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NEW PRENYLATED XANTHONE FROM THE BRANCH OF *GARCINIA COSTATA*

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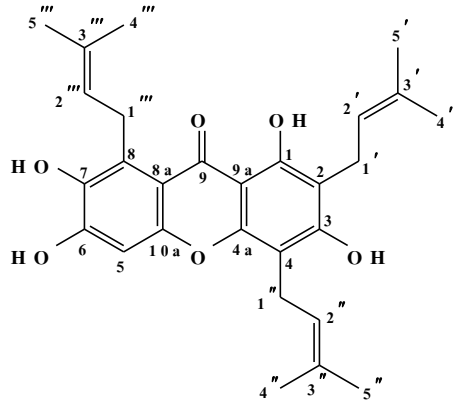
Abstract – A new triprenylated xanthone, costatin (**1**), together with five known xanthones (**2**-**6**), was isolated from a branch of *Garcinia costata* (Guttiferae). The structure was elucidated on the basis of spectroscopic data. All xanthone derivatives were evaluated for antimalarial, anti-tuberculosis (TB), cytotoxicity against human breast adenocarcinoma cell line (MCF-7), anti-human KB-cell line and cytotoxicity against African green monkey kidney (Vero) cells. Costatin (**1**) exhibited significant antimalarial activity (IC_{50} 1.57 μg/mL); however, it also showed comparable cytotoxic activity (IC_{50} 1.12 μg/mL).

Plants in the genus *Garcinia* are rich sources of bioactive compounds. A number of new compounds with diverse chemical structures have been isolated from several major species such as *G. mangostana*,[1] *G. hanburyi*,[2] *G. dulcis*,[3] *G. lancilimba*,[4] *G. subelliptica*[5] and *G. scortechinii*.[6,7] One of the major types of compounds present in *Garcinia* constituents are polyprenylated xanthones, which have been found to possess a broad range of pharmacological properties: antimycobacterial,[8] antioxidant,[9] HIV-1 protease inhibitory,[10] anti-inflammatory,[11] antifungal,[12] antimalarial,[13,14] anti-cancer and cytotoxic activities.[15]

Garcinia costata Hemsley ex King, a small tree with orange-brown twigs and large ovate-oblong leaves, is commonly known in Thailand as Mangkhut Paa (wild mangosteens). So far, there has been no report

on the phytochemical studies of this species. We found that a crude dichloromethane extract from the branches of *G. costata* showed significant biological activities against *Plasmodium falciparum* K1 and *Mycobacterium tuberculosis* H37Ra. An investigation of a branch of *G. costata* led to the isolation of 6 xanthones, numbered (**1**-**6**), among which compound **1** was a new prenylated xanthone derivative. We report herein the isolation and structural elucidation of the new compound as well as biological activities of these xanthones.



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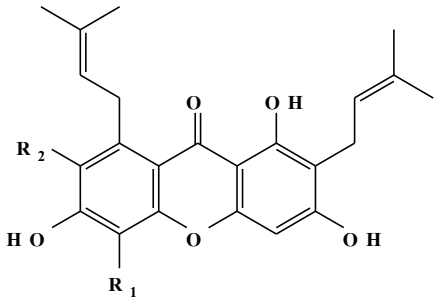
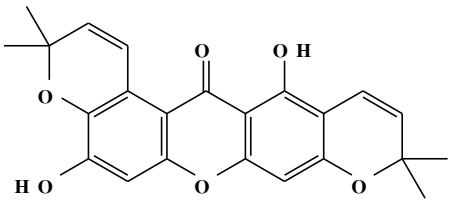


	R_1	R_2
2	H	OMe
4	prenyl	OH
6	H	OH



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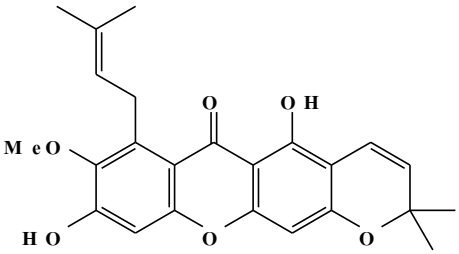

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Figure 1. Compounds isolated from *G. costata*

Garcinia costata was collected at Phukradung National Park, Loei Province, Thailand, in July 2006. The crude dichloromethane extract was subjected to isolation by successive column chromatography on Sephadex LH-20 and silica gel to yield compounds **1**-**6** (Figure 1). The structural elucidation was carried out by means of the spectroscopic analysis and compared with previous reports of the five known compounds.

