Antibacterial Caged-Tetraprenylated Xanthones from the Fruits of *Garcinia hanburyi*

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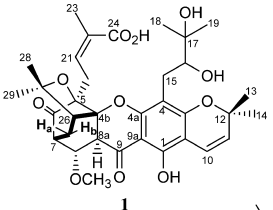
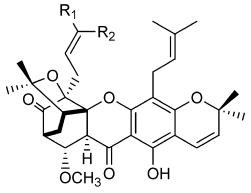
A new caged-tetraprenylated xanthone, hanburinone (1), was isolated from the fresh fruits of *Garcinia hanburyi* together with four known caged-tetraprenylated xanthones; isomoreollin B (2), morellin (3), moreollic acid (4) and morellic acid (5). Their structures were elucidated by analysis of spectroscopic data and comparison of the NMR data with those reported previously. Compounds 4 and 5 showed moderately antibacterial activity against methicillin-resistant *Staphylococcus aureus* with a MIC value of 25 μg/ml.

Key words *Garcinia hanburyi*; Guttiferae; caged-tetraprenylated xanthone; antibacterial activity

Garcinia hanburyi (Guttiferae, locally named "Rong Thong") is a small to medium-sized tree found in Central and Southern part of Thailand. The latex is used as a dye and folk medicine for potent purgative and infected wounds.[1)] Previous phytochemical investigation on the latex of *G. hanburyi*[2)] led to the identification of eleven new and four known caged-xanthones. Caged-polyprenylated xanthones have been isolated from several *Garcinia* species, *e.g.*, *G. bracteata*,[3)] *G. gaudichaudii*,[4—7)] *G. morella*[8—10)] and *G. scortechinii*.[11—13)] Compounds of this type exhibited cytotoxic[2—6)] and antibacterial[11,13)] activities. During our search for antibacterial substances from *Garcinia* plants, we found that the crude methanol extract from the fruits of *G. hanburyi* exhibited a significant antibacterial activity against methicillin-resistant *Staphylococcus aureus* (MRSA) with a minimum inhibition concentration (MIC) of 16 μg/ml. There is no report on the constituents from the fruits and no information on antibacterial activity of caged-xanthones isolated from *G. hanburyi* against MRSA. The crude methanol extract was separated into two parts by dissolving with $CHCl_3$. The $CHCl_3$-soluble part was then subjected to various chromatography to obtain one new (**1**) and four known caged-tetraprenylated xanthones; isomoreollin B (**2**),[2)] morellin (**3**),[8)] moreollic acid (**4**)[2)] and morellic acid (**5**).[2)] All structures were elucidated using 1D and 2D NMR spectroscopic data. The ^{1}H- and ^{13}C-NMR signals were assigned from DEPT, HMQC and HMBC spectra.

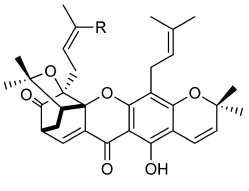


1



2: R_1 = CHO, R_2 = CH_3

4: R_1 = CH_3, R_2 = CO_2H



3: R = CHO

5: R = CO_2H

Hanburinone (**1**) had a molecular formula of $C_{34}H_{42}O_{11}$ from HR-MS of $[M-H_2O]^+$. The IR spectrum exhibited strong bands due to a hydroxyl group of carboxylic acid (3600—3200 cm^{-1}), an unconjugated carbonyl group (1739 cm^{-1}), an α,β-unsaturated carboxyl group (1697 cm^{-1}) and a chelated *ortho*-hydroxyl carbonyl group (1633 cm^{-1}). The presence of three carbonyl groups was confirmed by the signals at δ_C 208.0, 194.2 and 170.7 in the ^{13}C-NMR spectrum (Table 1). The UV absorption bands at λ_{max} 363, 316, 276 and 204 nm were similar to those of moreollic acid (**4**),[2)] suggesting that **1** had a caged-polyprenylated xanthone moiety without a C8/C8a double bond. The ^{1}H- and ^{13}C-NMR spectral data were similar to those of **4** except for the fact that signals of a 3-methylbut-2-enyl group at C-4 were replaced by signals for a 2,3-dihydroxy-3-methylbutyl group [δ_H 3.00 (1H, dd, J=15.0, 10.5 Hz, H_a-15), 2.86 (1H, dd, J=15.0, 2.4 Hz, H_b-15), 4.05 (1H, dd, J=10.5, 2.4 Hz, H-16), 1.71 (3H, s, Me-18) and 1.70 (3H, s, Me-19); δ_C 25.6 (C-15), 78.2 (C-16), 73.6 (C-17), 28.8 (C-18) and 28.6 (C-19)]. This group was assigned to be at C-4 (δ_C 105.3) by ^3J HMBC correlations of the methylene protons ($H_{a,b}$-15) with C-3 (δ_C 160.5) and C-4a (δ_C 157.0). The attachment of other substituents was identical to **4** based on HMBC data (Table 1). The NOEDIFF data (Table 1) confirmed the α-configuration of H-8a (δ_H 3.29, d, J=1.2 Hz), the β-configuration of H-8 (δ_H 4.30, dd, J=4.5, 1.2 Hz) and the *Z*-configuration of the C21/22 double bond which were identical to those of **4**. From these results, **1** is a new naturally occurring caged-tetraprenylated xanthone, having a 2,3-dihydroxy-3-methylbutyl unit at C-4.

