

Xanthenes from the Green Fruit Hulls of *Garcinia mangostana*

Sunit Suksamrarn,^{*,†} Narisara Suwannapoch,[†] Pinit Ratananukul,[†] Nantana Aroonlerk,[‡] and Apichart Suksamrarn[§]

Department of Chemistry, Faculty of Science, Srinakharinwirot University, Bangkok 10110, Thailand, Department of Stomatology, Faculty of Dentistry, Srinakharinwirot University, Bangkok 10110, Thailand, and Department of Chemistry, Faculty of Science, Ramkhamhaeng University, Bangkok 10240, Thailand

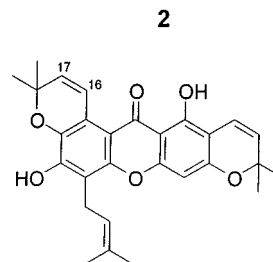
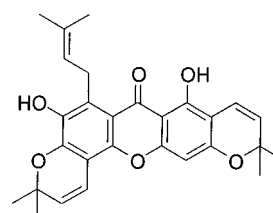
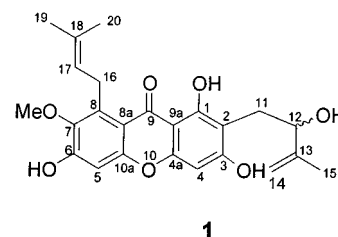
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Three new xanthenes, mangostenol (**1**), mangostenone A (**2**), and mangostenone B (**3**), were isolated from the green fruit hulls of *Garcinia mangostana*, along with the known xanthenes, trapezifolixanthone, tovoephyllin B (**4**), α - and β -mangostins, garcinone B, mangostinone, mangostanol, and the flavonoid epicatechin. The structures of the new xanthenes were elucidated by analysis of their spectroscopic data.

Phytochemical studies have shown that *Garcinia mangostana* L. (Clusiaceae) is rich in a variety of oxygenated and prenylated xanthenes.^{1,2} Biological studies on the constituents of the fruit hulls have demonstrated antibacterial,^{3–5} antifungal,⁶ antitumor-promotion,⁷ and other biological activities.^{8–10} In Thai folk medicine, the fruit hulls of *G. mangostana* are used for healing skin infections and wounds and for the relief of diarrhea.¹¹ The present work on the green fruit hulls of *G. mangostana*, collected from Chanthaburi Province of Thailand, was initiated to determine whether any phytochemical differences exist between the ripe and the green fruit hulls. This led to the isolation and characterization of three new xanthenes, mangostenol (**1**), mangostenone A (**2**), and mangostenone B (**3**), and several known xanthenes, trapezifolixanthone,¹² tovoephyllin-B (**4**),¹³ α -mangostin,¹⁴ β -mangostin,¹⁵ garcinone B,¹⁶ mangostinone,¹⁷ mangostanol,¹⁸ and the flavonoid epicatechin.¹⁹ Among these known compounds, trapezifolixanthone has not been isolated previously from this plant. The structures of compounds **1–3** were determined unambiguously by 1D and 2D ¹H and ¹³C NMR experiments in conjunction with spectral comparison with data for known related compounds. Moreover, the ¹³C NMR data of tovoephyllin-B (**4**) are reported for the first time in this paper.

The fresh green fruit hulls of *G. mangostana* were extracted with MeOH, and the dried residue obtained was then separated by column chromatography to afford three new xanthenes, **1–3**, and several known compounds.

Compound **1**, mangostenol, was obtained as a yellow powder, with mp 157–159 °C, and $[\alpha]_D^{25} -20.0^\circ$ (c 0.10, MeOH). The molecular formula C₂₄H₂₆O₇ (*m/z* 426.1680) was determined by HREIMS. Its UV and IR spectra were indicative of a xanthone derivative. The ¹H NMR spectrum showed one chelated phenolic hydroxyl group [δ 13.86 (s)], two aromatic singlets [δ 6.22 (1H, s) and 6.64 (1H, s)], and one methoxyl group [δ 3.69, (3H, s)]. Signals due to a prenyl group were observed [δ 1.59 (3H, s) and 1.75 (3H, s), 4.00 (2H, d, *J* = 6.1 Hz), 5.16 (1H, m)]. Olefinic signals of a terminal double bond [δ 4.73 (1H, br s) and 4.90 (1H, br s)], as well as resonances for a methyl group [δ 1.74 (3H, s)] and a hydroxy methine proton [δ 4.22 (1H, dd, *J* = 8.4 and ca. 2.2 Hz)], established a 2-hydroxy-3-methylbut-3-enyl group. The ¹H and ¹³C NMR spectral data of **1** closely



