mp 58–59°; $[\alpha]_D^{24.0}$ − 37° ($CHCl_3$; *c* 0.1); HRFABMS $[M + H]^+$ *m/z* 577.2815; calcd for $C_{34}H_{40}O_8$ $[M + H]^+$ 577.2801. EIMS *m/z* (rel. int.): 576 $[M]^+$ (12), 544 (100), 529 (76), 501 (71), 405 (66), 368 (92); UV λ_{max}^{EtOH} nm (log ε): 362 (3.66), 319 (4.16), 278 (4.55), 269 (4.54); ^1H NMR: δ1.16 (3H, *s*), 1.37 (3H, *s*), 1.40 (3H, *s*), 1.41 (1H, *dd*, *J* = 8.5, 14.7 Hz), 1.47 (3H, *s*), 1.64 (3H, *s*), 1.76 (6H, *s*), 2.00 (1H, *dd*, *J* = 6.0, 14.7 Hz), 2.55 (1H, *d*, *J* = 8.5 Hz), 2.91 (1H, *dd*, *J* = 4.6, 6.0 Hz), 2.92 (1H, *dd*, *J* = 6.2, 16.4 Hz), 3.06 (1H, *dd*, *J* = 7.5, 16.4 Hz), 3.08 (1H, *d*, *J* = 1.1 Hz), 3.19 (1H, *dd*, *J* = 5.7, 14.1 Hz), 3.31 (1H, *dd*, *J* = 7.6, 14.1 Hz), 3.33 (3H, *s*), 4.37 (1H, *dd*, *J* = 1.1, 4.6 Hz), 4.97 (1H, *m*), 5.53 (1H, *d*, *J* = 9.9 Hz), 6.62 (1H, *d*, *J* = 9.9 Hz), 6.97 (1H, *m*), 9.54 (1H, *s*), 11.75 (1H, *s*).

Moreollic acid (**8**). Powder (4.3 mg), mp 97°; $[\alpha]_D^{27.0}$ − 31° ($CHCl_3$; *c* 0.1); HRFABMS $[M + H]^+$ *m/z* 593.2732; calcd for $C_{34}H_{40}O_9$ $[M + H]^+$ 593.2751. EIMS *m/z* (rel. int.): 592 $[M]^+$ (31), 560 (37), 529 (46), 451 (51), 429 (38), 405 (37), 355 (62), 281 (52), 236 (100); UV λ_{max}^{EtOH} nm (log ε): 365 (3.47), 318 (4.00), 277 (4.43), 267 (4.41); ^1H NMR: δ1.15 (3H, *s*), 1.36 (3H, *s*), 1.40 (3H, *s*), 1.41 (1H, *m*), 1.46 (3H, *s*), 1.63 (3H, *s*), 1.74 (3H, *s*), 1.95 (1H, *m*), 1.96 (3H, *s*), 2.51 (1H, *d*, *J* = 9.4 Hz), 2.84 (1H, *t*, *J* = 4.6 Hz), 3.02–3.31 (4H, *m*), 3.19 (1H, *s*), 3.32 (3H, *s*), 4.35 (1H, *d*, *J* = 4.6 Hz), 5.02 (1H, *m*), 5.51 (1H, *d*, *J* = 9.9 Hz), 6.59 (1H, *m*), 6.61 (1H, *d*, *J* = 9.9 Hz), 11.93 (1H, *s*).

Gambogenic acid (**9**). Yellow powder (2.1 g), mp 77–78°; $[\alpha]_D^{26.0}$ − 246° ($CHCl_3$; *c* 0.1); HRFABMS $[M + H]^+$ *m/z* 631.3248; calcd for $C_{38}H_{46}O_8$ $[M + H]^+$ 631.3271. EIMS *m/z* (rel. int.): 630 $[M]^+$ (100), 602 (32), 545 (51), 507 (39), 476 (21), 368 (98), 351 (22), 295 (31), 256 (46), 236 (51), 213 (27), 213 (27), 185 (31), 129 (61); UV λ_{max}^{EtOH} nm (log ε): 360 (4.03); ^1H NMR: δ1.27 (3H, *s*), 1.36 (1H, *dd*, *J* = 9.1, 13.4 Hz), 1.56 (3H, *s*), 1.65 (9H, *s*), 1.67 (3H, *s*), 1.70 (3H, *s*), 1.74 (3H, *s*), 1.96–2.16 (4H, *m*), 2.31 (1H, *dd*, *J* = 4.6, 13.4 Hz), 2.48 (1H, *d*, *J* = 9.1 Hz), 2.88 (1H, *dd*, *J* = 5.6, 15.7 Hz), 3.13–3.36 (5H, *m*), 3.48 (1H, *dd*, *J* = 4.6, 6.7 Hz), 5.03 (1H, *m*), 5.05 (1H, *m*), 5.17 (1H, *m*), 5.83 (1H, *m*), 6.41 (1H, *br s*), 7.52 (1H, *d*, *J* = 6.7 Hz), 12.56 (1H, *s*); ^{13}C NMR: δ16.1, 17.6, 17.9, 20.6, 21.0, 22.0, 25.2, 25.6, (2C), 26.3, 28.9, 29.5, 29.8, 39.7, 46.9, 48.9, 83.7, 84.0, 90.4, 100.6, 106.4, 107.4, 121.4, 122.0, 123.8, 128.1, 131.7, 133.6 (2C), 135.0, 138.1, 138.9, 155.9, 160.3, 163.5, 172.1, 179.1, 203.5.

