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Xanthones from *Garcinia cowa* flowers and their cytotoxicity

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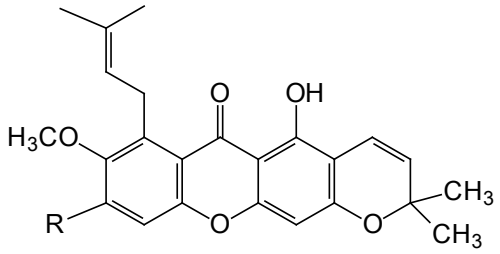
Five xanthones including 6-*O*-methylmangostanin (**1**), mangostanin (**2**), garcinianone A (**3**), mangostanol (**4**) and α-mangostin (**5**) were isolated from the flowers of *Garcinia cowa* Roxb. (Cha-muang, family Clusiaceae). This is the first report of mangostanol (**4**) as a constituent of *G. cowa*. Their identification was performed using one- and two-dimensional ^{1}H and ^{13}C NMR techniques and comparison with literatures. Cytotoxicity of these compounds against SW620, BT474, HepG2, KATO-III and CHAGO-K1 cell lines was evaluated. α-Mangostin was strongly cytotoxic against all cell lines tested, whereas mangostanin exhibited cytotoxicity specifically against CHAGO-K1 cells.



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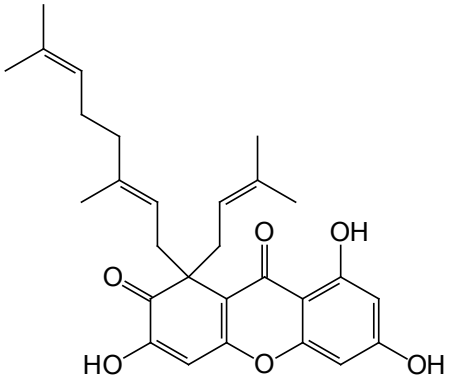
2 R = OH



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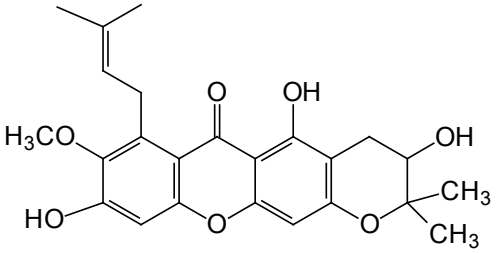

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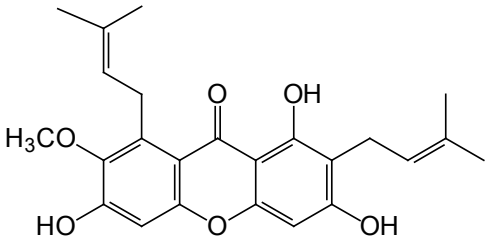

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Introduction

Garcinia is a major genus of tropical trees and shrubs belonging to the family Clusiaceae. More than twenty *Garcinia* species can be found growing in Thailand. The genus is a rich source of secondary metabolites, several of which have been shown to possess diverse biological activities[1], [2]. *Garcinia cowa* Roxb. (Thai name: Cha-muang) is small to medium tree with sour-tasting, edible leaves. A number of chemical constituents have been isolated from the plant and some of them, especially the xanthones, exhibited interesting biological activities including cytotoxicity to cancer cells, antibacterial, antimalarial and anti-inflammatory activities.[3] In this study, five xanthones were isolated from the flowers of *G. cowa* and their *in vitro* cytotoxicity against human colon carcinoma (SW620), breast carcinoma

(BT474), hepatocarcinoma (HepG2), gastric carcinoma (KATO-III) and lung carcinoma (CHAGO-K1) cell lines was evaluated.