All caged-xanthones were tested for antibacterial activity against MRSA. Moreollic acid (**4**) and morellic acid (**5**) exhibited moderate activity with a MIC of 25 μg/ml while hanburinone (**1**), isomoreollin B (**2**) and morellin (**3**) showed less activity with an equal MIC value of 200 μg/ml. From these results, caged-xanthones with the C-5 substituent with a terminal carboxyl group, such as **4** and **5**, played better activity than **2** and **3** with a formyl group. This was in agreement with the results obtained from our investigation on 7-methoxy caged-polyprenylated xanthones from *G. scortechinii*.[13)] In addition, **4** with the C-4 prenyl group had better activity than **1** with a more polar group at C-4. To explain the activity of the crude extract, further work on the $CHCl_3$-in-

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Table 1. ^{1}H- and ^{13}C-NMR Data of Hanburinone (**1**)

Position	C-type	δ_H	δ_C	HMBC	NOE
1	C	12.10 (s, OH)	157.2	C-1, C-9a	
2	C		103.0		
3	C		160.5		
4	C		105.3		
4a	C		157.0		
4b	C		88.1		
5	C		86.1		
6	C=O		208.0		
7	CH	2.83 (dd, 6.0, 4.5)	44.5	C-5, C-6, C-8, C-8a, C-26	H-8, H-8a, H-25a
8	CH	4.30 (dd, 4.5, 1.2)	74.8	C-4b, C-6, C-7, C-8a, C-9, C-25	H-7, H-8a, H-25b, 8-OMe
8-OMe	CH_3	3.31 (s)	55.9	C-8	H-8, Me-23
8a	CH	3.29 (d, 1.2)	47.6	C-4b, C-5, C-9, C-26	H-7, H-8, Me-23
9	C=O		194.2		
9a	C		102.1		
10	CH	6.64 (d, 10.0)	115.2	C-1, C-2, C-3, C-12	H-11
11	CH	5.53 (d, 10.0)	126.0	C-2, C-12, C-14	H-10
12	C		79.0		
13	CH_3	1.46 (s)	28.7	C-11, C-12, C-14	
14	CH_3	1.50 (s)	28.5	C-11, C-12, C-13	
15	CH_2	a: 3.00 (dd, 15.0, 10.5)	25.6	C-3, C-4, C-4a, C-16	
		b: 2.86 (dd, 15.0, 2.4)			
16	CH	4.05 (dd, 10.5, 2.4)	78.2	C-15	Me-18, Me-19
17	C		73.6		
18	CH_3	1.71 (s)	28.8	C-16, C-17, C-19	
19	CH_3	1.70 (s)	28.6	C-16, C-17, C-18	
20	CH_2	a: 3.54 (dd, 13.8, 10.5)	28.2	C-4b, C-5, C-6, C-21, C-22	
		b: 2.99 (dd, 13.8, 6.0)			
21	CH	5.91 (ddq, 10.5, 6.0, 1.5)	132.5	C-23, C-24	Me-23
22	C		131.4		
23	CH_3	1.88 (br s)	20.5	C-21, C-22, C-24	H-8a, H-21, 8-OMe
24	C=O		170.7		
25	CH_2	a: 1.98 (dd, 15.0, 6.0)	20.1	C-4b, C-7, C-8, C-27	H-7, H-25b, Me-29
		b: 1.35 (dd, 15.0, 8.7)		C-6, C-7, C-8, C-27	H-8, H-25a, H-26
26	CH	2.51 (d, 8.7)	43.9	C-4b, C-7, C-25, C-28	H-25b
27	C		81.5		
28	CH_3	1.29 (s)	29.5	C-26, C-27, C-29	
29	CH_3	1.14 (s)	27.5	C-26, C-27, C-28	

soluble part is required.

Experimental

General Procedures IR spectra were obtained on FTS165 FT-IR spectrometer. UV spectra were measured with UV-1601 spectrophotometer (Shimadzu). ^{1}H- and ^{13}C-NMR spectra were recorded on 300 MHz Bruker AVANCE spectrometer using deuterochloroform solution with tetramethylsilane (TMS) as an internal standard. Optical rotations were measured with sodium D line (589 nm) on a JASCO P-1020 polarimeter. EI-MS and HR-EI-MS data were determined on a MAT 95 XL mass spectrometer. Column chromatography was performed on silica gel (Merck) type 100 (70—230 mesh ASTM) or Sephadex LH-20. Thin-layer chromatography (TLC) and precoated TLC were performed on silica gel 60 F_{254} (Merck).