3 16,17-Dihydro analogue of **4**

resembled those of α -mangostin,^{10,14} except for the presence in **1** of a 2-hydroxy-3-methylbut-3-enyl moiety instead of a prenyl group. The structural assignments were substantiated by 2D NMR techniques (HMQC and HMBC) and NOE experiments. In the HMBC spectrum (Table 1), the protons resonating at δ 2.78 and 3.01 (H-11) showed long-range heteronuclear connectivities with C-1 (δ 160.6), C-2 (δ 107.7), and C-3 (δ 162.7) as well as with C-12 (δ 76.1) and C-13 (δ 146.9), whereas H-12 (δ 4.22) was coupled with C-2, C-11 (δ 28.5), C-13, C-14 (δ 109.8), and C-15 (δ 17.9). The H-16 proton, resonating at δ 4.00, exhibited HMBC interactions with C-7 (δ 143.0), C-8 (δ 137.2), C-8a (δ 111.3), C-17 (δ 123.2), and C-18 (δ 131.5). The methoxyl signal at δ 3.69 showed a cross-peak with a quaternary aromatic carbon signal at δ 143.0. In an NOE experiment, irradiation of H-16 (δ 4.00) gave rise to the enhancement of the methoxyl signal at δ 3.69. Similarly, irradiation of H-12 also resulted in NOE enhancements of the H-11, H-14, and

* To whom correspondence should be addressed. Tel: 66 2-6641000, ext. 8217. Fax: 66 2-2592097. E-mail: sunit@psm.swu.ac.th.

[†] Faculty of Science, Srinakharinwirot University.

[‡] Faculty of Dentistry, Srinakharinwirot University.

[§] Ramkhamhaeng University.

Table 1. ^1H NMR and HMBC Data of Compounds **1–3**

position	mangostenol (1)		mangostenone A (2)		mangostenone B (3)	
	H^a	HMBC ^c	H^b	HMBC ^c	H^b	HMBC ^c
OH-1	13.86 s		13.82 s	1, 2, 9a	13.79 s	1
4	6.22 s	2, 3, 4a, 9, 9a	6.24 s	2, 3, 4a, 8a, 9, 9a	6.28 s	4a, 9a
5	6.64 s	6, 7, 8a, 9, 10a				
6 or OH-6					6.47 s	5, 6
OH-7 or OMe-7	3.69	7	5.52	6, 7, 8		
11	2.78 dd (14.6, 8.4) 3.01 dd (14.6, 2.2)	1, 2, 3, 12, 13	6.72 d (10.0)	1, 2, 3, 12, 13	6.71 d (10.0)	1, 3
12	4.22 dd (8.4, ca. 2.2)	2, 11, 13, 14, 15	5.55 d (10.0)	2, 11, 13, 14, 15	5.54 d (10.0)	13
14	4.73 br s, 4.90 br s	12, 13, 15	1.44 s	11, 12, 13	1.45 s	12, 13
15	1.74 s	12, 13, 14	1.44 s		1.45 s	
16	4.00 d (6.1)	7, 8, 8a, 17, 18	4.10 d (6.7)	7, 8, 8a, 17, 18	3.44 t (6.7)	7, 8, 17, 18
17	5.16 m	16, 18, 19, 20	5.28 br t (6.7)	19, 20	1.84 t (6.7)	8, 16, 18
19, 20	1.59 s, 1.75 s	16, 17, 18	1.68 s, 1.84 s	17, 18	1.36 s	17, 18
21			6.85 d (10.1)	5, 6, 10a, 23	3.54 d (7.2)	5, 6, 10a, 22, 23
22			5.65 d (10.1)	5, 23, 24, 25	5.26 m	
24, 25			1.51 s	21, 22, 23	1.66 s, 1.86 s	22, 23

^a Recorded in $\text{CDCl}_3 + \text{MeOH}-d_4$. ^b Recorded in CDCl_3 . ^c Correlated carbons.

H-15 resonances. Thus, the observed NMR signals are characteristic of a 1,3,6,7-tetraoxygenated xanthone with methoxyl, prenyl, and 2-hydroxy-3-methylbut-3-enyl moieties at C-7, C-8, and C-2, respectively. Therefore, mangostenol (**1**) could be assigned as 1,3,5-trihydroxy-2-(2-hydroxy-3-methylbut-3-enyl)-7-methoxy-8-(3-methylbut-2-enyl)xanthone. However, the data obtained did not permit the assignment of absolute configuration at C-12 for this compound.