Compound **1** was obtained as a yellow solid. The molecular formula was determined to be $C_{28}H_{32}O_6$ by HRMS (ESI-TOF), with an observation of an $[M + H]^+$ ion peak at *m/z* 465.2278. The UV spectrum

showed characteristic absorption bands of the xanthone skeleton at λ_{max} 242, 264, 318, and 376 nm. The IR spectrum showed the functionality of a conjugated carbonyl at ν_{max} 1640 cm^{-1} (C=O stretching) and a broad hydroxyl absorption band at 3401 cm^{-1}. The ^1H NMR spectrum (Table 1) showed a sharp signal at δ_H 13.70 assignable to a chelated hydroxyl group. A phenolic hydroxyl group and a signal of aromatic proton exhibited at δ_H 6.31 and 6.83, respectively.

Table 1. ^1H- and ^{13}C-NMR spectral data of Costatin (1) in $CDCl_3$

Position	δ_H (mult, *J* in Hz)	δ_C
1	-	158.0
2	-	108.0
3	-	159.3
4	-	103.8
4a	-	151.6
5	6.83 (*s*)	100.7
6	-	[a]150.2
7	-	139.0
8	-	126.9
8a	-	110.8
9	-	182.5
9a	-	103.2
10a	-	[a]153.1
1′	3.45 (*d*, 7.2)	[b]21.1
2′	5.28 (*m*)	[c]121.3
3′	-	134.5
4′	[d]1.84 (*s*)	17.4
5′	[e]1.72 (*s*)	25.3
1″	3.49 (*d*, 7.2)	[b]21.2
2″	5.28 (*m*)	[c]121.0
3″	-	133.0
4″	[d]1.86 (*s*)	17.4
5″	[e]1.76 (*s*)	25.3
1‴	4.32 (*d*, 6.0)	25.5
2‴	5.28 (*m*)	121.5
3‴	-	135.1
4‴	[d]1.88 (*s*)	17.5
5‴	[e]1.78 (*s*)	25.3
1-OH	13.70 (*s*)	-
3-OH	6.31 (*s*)	-

[a–c] Assignments of carbons are interchangeable. [d and e] Assignments of protons are interchangeable.

The ^{13}C NMR spectrum (Table 1) and DEPT experiments indicated the presence of a carbonyl carbon (δ_C 182.5), six methyl carbons (δ_C 25.3 × 3, 17.5, and 17.4 × 2), three methylene carbons (δ_C 25.5, 21.2 and 21.1), four sp^2 methine carbons (δ_C 121.5, 121.3, 121.0 and 100.7), and fourteen sp^2 quaternary carbons (δ_C 159.3, 158.0, 153.1, 151.6, 150.2, 139.0, 135.1, 134.5, 133.0, 126.9, 110.8, 108.1, 103.8 and 103.2).

The chelated OH (δ_H 13.70) showed HMBC correlations (Figure 2) to C-1 (δ_C 158.0), C-2 (δ_C 108.0) and C-9a (δ_C 103.2), whereas the aromatic proton (δ_H 6.83) was located on C-5 based on its 2-bond (C-6 and C-10a) and 3-bond (C-7 and C-8a) HMBC correlations.