Oxidative cyclicization of compound (**9**). A soln of the compound **9** (20.0 mg) and DDQ (26.0 mg, 3.0 mol equiv.) in dry benzene (15 ml) was refluxed under Ar for 1 hr. The reaction mixture was cooled and filtered, and the solution was evaporated. The residue was chromatographed by HPLC (ODS; MeOH–H_2O) to give **1** (10.6 mg, 53.2%).

Gambogenin (**10**). Yellow gum (3.0 mg); $[\alpha]_D^{27.0}$ − 440° ($CHCl_3$; *c* 0.1); HRFABMS $[M + H]^+$ *m/z* 615.3365; calcd for $C_{38}H_{46}O_7$ $[M + H]^+$ 615.3322. EIMS *m/z* (rel. int.): 614 $[M]^+$ (32), 529 (36), 491 (34), 368 (100), 353 (35), 243 (41), 236 (34), 125 (59); UV λ_{max}^{EtOH} nm (log ε): 362 (4.22); ^1H NMR: δ1.30 (3H, *s*), 1.41 (1H, *dd*, *J* = 9.4, 13.4 Hz), 1.51 (3H, *s*), 1.60 (3H, *s*), 1.68 (3H, *s*), 1.71 (6H, *s*), 1.76 (3H, *s*), 1.82 (3H, *s*), 2.01–2.16 (4H, *m*), 2.36 (1H, *dd*, *J* = 4.5, 13.4 Hz), 2.54 (1H, *d*, *J* = 9.4 Hz), 2.87 (1H, *dd*, *J* = 6.4, 14.3 Hz), 3.13 (1H, *dd*, *J* = 9.8, 14.3 Hz), 3.24–3.41 (4H, *m*), 3.54 (1H, *dd*, *J* = 4.5, 7.0 Hz), 5.07 (1H, *m*), 5.18 (1H, *m*), 5.27 (1H, *m*), 6.03 (1H, *dd*, *J* = 6.4, 9.8 Hz), 6.64 (1H, *s*), 7.54 (1H, *d*, *J* = 7.0 Hz), 9.71 (1H, *s*), 12.80 (1H, *s*); ^{13}C NMR: δ16.3, 16.5, 17.7, 18.1, 21.2, 22.0, 25.4, 25.7 (2C), 26.3, 26.9, 29.0, 29.9, 39.7, 47.0, 49.0, 83.9, 84.0, 90.3, 100.6, 106.9, 107.6, 121.2, 121.8, 123.7, 132.1, 133.6, 133.8, 135.2, 137.7, 139.8, 140.1, 155.8, 160.3, 163.9, 179.1, 189.4, 202.9.

Isogambogenin (**11**). Yellow gum (9.0 mg); $[\alpha]_D^{24.0}$ − 425° ($CHCl_3$; *c* 0.1); HRFABMS $[M + H]^+$ *m/z* 615.3358; calcd for $C_{38}H_{46}O_7$ $[M + H]^+$ 615.3474. EIMS *m/z* (rel. int.): 614 $[M]^+$ (16), 529 (18), 368 (100), 353 (23), 256 (25), 236 (37), 213 (24), 185 (20), 129 (40); UV λ_{max}^{EtOH} nm (log ε): 361 (4.19); ^{1}H NMR: δ1.31 (3H, *s*), 1.40 (1H, *dd*, *J* = 9.3, 13.4 Hz), 1.56 (6H, *s*), 1.67 (3H, *s*), 1.69 (3H, *s*), 1.71 (3H, *s*), 1.74 (3H, *s*), 1.80 (3H, *s*), 2.04–2.15 (4H, *m*), 2.35 (1H, *dd*, *J* = 4.6, 13.4 Hz), 2.54 (1H, *d*, *J* = 9.3 Hz), 2.68 (2H, *d*, *J* = 7.2 Hz), 3.31 (2H, *d*, *J* = 7.1 Hz), 3.36 (2H, *d*, *J* = 7.2 Hz), 3.52 (1H, *dd*, *J* = 4.6, 6.8 Hz), 5.06 (1H, *m*), 5.12 (1H, *m*), 5.22 (1H, *m*), 6.36 (1H, *m*), 6.57 (1H, *br s*), 7.56 (1H, *d*, *J* = 6.8 Hz), 9.24 (1H, *s*), 12.82 (1H, *s*); ^{13}C NMR: δ8.7, 16.3, 17.7, 18.1, 21.2, 22.1, 25.3, 25.7, 25.8, 26.3, 28.9, 29.1, 30.0, 39.7, 46.9, 49.0, 83.3, 83.9, 90.6, 100.6, 106.7, 107.5, 121.1, 121.7, 123.7, 132.1, 133.5, 134.0, 135.7, 139.9, 140.3, 146.5, 155.9, 160.4, 163.9, 179.0, 194.5, 230.2.

