ANTIOXIDANT XANTHONES FROM THE PERICARP OF *GARCINIA MANGOSTANA* (MANGOSTEEN)

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Introduction

Garcinia mangostana L.(Guttiferae), commonly known as "Mangosteen" (**Figure 1**), is one of the most widely recognized tropical fruits and has great appeal because of its quality of color, shape, and flavor. In Thai folk medicine, the pericarp of Mangosteen has been used for many years to healing skin infections and wounds and for relief of diarrhea. The antioxidant and anticancer activities of an extract of the pericarp of Mangosteen have been reported.[1] Mangosteen is used as a constituent of an herbal remedy sold in the United States for its antioxidant effects. Previously phytochemical investigations have indicated that the major components of Mangosteen are prenylated xanthones.[2]

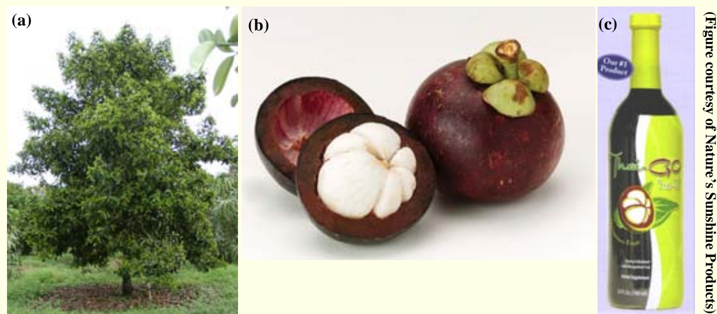

Figure 1. **(a) Whole tree; (b) Fruit; (c) Product containing *G. mangostana.***

Isolation and Identification of Compounds

As part of our ongoing research on cancer chemopreventive agents from botanical dietary supplements, the pericarp of Mangosteen was selected for detailed studies. Repeated chromatography of the CH_2Cl_2-soluble extract led to the isolation of three new highly oxygenated prenylated xanthones, 8-hydroxycudraxanthone G (**1**), 7-methoxy-2-(3-methyl-2-butenyl)-8-(3-methyl-2-oxo-3-butenyl)-1,3,6-trihydroxyxanthone (**2**) and 8-hydroxymangostinone (**3**), along with seven known xanthones, α-mangostin (**4**), γ-mangostin (**5**), 8-deoxygartanin (**6**), gartanin (**7**), mangostinone (**8**), tovophyllin A (**9**), and cudraxanthone G (**10**) (**Figure 2**). The structures of all isolates were elucidated by spectroscopic data analysis as well as by comparison with published data.