Mangostenone A (**2**) was isolated as a yellow powder, mp 110–112 °C, and HREIMS at m/z 460.1895 established a molecular formula of $\text{C}_{28}\text{H}_{28}\text{O}_6$. The UV and IR spectra suggested that **2** is a xanthone derivative. Analysis of the ^1H NMR, ^1H – ^1H COSY, and HMQC spectra of **2** revealed the presence of a chelated hydroxyl group (δ 13.82) which was placed at C-1, a phenolic hydroxyl [δ 5.52 (1H, s, D_2O exchangeable)], and a single aromatic proton at δ 6.24. The other series of signals could be attributed to a prenyl group [δ 4.10 (2H, d, $J = 6.7$ Hz), 5.28 (1H, br t, $J = 6.7$ Hz), 1.68 (3H, s), and 1.84 (3H, s)] and two 2,2-dimethylchromene rings [δ 6.72 (1H, d, $J = 10.0$ Hz), 5.55 (1H, d, $J = 10.0$ Hz), 1.44 (6H, s) and δ 6.85 (1H, d, $J = 10.1$ Hz), 5.65 (1H, d, $J = 10.1$ Hz), 1.51 (6H, s)]. The ^1H and ^{13}C NMR chemical shifts of **2** were very similar to those obtained from garcimangosone A,²⁰ with the only difference being the occurrence of a second dimethylchromene ring. Careful comparison of the ^{13}C NMR data of these compounds, particularly the chemical shifts of C-5 (δ 106.9) and C-6 (δ 144.7), indicated that the second dimethylchromene substituent in **2** should be affixed at C-5/C-6 with the ether oxygen at C-6. Conclusive proof of the structure of **2** came from the extensive analysis of the HMBC (Table 1) and NOE spectra. The most important observations in the HMBC spectrum were the connectivities observed between the doublet proton signal at H-21 (δ 6.85) and C-10a (δ 147.3) and C-23 (δ 79.2) and between C-23 and the *cis* olefinic proton at δ 5.65 (H-22). An NOE observed for H-16 (δ 4.10) and OH-7 (δ 5.52), as well as the HMBC correlations between the phenolic signal at δ 5.52 and C-6, C-7, and C-8, further confirmed the placement of the prenyl moiety at C-8 and the hydroxyl group at C-7, respectively. An NOE between the chelated hydroxyl and H-11 together with the HMBC cross-peaks between H-11 and C-1, C-2, and C-3 established the presence of a 2,2-dimethylchromene ring at C-2/C-3 of the xanthone with the ether oxygen affixed to position C-3. The structure of mangostenone A (**2**) was thus elucidated as 1,7-dihydroxy-8-(3-

methylbut-2-enyl)-6',6'-dimethylpyrano(2',3':3,2)-6'',6''-dimethylpyrano(2'',3'':6,5)xanthone.

Mangostenone B (**3**), isolated as a yellow amorphous resin, was found to have the molecular formula $\text{C}_{28}\text{H}_{30}\text{O}_6$ by HREIMS (m/z 462.2050). Its UV and IR data suggested that **3** also possesses a xanthone skeleton. In the ^1H NMR spectrum, signals for an aromatic proton [δ 6.28 (1H, s)], a prenyl group [δ 3.54 (2H, d, $J = 7.2$ Hz), 5.26 (1H, m), 1.66 (3H, s), and 1.86 (3H, s)], and a 2,2-dimethylchromene ring [δ 3.44 (2H, t, $J = 6.7$ Hz), 1.84 (2H, t, $J = 6.7$ Hz), and 1.36 (6H, s)], together with a dimethylchromene ring [δ 6.71 (1H, d, $J = 10.0$ Hz), 5.54 (1H, d, $J = 10.0$ Hz), and 1.45 (6H, s)], were observed, in addition to two hydroxyl groups [δ 6.47 (1H, s) and 13.79 (1H, s, chelated OH)]. The ^1H NMR data of **3** were similar to those of tofophyllin B (**4**), a compound isolated from *Tovomita pyrifolium*.¹³ The only difference was in one of the dimethylchromene rings in **3**, when the two doublets at H-16 and H-17 in **4** were replaced by the two triplet methylenes at H-16 and H-17. This structure assignment was confirmed by its HMBC spectra (Table 1). The ^{13}C NMR data for **4** are reported herein for the first time (Table 2). The structure of mangostenone B (**3**) was therefore concluded to be 1,6-dihydroxy-5-(3-methylbut-2-enyl)-6',6'-dimethylpyrano-(2',3':3,2)-6'',6''-dimethyl-4'',5''-dihydropyrano(2'',3'':7,8)xanthone.