Materials and Methods

Plant materials: The flowers of *G. cowa* were collected from the medicinal plant garden of the Faculty of Pharmaceutical Sciences, Chulalongkorn University in February 2014. A voucher specimen of the plant has been deposited at the herbarium of the Faculty of Pharmaceutical Sciences, Chulalongkorn University.

Extraction and isolation: Fresh flowers of *G. cowa* (1.8 kg) were macerated with EtOH (3 x3 L). The combined EtOH extract was concentrated by rotary evaporation, added water to make 70% EtOH and partitioned with *n*-hexane, CH_2Cl_2, EtOAc and *n*-BuOH, respectively, to give hexane (20.84 g), CH_2Cl_2 (1.16 g), EtOAc (3.75 g) and BuOH (9.57 g) extracts after solvent evaporation. *n*-Hexane extract (15 g) was separated on a silica gel column, eluted with *n*-hexane-EtOAc gradient (1:0 → 0:1), into nine fractions (A–I). Sephadex LH-20 column chromatography (CC) of fractions C and F, eluted with MeOH, afforded compounds **1** (3.1 mg) and **2** (11.7 mg), respectively. Sephadex LH-20 CC of fraction G, washed down with MeOH, yielded five subfractions (G1–G5). Silica gel CC of subfraction G4 with *n*-hexane-acetone (19:1) as the solvent system gave **3** (9.7 mg). Sephadex LH-20 CC of fraction H, eluted with MeOH, gave seven subfractions (H1–H7). Subfraction H6 was further purified through a silica gel column, using *n*-hexane-acetone (9:1) as the eluent, to give twelve subfractions (H601–H612). Compounds **4** (8.4 mg) and **5** (15.3 mg) were obtained from subfraction H609 and H612, respectively.

6-O-Methylmangostanin (**1**)**:** Yellow amorphous powder. ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 6.24 (1H, s, H-4), 6.76 (1H, s, H-5), 6.74 (1H, d, *J* = 10.0 Hz, H-11), 5.57 (1H, d, *J* = 10.0 Hz, H-12), 1.47 (6H, s, H-14 and H-15), 4.13 (2H, d, *J* = 6.5 Hz, H-16), 5.24 (1H, br t, *J* = 6.5 Hz, H-17), 1.68 (3H, s, H-19), 1.85 (3H, s, H-20), 13.76 (1H, s, 1-OH), 3.96 (3H, s, 6-OCH_3), 3.79 (3H, s, 7-OCH_3). ^{13}C-NMR (125 MHz, $CDCl_3$): see **Table 1**.

Mangostanin (**2**)**:** Orange-yellow amorphous powder. ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 6.22 (1H, s, H-4), 6.82 (1H, s, H-5), 6.71 (1H, d, *J* = 10.1 Hz, H-11), 5.54 (1H, d, *J* = 10.1 Hz, H-12), 1.47 (6H, s, H-14 and H-15), 4.06 (2H, d, *J* = 6.3 Hz, H-16), 5.24 (1H, br t, *J* = 6.3 Hz, H-17), 1.67 (3H, s, H-19), 1.80 (3H, s, H-20), 13.68 (1H, s, 1-OH), 3.82 (3H, s, 7-OCH_3). ^{13}C-NMR (75 MHz, $CDCl_3$): see **Table 1**.

Garcinianone A (**3**)**:** Yellow amorphous powder. ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 6.26 (1H, br s, H-2), 6.31 (1H, br s, H-4), 2.77 (2H, m, H-11a and H-16a), 3.43 (2H, m, H-11b and H-16b), 4.62 (2H, m, H-12 and H-17), 1.46 (12H, s, H-14, H-15, H-19 and H-24), 1.79 (1H, m, H-20a), 1.99 (1H, m, H-20b), 1.89 (1H, m, H-21a), 2.06 (1H, m, H-21b), 4.84 (1H, br t, H-22), 1.58 (3H, s, H-25), 13.23 (1H, s, 1-OH). ^{13}C-NMR (75 MHz, $CDCl_3$): see **Table 1**.

