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Absolute Configuration of (−)-Gambogic Acid, an Antitumor Agent

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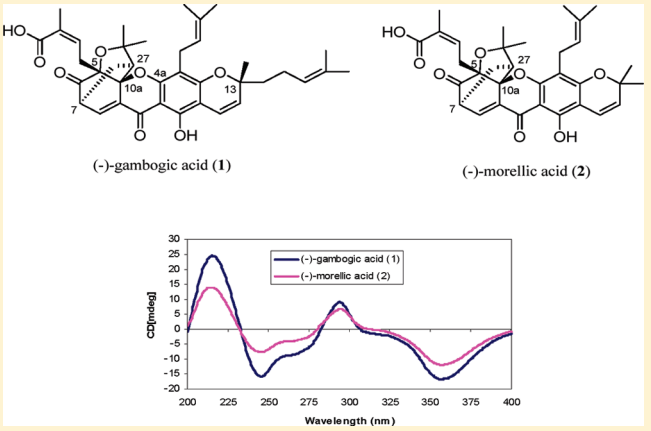
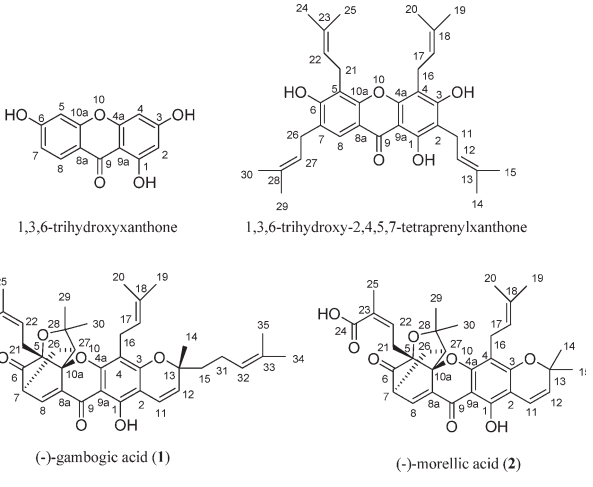
Supporting Information

ABSTRACT: (−)-Gambogic acid (**1**), a biologically active "caged xanthone" from gamboge, the dried resin of *Garcinia hanburyi*, is of interest as a potential anticancer agent. The planar structure of (−)-gambogic acid has been determined previously by analysis of its detailed NMR data and confirmed by single-crystal X-ray diffraction, with the absolute configuration at C-13 deduced as *R* through a series of chemical degradations. Using (−)-morellic acid (**2**), an analogue of (−)-gambogic acid, as a model compound, the 5*R*, 7*S*, 10a*S*, 13*R*, 27*S* absolute configuration of (−)-gambogic acid was determined for the first time by comparison of physical and spectroscopic data, especially experimental and calculated electronic circular dichroism.



(−)-Gambogic acid (**1**) is a major constituent of gamboge, a resin exuded by *Garcinia hanburyi* Hook.f (Clusiaceae) used as a folk medicine to treat infections and tumors.[1−5] This compound was isolated from *G. hanburyi* for the first time in 1949,[6] and its planar structure, based on the same carbon skeleton as (−)-morellin, was established through several chemical reactions and NMR spectroscopy in 1965[7] and confirmed by X-ray diffraction analysis in 2001.[8] The ^{1}H and ^{13}C NMR spectra of (−)-gambogic acid were assigned with the aid of HMBC and ROESY data,[9] and its 13*R* absolute configuration was determined by a series of chemical degradations[10] and supported by the later isolation of an epimer, (13*S*)-epigambogic acid.[11]

(−)-Gambogic acid has been found to be cytotoxic for various human cancer cell lines, such as BCG-823 gastric carcinoma,[12] SMMC-7721 hepatoma,[13] and SPC-A1 lung cancer[14] cells, and has shown inhibitory activity in tumor-bearing mouse models.[12−15] The mechanism of action of (−)-gambogic acid has been related to factors such as apoptosis induction,[16] inhibition of human-topoisomerase-IIα[17] and telomerase,[18] and modulation of angiogenesis.[19] The nontoxic dose of (−)-gambogic acid for Sprague−Dawley rats was established as 60 mg/kg, when administered by gavage once every other day for 13 weeks.[20] This compound has been subjected to a phase I clinical trial as an anticancer agent in the People's Republic of China, with a dose regimen developed for subsequent phase II testing.[21]