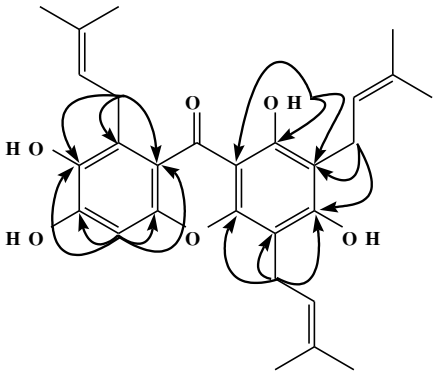

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Figure 2. Selected HMBC Correlations for Costatin (**1)**

The remaining signals, olefinic protons at δ_H 5.28 (3H, overlapped, H-2′, H-2″and H-2‴, methyl groups at δ_H 1.84 (H-4′), 1.86 (H-4″), 1.88 (H-4‴), 1.72 (H-5′), 1.76 (H-5″), 1.78 (H-5‴) and allylic methylene protons at δ_H 3.45 (H-1′), 3.49 (H-1″) and 4.32 (H-1‴), constitute three prenyl groups which are all attached to quaternary aromatic carbons (C-2, C-4 and C-8) of the xanthone. These were confirmed by the HMBC correlations: from H-1′ to C-2, C-3, C-2′, and C-3′, from H-1″ to C-3, C-4, C-4a, C-2″, and C-3″, and from H-1‴ to C-7, C-8, C-8a, C-2‴, and C-3‴. Consequently, the structure of **1** was determined to be costatin (1,3,6,7-tetrahydroxy-2,4,8-tri-(3-methyl-2-butenyl)xanthone).

Compounds **2**–**6** were identified respectively as *α*-mangostin,[16-18] brasilixanthone B,[19] garcinone E,[20] 5,9-dihydroxy-8-methoxy-2,2-dimethyl-7-(3-methylbut-2-enyl)-2*H*,6*H*-pyrano[3,2-*b*]xanthen-6-one[21] and *γ*-mangostin[10,20] by spectroscopic methods. The ^{1}H and ^{13}C NMR spectroscopic data were identical to those reported in the literature.

Compounds **1**-**6** were tested for the following biological activities: antimalaria (*Plasmodium falciparum* K1) with the use of the microculture radioisotope technique, antituberculosis (*Mycobacterium tuberculosis* H37Ra) and cytotoxicity against Vero cells (African green monkey kidney fibroblasts) with the use of the green fluorescent protein microplate assay (GFPMA), and cytotoxicity against two cancer cell lines (MCF-7 and KB) and nonmalignant Vero cells (Aftrican green monkey kidney fibroblasts) with the use of the resazurin microplate assay. The biological activity test results are summarized in Table 2.

Table 2. Biological Activities of Compounds **1**–**6**

compound	Anti-malaria[a]	Anti-TB [b]	Cytotoxicity,[c] IC_{50} (μg/mL)		
	IC_{50} (μg/mL)	MIC (μg/mL)	MCF-7	KB	Vero
1	1.57	50.00	0.24	0.41	1.12
2	>10	3.13	3.91	8.56	5.80
3	>10	>200	>50	>50	– [d]
4	2.66	25.00	0.65	0.51	2.04
5	3.15	12.50	20.08	29.87	10.00
6	2.88	100.00	1.62	0.90	2.91

[a] In vitro anti-malarial activity against *Plasmodium falciparum* K1. The IC_{50} value of a standard antimalarial compound, dihydroartemisinin, was 0.0031 μg/mL. [b] Growth inhibitory activity against *Mycobacterium tuberculosis* H37Ra. The MIC value of a standard anti-TB compound, isoniazid, was 0.050 μg/mL. [c] The standard compound, ellipticine, exhibited cytotoxic activities against MCF-7, KB and Vero cell lines with respective IC_{50} values of 0.087, 0.34 and 0.39 μg/mL. [d] Not tested.