The known xanthone trapezifolixanthone¹² or toxyl-xanthone A²¹ was isolated as a minor constituent, and this compound has not been reported previously from this plant.

Although many xanthenes alkylated with a variation of structure in C_5 unit(s) have been reported,^{1,2} those with a 2-hydroxy-3-methylbut-3-enyl group in the C_5 unit have been found only in caged-polyprenylated xanthenes.^{22,23} Mangostenol (**1**) represents the first simple xanthone with a C_5 moiety of this type. A number of tri- C_5 -oxygenated xanthone derivatives have been isolated from *Garcinia* species. However, there have been only two reports^{20,24} on the isolation of compounds of this type from *G. mangostana*. It also appears that tri- C_5 -oxygenated xanthenes such as **2** and **3** are rare constituents of *G. mangostana*.

Experimental Section

General Experimental Procedures. Melting points were determined on a Griffin melting point apparatus and are uncorrected. Optical rotations were measured with a JASCO DIP-370 digital polarimeter. The UV spectra were run on a Shimadzu UV-2401 PC spectrophotometer. IR spectra were taken as KBr pellets on a Perkin-Elmer FT-IR Spectrum BX

Table 2. ^{13}C NMR Assignments for Compounds 1–4

carbon	1 ^a	2 ^b	3 ^b	4 ^b
1	160.6	157.9	157.8	157.7
2	107.7	104.3	104.2	104.3
3	162.7	159.6	159.5	159.7
4	93.5	93.9	94.1	94.1
4a	155.8	156.0	156.5	156.4
5	101.6	106.9	113.0	115.2
6	155.4	144.7	149.2	148.6
7	143.0	139.4	137.7	136.5
8	137.2	127.9	118.7	117.1
8a	111.3	111.9	111.2	108.3
9	181.8	182.4	183.0	182.8
9a	102.9	103.8	104.0	103.8
10a	155.2	147.3	151.0	150.9
11	28.5	115.8	115.8	115.6
12	76.1	127.0	127.0	127.0
13	146.9	77.8	77.2	77.9
14	109.8	28.2	28.3	28.3
15	17.9	28.2	28.3	28.3
16	26.2	25.7	22.2	120.9
17	123.2	122.4	33.0	131.3
18	131.5	132.4	75.5	76.8
19, 20	18.1, 25.6	18.1, 26.0	26.5	27.4
21		115.5	22.4	22.5
22		129.0	121.3	120.9
23		79.2	132.5	132.6
24, 25		28.4	18.0, 25.8	17.9, 25.8
OMe	60.9			

^a Recorded in $\text{CDCl}_3 + \text{CD}_3\text{OD}$. ^b Recorded in CDCl_3 .

spectrophotometer. ^1H and ^{13}C NMR spectra were recorded on a Bruker Avance 300 FT-NMR spectrometer, operating at 300 MHz (^1H) and 75 MHz (^{13}C). Low- and high-resolution EIMS were measured with a Finnigan MAT 90 instrument. Column chromatography and TLC were carried out using Merck silica gel 60 (>230 mesh) and precoated silica gel 60 F₂₅₄ plates, respectively. Plates of silica gel PF₂₅₄, thickness 1.25 mm, were utilized for preparative TLC. Spots on TLC were visualized under UV light and by spraying with anisaldehyde– H_2SO_4 reagent followed by heating.

Plant Materials. Green fruit hulls of *G. mangostana* were collected from Bahnkai District, Chanthaburi Province, Thailand, in April 1999. A voucher specimen [voucher #0032(RU)] of this plant is deposited at the Plant Collection Center, Faculty of Science, Ramkhamhaeng University, Bangkok, Thailand.