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Previous studies have demonstrated that the bridged cage moiety of (−)-gambogic acid (**1**) plays a key role in mediating the cytotoxicity of this compound.[22−24] However, the importance of the individual stereogenic centers present in the caged unit is unknown, and the CD spectrum of (−)-gambogic acid has not been reported. Even though the planar structure of (−)-gambogic acid was fully determined by X-ray diffraction analysis, and the absolute configuration of C-13 was deduced as *R* by a series of chemical degradations in a 1976 study, the absolute configuration at C-5, -7, -10a, and -27 is still undetermined because insufficient data were provided by the X-ray crystallographic experiment.[8] Several efforts at total synthesis of the caged part of (−)-gambogic acid have been successful,[25−28] but they have not resolved the absolute configuration at C-5, -7, -10a, and -27 of (−)-gambogic acid, as stated categorically in a recent review paper concerning caged xanthones.[5]

In our previous collaborative study using electronic circular dichroism (ECD), the absolute configuration at carbons 5, 7, 10a, and 27 of the structurally related caged xanthone (−)-morellic acid was determined.[29] By comparison of the specific rotation, NMR, and CD spectra of (−)-gambogic acid (**1**) with those of (−)-morellic acid (**2**), the absolute configuration of (−)-gambogic acid (**1**) has been determined in the present investigation.

The numbering system used for (−)-gambogic acid (**1**) and its analogues is not uniform in the literature.[2−5] The compound has been numbered from the oxygen atom of a pyran ring linked to the xanthone core.[9] (−)-Gambogic acid is a naturally prenylated xanthone, containing a 1,3,6-trihydroxyxanthone core, which may be numbered on the basis of IUPAC provisional recommendations.[30] It is recommended that (−)-gambogic acid is numbered in the same manner, on the basis of a prenyl xanthone, 1,3,6-trihydroxy-2,4,5,7-tetraprenylxanthone.

Although the ^{1}H and ^{13}C NMR signals of (−)-gambogic acid (**1**) were assigned by previous investigators,[9] several carbons showed two signals each, indicating that the sample used earlier might be a mixture of two epimers. The ^{1}H and ^{13}C NMR spectra of our (−)-gambogic acid showed one signal for each proton and carbon, respectively, and all protons and carbons were assigned by analysis of its DEPT 90, DEPT 135, COSY, HMQC, and HMBC spectra (Table 1). A boat conformation for the 4-oxatricyclo[4.3.1.0^{3,7}]dec-2-one moiety of (−)-gambogic acid was proposed by Rao's group when they attempted the total synthesis of morellin.[31] This proposal was confirmed as a result of the X-ray diffraction data of (−)-gambogic acid,[8] which indicated an inverted boat conformation for the C-7, C-6, C-5, C-10a, C-26, and C-27 array, with C-27, C-28, the oxygen between C-28 and C-5, and C-5 being essentially coplanar with the C-7, C-8, C-8a, and C-10a arrangement. In turn, C-27 lies below the plane defined by C-5, C-6, and C-26.[8] Such a configurational diagram is shown in Figure 2, also indicating the NOESY correlations that were observed for **1**.

The NMR spectra for (−)-gambogic acid (**1**) and (−)-morellic acid (**2**) were measured under the same conditions. All protons and carbons of both compounds were assigned and confirmed by their 2D NMR spectra, and both compounds provided identical ^{1}H and ^{13}C NMR data at H-5, -7, -10a, and -27 and at C-5, -7, -10a, and -27. Further identical HMBC and NOESY correlation profiles of both (−)-gambogic acid and (−)-morellic acid indicated the same relative configurations for both compounds. Also, these two compounds exhibited well-matched CD spectra (Figure 3), displaying negative and positive Cotton effects (CEs) near 360 and 290 nm, respectively. They additionally displayed sequential negative and positive CEs at 246 and 215 nm, arising from exciton coupling of the α,β-unsaturated carbonyl and carboxylic acid chromophores. Critically, the absolute configuration of (−)-morellic acid (**2**) was defined unambiguously by a combination of experimental and theoretically calculated ECD spectra.[29] Therefore, the absolute