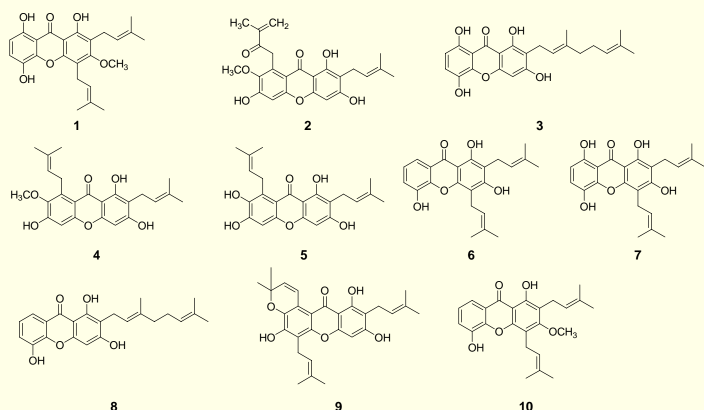

Figure 2. **Structures of compounds 1-10 from *G. mangostana.***

8-Hydroxycudraxanthone G (1) Yellow solid. UV (MeOH) λ_{max} nm (log ε): 238 (4.28), 263 (4.38), 279 (4.34), 351(3.97) nm. IR (dried film) ν_{max} 1623, 1584, 1490, 1217, 1098 cm^{-1}. HRESIMS *m/z* 433.16114 [M+Na]$^+$ (calcd for $C_{24}H_{26}O_6Na^+$, 433.16216). ^{1}H NMR (300 MHz, $CDCl_3$) δ 12.22 (OH), 11.22 (OH), 7.25 (1H, d, *J* = 9.0 Hz, H-6), 6.69 (1H, d, *J* = 9.0 Hz, H-7), 5.24 (2H, m, H-2′ and H-2″), 3.81 (3H, s, 3-OCH_3), 3.54 (2H, d, *J* = 6.2 Hz, H-1″), 3.41 (2H, d, *J* = 6.9 Hz, H-1′), 1.87 (3H, s, H-5″), 1.81 (3H, s, H-5′), 1.74 (3H, s, H-4″), 1.71 (3H, s, H-4′). ^{13}C NMR (75 MHz, $CDCl_3$) δ 185.4 (C-9), 164.4 (C-3), 158.8 (C-1), 153.8 (C-8), 152.7 (C-4a), 142.8 (C-10a), 135.9 (C-5), 132.3 (C-3′), 132.2(C-3″), 123.0 (C-6), 123.0 (C-2″), 122.2 (C-2′), 118.2 (C-2), 113.0(C-4), 109.8 (C-7), 107.3 (C-8a), 104.9 (C-9a), 62.1 (3-OCH_3), 25.7 (C-4′), 25.5 (C-4″), 23.0 (C-1″), 22.5 (C-1′), 18.0 and 17.9 (C-5′ or C-5″).[3]

7-Methoxy-2-(3-methyl-2-butenyl)-8-(3-methyl-2-oxo-3-butenyl)-1,3,6-trihydroxyxanthone (2) Yellow solid. UV (MeOH) λ_{max} nm (log ε) 243 (3.84), 320 (3.65), 354 (3.32) nm. IR (dried film) ν_{max} 1608, 1578, 1465, 1284, 1162, 1081 cm^{-1}. HRESIMS *m/z* 447.14323 [M+Na]$^+$ (calcd for $C_{24}H_{24}O_7Na^+$, 447.14142). ^{1}H NMR (300 MHz, acetone-d_6) δ 13.50 (OH), 6.86 (1H, s, H-5), 6.39 (1H, s, H-4), 6.23 (1H, s, H-4″a), 5.86 (1H, s, H-4″b), 5.24 (1H, t, H-2′), 4.75 (2H, s, H-1″), 3.73 (3H, s, 3-OCH_3), 3.30 (2H, d, *J* = 7.2 Hz, H-1″), 1.92 (3H, s, H-5″), 1.75 (3H, s, H-4′), 1.61 (3H, s, H-5′). ^{13}C NMR (75 MHz, acetone-d_6) δ 199.1 (C-2″), 182.2 (C-9), 163.3 (C-3), 161.4 (C-1), 161.1 (C-6), 156.1 (C-4a), 155.8 (C-10a), 145.8 (C-3″), 145.7 (C-7), 131.4 (C-8), 131.2 (C-3′), 123.6 (C-2′), 123.6 (C-4″), 111.0 (C-8a), 111.0 (C-2), 103.3 (C-5), 103.2 (C-9a), 93.4 (C-4), 61.3 (3-OCH_3), 37.9 (C-1″), 25.9 (C-4′), 22.0 (C-1′), 18.1 (C-5″), 17.9 (C-5′).[4]

8-Hydroxymangostinone (3) Yellow solid. UV ($CHCl_3$) λ_{max} nm (log ε) 243 (3.84), 255 (3.86), 281 (3.18), 341 (3.60) nm. IR (dried film) ν_{max} 1634, 1592, 1505, 1447, 1253, 1086, 1054 cm^{-1}: HRESIMS *m/z* 419.14466 [M+Na]$^+$ (calcd for $C_{23}H_{24}O_6Na^+$, 419.14651). ^{1}H NMR (300 MHz, acetone-d_6) δ 12.30 (OH), 11.25 (OH), 7.27 (1H, d, *J* = 8.7 Hz, H-5), 6.61 (1H, d, *J* = 8.7 Hz, H-6), 6.57 (1H, s, H-4), 5.29 (1H, t, 6.3, H-2′), 5.06 (1H, t, 6.9, H-2″), 3.37 (2H, d, *J* = 7.2 Hz, H-1′), 2.04 (2H, m, H-1″), 1.96 (2H, t, 7.5, H-4′), 1.79 (3H, s, H-5′), 1.59 (3H, s, H-4″), 1.54 (3H, s, H-5″). ^{13}C NMR (75 MHz, acetone-d_6) δ 185.4 (C-9), 165.0 (C-3), 160.9 (C-1), 156.6 (C-4a), 154.1 (C-8), 144.5 (C-10a), 137.9 (C-5), 135.5 (C-3′), 131.6 (C-3″), 125.1 (C-2″), 124.3 (C-6), 122.9 (C-2′), 112.1 (C-2), 110.2 (C-7), 108.5 (C-8a), 102.5 (C-9a), 94.6 (C-4), 40.5 (C-4′), 27.4 (C-1″), 25.8 (C-4″), 21.8 (C-1′), 17.7 (C-5″), 16.2 (C-5′).[5]