Mangostanol (**4**)**:** Orange-yellow amorphous powder. ^{1}H-NMR (300 MHz, acetone-d_6) δ: 6.21 (1H, s, H-4), 6.83 (1H, s, H-5), 2.55 (1H, dd, *J* = 17.0, 7.4 Hz, H-11a), 2.90 (1H, dd, *J* = 17.0, 5.5 Hz, H-11b), 3.81 (1H, dd, *J* = 7.4, 5.5 Hz, H-12), 1.36 (3H, s, H-14), 1.28 (3H, s, H-15), 4.11 (2H, d, *J* = 6.8 Hz, H-16), 5.23 (1H, t, *J* = 6.8 Hz, H-17), 1.62 (3H, s, H-19), 1.80 (3H, s, H-20), 13.89 (1H, s, 1-OH), 3.77 (3H, s, 7-OCH_3). ^{13}C-NMR (75 MHz, acetone-d_6): see **Table 1**.

α-Mangostin (**5**)**:** Orange-yellow amorphous powder. ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 6.19 (1H, s, H-4), 6.66 (1H, s, H-5), 3.35 (1H, d, *J* = 6.6 Hz, H-11), 5.24 (1H, t, *J* = 6.6 Hz, H-12), 1.68 (3H, s, H-14), 1.78 (6H, s, H-15 and H-20), 4.00 (2H, d, *J* = 6.6 Hz, H-16), 5.20 (1H, t, *J* = 6.6 Hz, H-17), 1.64 (3H, s, H-19), 13.68 (1H, s, 1-OH), 3.75 (3H, s, 7-OCH_3). ^{13}C-NMR (75 MHz, $CDCl_3$): see **Table 1**.

Assay for cytotoxic activity: Cytotoxicity of the isolated compounds against SW620, BT474, HepG2, KATO-III and CHAGO-K1 cell lines was measured by the tetrazolium dye (MTT) method.[4] Their IC_{50} values were calculated and compared with the positive control, doxorubicin.

Result and Discussion

Five xanthones (**Figure 1**) including 6-*O*-methylmangostanin (**1**),[5] mangostanin (**2**),[6] garcinianone A (**3**),[7] mangostanol (**4**)[8] and α-mangostin (**5**)[9] were isolated and identified from the flowers of *G. cowa*. Compounds **2** and **3** were previously found in the inflorescences of this plant and were subjected to antibacterial assay[7]. The xanthones **1**, **2** and **5** were also found in *G. cowa* fruits and bark[3]. However, the presence of **4** in *G. cowa* is reported herein for the first time. Cytotoxicity of these xanthones against five cancer cell lines and their IC_{50} values are shown in **Table 2**. Mangostanin (**2**) was strongly cytotoxic to CHAGO-K1 cell line with an IC_{50} value of 5.68 μg/ml, whereas α-mangostin (**5**) exhibited strong cytotoxicity against all cell lines tested with IC_{50} values between 2.07-4.10 μg/ml.

Conclusion

Five xanthones from the flowers of *G. cowa* were evaluated for their cytotoxicity against five cancer cell lines. α-Mangostin (**5**) displayed strong cytotoxicity against all cell lines tested, while mangostanin (**2**) was specifically toxic to CHAGO-K1 cell line.