The phytochemical study of *Garcinia costata* (Guttiferae) is reported here for the first time. Costatin (**1)** exhibited a significant antimalarial activity (IC_{50} 1.57 μg/mL); however, it also showed comparable cytotoxicity (IC_{50} 1.12 μg/mL). These data were similar to those of other analogues except for the lack of biological activity of **3**. Antimalarial activities of these xanthones were well correlated to their cytotoxic activities. In contrast, the structural relationship of the antituberculosis activities among the 6 compounds greatly varied, suggesting a possibility of designing selective anti-TB xanthone drugs.

EXPERIMENTAL

GENERAL

Melting points were measured with the use of a Bibby Stuart Scientific melting point apparatus SMP3. UV spectra were measured with a UNICAM UV-310 spectrophotometer. ESI-TOF mass spectra were recorded with a Micromass LCT spectrometer. NMR spectra (^{1}H, ^{13}C, DEPT, ^{1}H-^{1}H COSY, NOESY, HMQC and HMBC) were recorded with Bruker AV300 spectrometers. Column chromatography was performed with the use of Merck silica gel 60 and Sephadex LH-20.

PLANT MATERIAL

Garcinia costata was collected at Phukradung National Park, Loei Province, Thailand, in July 2006. The plant identification was confirmed by Assistant Professor Dr. Maruay Mekanawakul, a specialist in the field of botanical study. A voucher specimen (WU 1414) is deposited at the botanic garden, Walailak University, Nakhon Si Thammarat, Thailand.

EXTRACTION AND ISOLATION

Dried branches of *G. costata* (3.10 kg) were macerated in CH_2Cl_2 (40 L) at room temperature for 3 days and the extract was concentrated under reduced pressure. The evaporated CH_2Cl_2 extract (26.89 g) was dissolved in MeOH (250 mL), filtered and concentrated under reduced pressure. The MeOH soluble portion (13.33 g) was subjected to Sephadex LH-20 column chromatography (MeOH) to afford seven fractions, 1–7, upon TLC profile. Fraction 6 (1.79 g) was chromatographed again with the use of a Sephadex LH-20 column, yielding nine fractions (6A–6I). Fraction 6F (0.38 g) was chromatographed with Sephadex LH-20 (MeOH) to give six fractions (6F1–6F6). Fraction 6F4 (0.19 g) was subjected to column chromatography with silica gel (CH_2Cl_2-MeOH, 99:1) to furnish compounds **1** (25 mg), **2** (10 mg), **3** (1 mg), and **4** (13 mg). Fraction 6E (0.42 g) was purified on a silica gel column by elution with hexane-CH_2Cl_2 (60:40) giving **5** (2 mg). Compound **6** (5 mg) was isolated from fraction 6H by successive column chromatography on Sephadex LH-20 (MeOH) and silica gel (CH_2Cl_2-MeOH, 98:2) columns.

Costatin [1,3,6,7-tetrahydroxy-2,4,8-tri-(3-methyl-2-butenyl)xanthone] (**1**): Yellow solid. mp 145–146 °C. UV λ_{max} (MeOH) (log ε): 264 (4.59), 242 (4.51), 318 (4.42), 376 (4.04) nm. IR (neat) ν_{max}: 3401, 1640, 1616, 1581, 1464, 1238, 1172, 1055 cm^{-1}. ^{1}H-NMR (300 MHz, $CDCl_3$) and ^{13}C-NMR (75 MHz, $CDCl_3$) see Table 1. HRMS (ESI-TOF) $[M+H]^+$ *m/z*: 465.2278 for $C_{28}H_{33}O_6$ (calcd. 465.2277).

BIOLOGICAL ASSAY

An assay for the activity against *Plasmodium falciparum* K1 was performed with the use of the microculture radioisotope technique.[22] The growth inhibitory activity against *Mycobacterium tuberculosis* H37Ra and the cytotoxicity against Vero cells (African green monkey kidney fibroblasts) were performed with the use of the green fluorescent protein microplate assay (GFPMA).[23] Anticancer activities against KB cells (oral human epidermoid carcinoma) and MCF7 cells (human breast cancer) were evaluated with the use of the resazurin microplate assay.[24]

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