Antioxidant Activity Evaluation

Measurement of the ONOO$^-$ Scavenging Activity

DL-Penicillamine (DL-2-amino-3-mercapto-3-methylbutanoic acid) were purchased from Sigma Chemical Company (St. Louis, MO). DHR 123 (dihydrorhodamine 123) was purchased from Molecular Probes (Eugene, OR), and ONOO$^-$ and morpholinosydnonimine (SIN-1) were purchased from Cayman Chemicals Co. (Ann Arbor, MI).

The ONOO$^-$ scavenging activity was measured by monitoring the oxidation of DHR 123, by modification of the method of Kooy et al.[6] In brief, DHR 123 (5 mM) in dimethylformamide, purged with nitrogen, was stored at –80°C as a stock solution. The rhodamine buffer used consisted of 90 mM sodium chloride, 50 mM sodium phosphate dibasic, 50 mM sodium phosphate monobasic, 5 mM potassium chloride at pH 7.4, and 100 μM diethylenetriaminepentaacetic acid (DTPA), each of which was prepared with high quality deionized water and purged with nitrogen. The final concentration of DHR 123 was 5 μM by dilution of this buffer. The background and final fluorescent intensities of oxidized DHR 123 were measured at 5 min after treatment with and without the addition of authentic ONOO$^-$ and SIN-1 derived ONOO$^-$ (10 μM) by a microplate fluorescence reader FL 500 (Bio-Tek Instruments Inc.) at the excitation and emission wavelengths of 480 and 530 nm, respectively. Results were expressed as the mean (n = 3) for the final fluorescence intensity minus background fluorescence. DL-Penicillamine was used as a positive control.

Results

Peroxynitrite (ONOO$^-$), generated from nitric oxide (NO·) and superoxide anion (·O_2^-) *in vivo*, has been reported to act as an oxidizing and nitrating agents and involve in the initiation of carcinogenesis, along with NO·.

Except for compound **2**, which was isolated as a very minor compound, the antioxidant activities of the other nine isolates (**1**, and **3-10**) obtained in the present study were determined by using authentic and morpholinosydnonimine (SIN-1) derived ONOO$^-$. Five compounds (**1, 3, 4, 5** and **7**) were demonstrated to possess pronounced antioxidant activity in the assays utilized (Table 1).

Table 1. Antioxidant activities of isolates from *G. mangostana* on ONOO$^-$ assays

Compounds	IC_{50} (μM)[a]	
	Authentic ONOO$^-$	SIN-1 derived ONOO$^-$
1	4.61	10.03
3	2.20	9.65
4	12.20	<0.49
5	7.95	3.14
6	N.A.[c]	11.90
7	9.09	9.27
8	N.A.	N.A.
9	N.A.	N.A.
10	N.A.	3.20
DL-penicillamine[b]	3.14	7.35

[a]ONOO$^-$ is the scavenging activity of authentic peroxynitrite and generated peroxynitrite from SIN-1 (IC_{50}: μM). Values of ONOO$^-$ scavenging/inhibitory activities expressed as the mean of three experiments. [b]DL-Penicillamine used as positive control. [c]N.A.: no activity within the tested concentration (5-100 μM).

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References

1. Primchanien, M.; Nuttavut, K.; Sineenart, K.; Omboon, L.; Narongchai, P.; Neelobol, N. *J. Ethnopharmacol.* **2004**, *90*, 161–166.
2. Peres, V.; Nagem, T. J.; Faustino de Oliveira, F. *Phytochemistry* **2000**, *55*, 683–710.
3. Hano, Y.; Matsumoto, Y.; Sun, J.-Y.; Nomura, T. *Planta Med.* **1990**, *56*, 399–402.
4. Nilar, L.J. H. *Phytochemistry* **2002**, *60*, 541–548.
5. Asai,F.; Tosa, H.; Tanaka, T.; Iinuma, M. *Phytochemistry* **1995**, *39*, 943–944.
6. Kooy, N. W.; Royall, J. A.; Ischiropoulos, H.; Beckman, J. S. *Free Radic. Biol. Med.*, **1994**, *16*, 149–156.