Table 1. ^{1}H and ^{13}C NMR Spectroscopic Data of (−)-Gambogic Acid (1)

position	δ_H[a] (*J* in Hz)	δ_C[b]
1		157.3 C
2		102.7 C
3		161.5 C
4		107.6 C
4a		157.6 C
5		84.0 C
6		203.2 C
7	3.48 (m)	46.8 CH
8	7.54 (d, 6.9)	135.3 CH
8a		133.5 C
9		178.8 C
9a		100.4 C
10a		90.9 C
11	6.60 (d, 10.2)	115.9 CH
12	5.38 (d, 10.2)	124.5 CH
13		81.3 C
14	1.36 (s)	27.7 CH_3
15	1.76 (m)	42.0 CH_2
	1.58 (m)	
16	3.27 (m)	21.6 CH_2
	3.16 (m)	
17	5.04 (m)	122.2 CH
18		131.6 C
19	1.63 (s)	25.6 CH_3
20	1.70 (s)	18.1 CH_3
21	2.93 (m)	29.3 CH_2
22	6.10 (m)	137.4 CH
23		127.9 C
24		170.1 C
25	1.73 (s)	20.8 CH_3
26	1.40 (m)	25.2 CH_2
	2.33 (m)	
27	2.52 (d, 9.3)	49.0 CH
28		83.8 C
29	1.68 (s)	29.9 CH_3
30	1.27 (s)	28.9 CH_3
31	2.03 (m)	22.7 CH_2
32	5.04 (m)	123.8 CH
33		131.8 C
34	1.61 (s)	25.7 CH_3
35	1.53 (s)	17.6 CH_3
OH-1	12.80 (s)	

[a] Data were measured in $CDCl_3$ at 300 MHz. Chemical shifts (δ) are in ppm from TMS. *J* values are in Hz and omitted if the signals were overlapped as multiplets. s = singlet, d = doublet, m = multiplet. [b] Data were measured in $CDCl_3$ at 75.5 MHz. Chemical shifts (δ) are in ppm from TMS.

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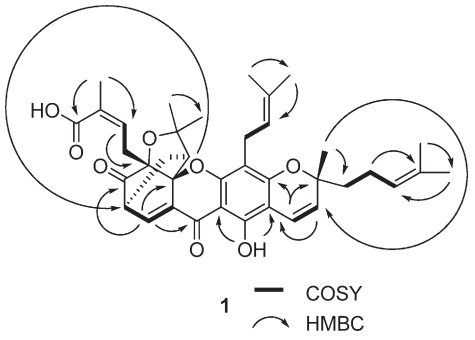

Figure 1. COSY and key HMBC correlations of (−)-gambogic acid (**1**).

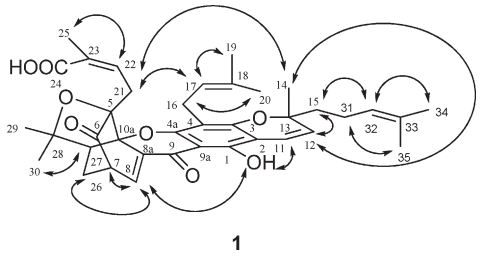

Figure 2. Selected NOESY correlations of (−)-gambogic acid (**1**).