Extraction and Isolation. Pulverized fresh fruit hulls of *G. mangostana* (7.06 kg) were extensively extracted with MeOH. The combined extracts were concentrated in vacuo to give a yellow solid (205.5 g), and the filtrate was partitioned between *n*-BuOH and H_2O to afford an *n*-BuOH-soluble portion (133.0 g). A portion (45.0 g) of the yellow solid was subjected to column chromatography, eluting with CHCl_3 followed by stepwise addition of EtOAc and MeOH to yield 12 fractions (A–L). β -Mangostin (24 mg), trapezifolixanthone (4 mg), and mangostanol (8 mg) were obtained after repeated column chromatography of fraction E (1.2 g, eluting with hexane– CHCl_3 , 70:30), fraction G (309 mg, using CHCl_3 as eluent), and fraction J (3.5 g, eluting with CHCl_3 –MeOH, 94:6), respectively. Fraction D (83 mg) was rechromatographed on a silica gel column (eluted with hexane, hexane– CHCl_3 , CHCl_3 –MeOH in order of increasing polarity) to afford seven fractions, in which mangostenol (1, 5 mg) was obtained from the fifth fraction (CHCl_3 –hexane, 1:4). Fraction H (13.9 g) was purified by column chromatography eluting with a hexane– CHCl_3 system to furnish α -mangostin (400 mg) and a mixture (73 mg) of tofophyllin B (4) and mangostenone B (3). The mixture was further separated on preparative TLC to yield 4 (7 mg) and 3 (1 mg). Fraction I (1.7 g) was subjected to column chromatography eluting with a CHCl_3 –MeOH system to afford 16 subfractions, in which two known compounds, garcinone B (32 mg) and mangostinone (4 mg), were identified from the subfractions 8 and 10, respectively. Subfraction 9, eluting with CHCl_3 –MeOH (94.4:0.6), was further separated by preparative TLC (CHCl_3 –MeOH, 97:3) to give mangostenone A (2, 8 mg).

A portion of the *n*-BuOH-soluble fraction (33.0 g), after being purified by column chromatography on silica gel (eluted with CHCl_3 –MeOH, 90:10), afforded (–)-epicatechin (1.38 g).

Mangostenol (1): yellow powder; mp 157–159 °C; $[\alpha]_{\text{D}}^{20} -20.0^\circ$ (c 0.10, MeOH); UV (EtOH) λ_{max} (log ϵ) 243 (4.40), 319 (4.08), 358 (4.00) nm; IR (KBr) ν_{max} 3420, 2920, 1645, 1610, 1580, 1465, 1286, 1192, 1102 cm^{-1} ; ^1H NMR ($\text{CDCl}_3 + \text{MeOH}-d_4$) and ^{13}C NMR ($\text{CDCl}_3 + \text{MeOH}-d_4$) data, Tables 1 and 2, respectively; EIMS m/z 426 $[\text{M}]^+$ (15), 408 (6), 393 (11), 356 (22), 355 (100); HREIMS m/z 426.1680 (calcd for $\text{C}_{24}\text{H}_{26}\text{O}_7$, 426.1678).

Mangostenone A (2): yellow powder; mp 110–112 °C; UV (EtOH) λ_{max} (log ϵ) 285 (4.77), 344 (4.29) nm; IR (KBr) ν_{max} 3439, 2974, 1649, 1611, 1441, 1402, 1373, 1292, 1177, 1127, 886 cm^{-1} ; ^1H NMR (CDCl_3) and ^{13}C NMR (CDCl_3) data, Tables 1 and 2, respectively; EIMS m/z 460 $[\text{M}]^+$ (37), 445 (58), 417 (100), 401 (14), 389 (12); HREIMS m/z 460.1895 (calcd for $\text{C}_{28}\text{H}_{28}\text{O}_6$, 460.1885).

Mangostenone B (3): yellow amorphous resin; UV (EtOH) λ_{max} (log ϵ) 293 (4.47), 334 (4.09), 366 (3.87) nm; IR (KBr) ν_{max} 3413, 2925, 2855, 1732, 1649, 1616, 1597, 1456, 1314, 1214, 1178, 1122, 757, 705 cm^{-1} ; ^1H NMR (CDCl_3) and ^{13}C NMR (CDCl_3) data, Tables 1 and 2, respectively; EIMS m/z 462 $[\text{M}]^+$ (35), 447 (100), 391 (22), 335 (15), 307 (10); HREIMS m/z 462.2050 (calcd for $\text{C}_{28}\text{H}_{30}\text{O}_6$, 462.2042).

Tovophyllin B (4): yellow powder; mp 185–186 °C (lit.²⁵ 190–191 °C); its UV, IR, ^1H NMR ($\text{Me}_2\text{CO}-d_6$), and MS spectra were in consistent with the reported values.²⁵ The ^{13}C NMR (CDCl_3) values are shown in Table 2.

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