Plant Material The fruits of *G. hanburyi* were collected at the Sri Pang Nga National Park, Kura Buri and Tagua Pa, Pang Nga, Thailand in May 2004. The plant was identified by Miss Katesarin Maneenoon, Department of Biology, Faculty of Science, Prince of Songkla University, Hat Yai, Songkhla, where a voucher specimen, No. VR 01-04, has been deposited.

Extraction and Isolation The fruits (16.13 g) of *G. hanburyi*, cut into small segments, were extracted with MeOH (3×0.6 l) at room temperature and concentrated under reduced pressure to give a brown-yellow gum (9.39 g). It was separated into two parts by dissolving with $CHCl_3$. The $CHCl_3$-soluble part was evaporated to dryness under reduced pressure to give a brown-yellow gum (1.15 g) which was further fractionated by column chromatography using gradient system of increasing polarity (MeOH–CH_2Cl_2) to yield five fractions. Fraction 3 (875 mg, eluted with 1—3% MeOH–CH_2Cl_2) was subjected to column chromatography on Sephadex LH-20 eluted with 50% MeOH–$CHCl_3$ to afford four fractions. The second fraction (284 mg) was separated by column chromatography with solvent mixtures of increasing polarity (70% $CHCl_3$–light petroleum to 50% MeOH–$CHCl_3$) to give two subfractions. The first subfraction (22 mg, eluted with 70% $CHCl_3$–light petroleum), upon column chromatography using 15% EtOAc–light petroleum, followed by purification on preparative TLC using 15% EtOAc–light petroleum (9 runs) gave **2** [8.7 mg, $[\alpha]_D^{28}$ −44° (c=0.11, $CHCl_3$); $[\alpha]_D^{27}$ −37° (c=0.1, $CHCl_3$)[2)]]. The third fraction (506 mg) was purified by column chromatography with solvent mixtures of increasing polarity (80% $CHCl_3$–light petroleum to 60% MeOH–$CHCl_3$) to afford ten subfractions. Compound **3** [3.1 mg, $[\alpha]_D^{28}$ −600° (c=0.04, $CHCl_3$); $[\alpha]_D^{27}$ −594° (c=4.5, $CHCl_3$)[8)]] was obtained from the second subfraction (11 mg, eluted with 80% $CHCl_3$–light petroleum) after purification on preparative TLC using 15% EtOAc–light petroleum (5 runs). Further separation of the fifth subfraction (16 mg, 90% $CHCl_3$–light petroleum) on column chromatography eluted with solvent mixtures of increasing polarity (20% EtOAc–light petroleum to 10% MeOH–EtOAc), followed by preparative TLC with 1% MeOH–CH_2Cl_2 (7 runs), afforded **1** (4 mg). Compound **4** [88 mg, $[\alpha]_D^{28}$ −39° (c=0.22, $CHCl_3$); $[\alpha]_D^{27}$ −31° (c=0.1, $CHCl_3$)[2)]] was obtained from the seventh subfraction. The ninth subfraction (71 mg, eluted with 1—3% MeOH–$CHCl_3$) was further purified by column chromatography using 1% MeOH–$CHCl_3$, followed by preparative TLC using 15% EtOAc–light petroleum (15 runs) to afford **5** [4 mg, $[\alpha]_D^{28}$ −541° (c=0.19, $CHCl_3$)].

Hanburinone (**1**): Yellow gum, $[\alpha]_D^{28}$ −62° (c=0.09, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 363 (3.45), 316 (4.02), 276 (4.51), 204 (4.35); IR (neat) ν_{max} 3600—3200, 1739, 1697, 1633 cm^{-1}; ^{1}H-NMR (300 MHz), Table 1; ^{13}C-NMR (125 MHz), Table 1; EI-MS m/z 608 $[M-H_2O]^+$ (9), 592 (15), 575 (23), 537 (59), 504 (100), 476 (52), 420 (55), 303 (37), 231 (55); HR-EI-MS m/z 608.2626 $[M-H_2O]^+$ (Calcd for $C_{34}H_{40}O_{10}$, 608.2621).

Antibacterial Activity Testing MICs were determined by the agar microdilution method.[14)] The test substances were dissolved in DMSO (Merck, Germany). Serial 2-fold dilutions of the test substances were mixed with

melted Mueller-Hinton agar (Difco) in the ratio of 1 : 100 in microtiter plates with flat-bottomed wells (Nunc, Germany). Final concentration of the test substances in agar was ranged from 200 to 0.39 μg/ml. MRSA isolated from a clinical specimen, Songklanakarin Hospital, was used as test strain. Inoculum suspensions (10 μl) were spotted on agar-filled wells. The inoculated plates were incubated at 35 °C for 18 h. MICs were recorded by reading the lowest substance concentration that inhibited visible growth. Vancomycin was used as a positive control drug and had a MIC value of 2 μg/ml. Growth controls were performed on agar containing DMSO.

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