Desoxygambogenin (**12**). Yellow gum (9.4 mg); $[\alpha]_D^{26.0}$ − 152° ($CHCl_3$; *c* 0.1); HRFABMS $[M + H]^+$ *m/z* 601.3474; calcd for $C_{38}H_{48}O_6$ $[M + H]^+$ 601.3529. EIMS *m/z* (rel. int.): 600 $[M]^+$ (67), 572 (100), 503 (79), 475 (40), 449 (46), 368 (69), 351 (44), 295 (58), 253 (61), 215 (64); UV λ_{max}^{EtOH} nm (log ε): 358 (4.10); ^{1}H NMR: δ1.01 (3H, *s*), 1.28 (3H, *s*), 1.34 (1H, *dd*, *J* = 9.1, 13.3 Hz), 1.36 (3H, *s*), 1.60 (3H, *s*), 1.69 (6H, *s*), 1.72 (3H, *s*), 1.78 (3H, *s*), 1.82 (3H, *s*), 2.02–2.15 (4H, *m*), 2.33 (1H, *dd*, *J* = 4.5, 13.3 Hz), 2.46 (1H, *d*, *J* = 9.1 Hz), 2.56 (2H, *d*, *J* = 7.1 Hz), 3.38 (4H, *d*, *J* = 6.8 Hz), 3.49 (1H, *dd*, *J* = 4.5, 6.9 Hz), 4.43 (1H, *m*), 5.06 (1H, *m*), 5.22 (2H, *m*), 6.47 (1H, *s*), 7.45 (1H, *d*, *J* = 6.9 Hz), 12.96 (1H, *s*); ^{13}C NMR: δ16.3, 16.8, 17.7, 18.1, 21.2, 22.1, 25.5, 25.6, 25.7, 25.8, 26.4, 28.9, 29.1, 30.1, 39.7, 47.0, 49.1, 83.2, 84.6, 90.3, 100.8, 106.4, 107.2, 117.8, 121.4, 121.9, 123.8, 132.0, 133.8, 133.9, 134.0, 135.0, 139.2, 156.3, 160.2, 163.1, 179.7, 203.6.

Gambogenin dimethyl acetal (**13**). Yellow gum (11.3 mg); $[\alpha]_D^{27.0}$ − 295°($CHCl_3$; *c* 0.1); HRFABMS $[M + H]^+$ *m/z* 661.3724; calcd for $C_{40}H_{52}O_8$ $[M + H]^+$ 661.3740. EIMS *m/z* (rel. int.): 660 $[M]^+$ (1.5), 599 (65), 585 (20), 531 (19), 461 (14), 368 (17), 295 (19), 253 (19), 243 (66), 125 (100); UV λ_{max}^{EtOH} nm (log ε): 358 (4.01), 277 (3.73), 263 (3.73); ^{1}H NMR: δ1.28 (3H, *s*), 1.35 (1H, *dd*, *J* = 9.1, 13.6 Hz), 1.44 (3H, *s*), 1.59 (3H, *s*), 1.68 (6H, *s*), 1.71 (3H, *s*), 1.77 (3H, *s*), 1.81 (3H, *s*), 2.03–2.15 (4H, *m*), 2.32 (1H, *dd*, *J* = 4.5, 13.6 Hz), 2.48 (1H, *d*, *J* = 9.1 Hz), 2.61 (2H, *d*, *J* = 7.1 Hz), 3.03 (3H, *s*), 3.10 (3H, *s*), 3.38 (4H, *d*, *J* = 6.8 Hz), 3.48 (1H, *dd*, *J* = 4.5, 6.8 Hz), 4.51 (1H, *s*), 5.00 (1H, *m*), 5.05 (1H, *m*), 5.19 (1H, *m*), 5.24 (1H, *m*), 6.52 (1H, *s*), 7.50 (1H, *d*, *J* = 6.8 Hz), 12.89 (1H, *s*); ^{13}C NMR: δ16.2, 17.7, 18.1, 18.3, 21.2, 22.1, 25.5, 25.7, 25.8, 26.3, 27.6, 29.0, 30.0, 39.8, 46.9, 49.2, 52.9, 53.7, 83.3, 83.8, 90.4, 100.8, 101.3, 106.5, 107.1, 121.3, 122.0, 122.7, 123.7, 132.0, 133.6, 133.9, 134.8, 135.3, 139.4, 156.1, 160.4, 163.5, 179.4, 203.4.