Figure 1. Xanthones isolated from the flowers of *G. cowa.*

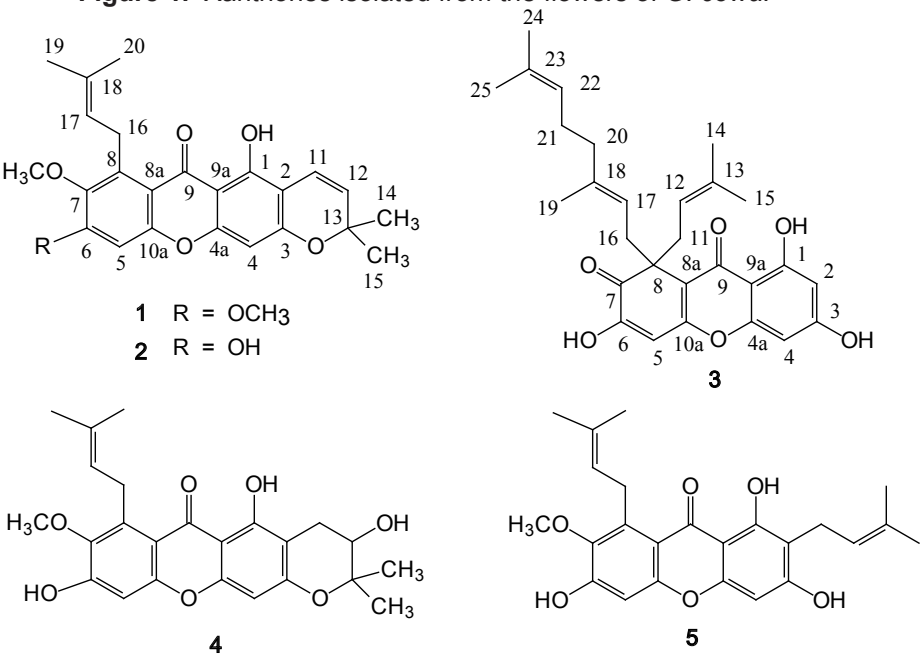

Table 1. ^{13}C NMR data of xanthones **1**-**5** from *G. cowa* flowers (δ in ppm).

C	1	2	3	4	5
1	158.0	157.9	162.8	158.1	160.4
2	104.5	104.5	99.5	103.7	109.2
3	159.7	159.9	162.0	161.7	161.4
4	94.0	94.1	93.6	94.2	93.0
4a	156.3	156.3	156.8	160.8	154.8
5	98.4	101.7	108.7	102.3	101.7
6	158.2	154.7	159.2	155.6	154.7
7	144.1	142.7	201.4	144.5	142.6
8	137.3	137.0	56.0	137.9	137.0
8a	111.9	112.1	116.8	111.4	111.8
9	182.1	182.0	179.3	182.8	181.8
9a	103.9	103.7	105.0	103.3	103.3
10a	155.4	155.7	151.8	156.3	155.4
11	115.8	115.7	38.0	25.8	21.3
12	127.1	127.1	117.7	68.8	121.8
13	77.9	77.9	135.3	79.7	133.9
14	28.3	28.3	25.5	21.0	25.7
15	28.3	28.3	17.6	25.9	17.8
16	26.2	26.5	37.8	26.7	26.4
17	123.1	123.1	117.6	124.6	123.2
18	131.9	132.2	139.0	131.4	131.8
19	25.9	25.8	16.2	25.9	25.7
20	18.2	18.2	39.6	18.2	18.1
21	-	-	26.5	-	-
22	-	-	123.8	-	-
23	-	-	131.4	-	-
24	-	-	25.8	-	-
25	-	-	17.9	-	-
6-OCH_3	56.0	-	-	-	-
7-OCH_3	61.0	62.0	-	61.0	61.6

Table 2. Cytotoxicity of xanthones isolated from the flowers of *G. cowa*.

Compound	IC_{50} (μg/mL)				
	BT474	**CHAGO-K1**	**HepG2**	**KATO-III**	**SW620**
6-*O*-Methylmangostanin (**1**)	>10	>10	>10	>10	>10
Mangostanin (**2**)	>10	5.68	>10	>10	>10
Garcinianone A (**3**)	>10	>10	>10	>10	>10
Mangostanol (**4**)	>10	>10	>10	>10	>10
α-Mangostin (**5**)	3.21	2.19	2.07	3.79	4.10
Doxorubicin	0.80	0.65	0.12	0.71	2.57

Acknowledgement
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