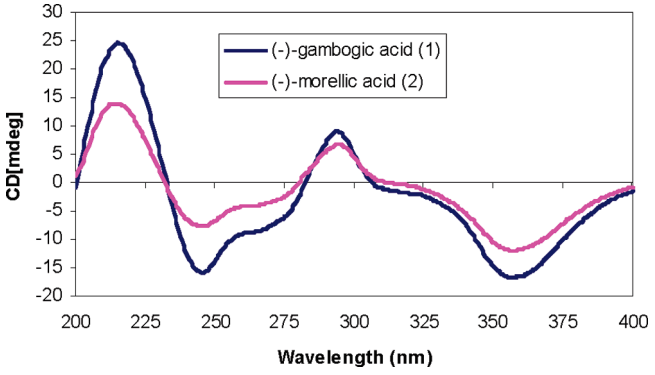

Figure 3. CD spectra of (−)-gambogic acid (**1**) and (−)-morellic acid (**2**). The data were obtained in MeOH (0.017 mg/mL for both compounds) on the average of three scans corrected by subtracting a spectrum of the appropriate solution in the absence of the samples recorded under identical conditions. Each scan in the range 200−400 nm was obtained by taking points every 0.5 nm with an integration time of 0.5 s and a 2 nm bandwidth.

configuration of the caged unit of (−)-gambogic acid (**1**) may be defined unequivocally as 5*R*, 7*S*, 10a*S*, and 27*S*.

The NOESY correlations between H-7/H-8 and H-7/H-22, H-14/H-8, H-14/H-16, and H-14/H-21, H-16/H-22, and H-17/H-21 indicated the same orientation of the methyl group (C-14) as the prenyl group linked at the C-5 position. This relative configuration of C-13 indicates a 13*R* absolute configuration, confirming the previous determination of the absolute configuration of C-13 by chemical degradations.[10] Consequently, the absolute configuration of (−)-gambogic acid (**1**) was determined as 5*R*, 7*S*, 10a*S*, 13*R*, and 27*S*.

Both (−)-gambogic acid (**1**) and (−)-morellic acid (**2**) were evaluated for their cytotoxicity against the HT-29 human colon cancer cell line, using paclitaxel as positive control, and they were cytotoxic with ED_{50} values of 0.48 and 0.36 μM, respectively.

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were measured with a Perkin-Elmer 343 polarimeter. UV spectra were recorded on a Shimadzu UV-2401 PC UV−vis recording spectrophotometer. CD measurements were performed using a JASCO J-810-401 instrument. IR spectra were recorded on a Nicolet 6700 FT-IR spectrometer. ^{1}H and ^{13}C NMR data, including DEPT, HMQC, HMBC, and COSY spectra, were recorded at room temperature on a Bruker Avance DPX-300 MHz spectrometer, and the NOESY or ROESY NMR spectra were recorded on a Bruker Avance DRX-600 NMR spectrometer, with TMS as internal standard for both the 300 and 600 MHz instruments. ESIMS and HRESIMS were recorded on a LCT-TOF mass spectrometer. (−)-Gambogic acid (**1**) was purchased from Sigma (G8171 5MG), and (−)-morellic acid (**2**) was isolated from *Garcinia lateriflora*.[29]

(−)-Gambogic Acid (1):. amorphous, orange powder showing a brown color under UV light at 365 nm; $[\alpha]^{20}_{D}$ −714.1 (*c* 0.17, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 290 (3.36), 362 (3.28) nm; CD (MeOH) λ_{max} ($\Delta\varepsilon$) 215 (+17.52), 245.5 (−10.82), 294 (+6.16), 355.5 (−11.45) nm; IR (dried film) ν_{max} 2970, 2927, 1738, 1693, 1634, 1594, 1456, 1383, 1261, 1176, 756 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 1; positive ESIMS *m*/*z* 651.4 $[M + Na]^+$; positive HRESIMS found *m*/*z* 651.2931, calcd 651.2934 for $C_{38}H_{44}O_8Na$.

Cytotoxicity Assay. Cytotoxicity of the samples was screened against HT-29 human colon cancer cells by a previously reported procedure, with paclitaxel used as a positive control (ED_{50}: 0.1 nM).[32]

ASSOCIATED CONTENT

Supporting Information. MS and ^{1}H, ^{13}C, DEPT 90, DEPT 135, COSY, NOESY, HMQC, and HMBC NMR spectra of (−)-gambogic acid (**1**), overlap profile of ROESY 2D NMR spectra of (−)-gambogic acid (**1**), and NOESY 2D NMR spectra of (−)-morellic acid (**2**). This information is available free of charge via the Internet at http://pubs.acs.org.

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DEDICATION

Dedicated to Dr. Koji Nakanishi of Columbia University for his pioneering work on bioactive natural products.

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