Gambogellic acid (**14**). Yellow powder (10.1 mg), mp 116–117°; $[\alpha]_D^{27.0}$ − 349° ($CHCl_3$; *c* 0.1); HRFABMS $[M + H]^+$ *m/z* 629.3038; calcd for $C_{38}H_{44}O_8$ $[M + H]^+$ 629.3114. EIMS *m/z* (rel. int.): 628 $[M]^+$ (100), 600 (74), 545 (25), 517 (20), 474 (73), 355 (19), 278 (52), 149 (70), 129 (27); UV λ_{max}^{EtOH} nm (log ε): 358 (4.25); ^{1}H NMR: δ1.29 (3H, *s*), 1.32 (3H, *s*), 1.65 (3H, *s*), 1.71 (6H, *s*), 1.72 (3H, *s*), 1.84 (3H, *s*), 1.24–1.95 (7H, *m*), 2.09 (1H, *d*, *J* = 12.2 Hz), 2.30 (1H, *dd*, *J* = 4.6, 13.2 Hz), 2.53 (1H, *d*, *J* = 9.2 Hz), 2.99 (2H, *d*, *J* = 7.3 Hz), 3.12 (1H, *dd*, *J* = 5.3, 14.1 Hz), 3.26 (1H, *dd*, *J* = 7.9, 14.1 Hz), 3.42 (1H, *br s*), 3.46 (1H, *dd*, *J* = 4.6, 6.9 Hz), 4.21 (1H, *br s*), 4.54 (1H, *br s*), 5.06 (1H, *m*), 6.03 (1H, *m*), 7.49 (1H, *d*, *J* = 6.9 Hz), 12.65 (1H, *s*); ^{13}C NMR: δ18.2, 20.8, 21.8, 22.8, 23.0, 25.2, 25.8, 28.4, 28.8, 28.9, 29.1, 29.9, 36.5, 39.3, 46.7, 48.1, 48.9, 77.0, 83.9, 84.3, 90.4, 99.6, 104.1, 106.2, 108.6, 122.4, 127.8, 131.1, 133.9, 134.3, 138.2, 147.9, 155.3, 160.8, 164.4, 171.6, 178.5, 203.8.

Hanburin (**15**). Yellow powder (4.0 mg), mp 176°; $[\alpha]_D^{24.0}$ − 199° ($CHCl_3$; *c* 0.1); HRFABMS $[M + H]^+$ *m/z* 533.2904; calcd for $C_{33}H_{40}O_6$ $[M + H]^+$ 533.2903. EIMS *m/z* (rel. int.): 504 $[M - 28]^+$ (80), 435 (100) 407 (31), 393 (20), 379 (16), 365 (16), 339 (32), 283 (22), 241 (22), 165 (15); UV λ_{max}^{EtOH} nm (log ε): 358 (3.87), 279 (3.50), 267 (3.57); ^{1}H NMR: δ1.04 (3H, *s*), 1.23 (3H, *s*), 1.37 (3H, *s*), 1.42 (1H, *dd*, *J* = 9.7, 13.0 Hz), 1.66 (3H, *s*), 1.71 (3H, *s*), 1.75 (6H, *s*), 1.81 (3H, *s*), 2.01 (1H, *d*, *J* = 13.0 Hz), 2.41–2.67 (5H, *m*), 3.42 (2H, *d*, *J* = 6.6 Hz), 4.41 (1H, *m*), 5.24 (2H, *m*), 6.04 (1H, *s*), 6.19 (1H, *br s*), 7.23 (1H, *s*), 12.63 (1H, *s*); ^{13}C NMR: δ16.8, 18.0, 18.1, 22.1, 25.5, 25.8, 26.0, 28.8, 29.0, 29.1, 30.2, 31.1, 50.7, 53.3, 83.2, 84.9, 90.4, 97.0, 101.1, 105.7, 117.9, 118.4, 121.3, 133.2, 134.9, 135.5, 135.7, 137.9, 158.2, 163.3, 164.2, 179.7, 204.2.

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