Middle-East Journal of Scientific Research 6 (2): 123-127, 2010
ISSN 1990-9233

Bioactive Xanthones from the Pericarp of *Garcinia mangostana*

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Abstract: Investigation on the constituents of the pericarp of *Garcinia mangostana* has led to the isolation of three prenylated xanthones; α-mangostin (**1**), β-mangostin (**2**) and 1, 6-dihydroxy-7-methoxy-8-isoprenyl-6',6'-dimethylpyrano(2',3',3,2) xanthone (**3**). The structures of these compounds were elucidated based on spectroscopic methods such as nuclear magnetic resonance (NMR-1D and 2D), UV, IR and mass spectrometry (MS). α-mangostin was found to be cytotoxic against HL-60, MCF-7 and HeLa cancer cell lines. This paper reports on the isolation, structural elucidation and cytotoxicity of the xanthones.

Key words: Guttiferae · *Garcinia mangostana* · Xanthones · Bioactive · Cytotoxic

INTRODUCTION

The Guttiferae is a family of plants including about 37 genera and 1610 species of trees and shrubs, often with milky sap and fruits or capsules for seeds. It is primarily tropical. The most commonly studied of genus within Guttiferae family is *Garcinia. Garcinia* is a large genus of polygamous trees or shrubs, distributed in the tropical Asia, Africa and Polynesia, which consists of 180 species. The genus is known to produce several xanthones [1]. *G. mangostana* which is known locally as "manggis" or mangosteen is one of the important tropical evergreen tree natives to Southeast Asia, with deep reddish purple fruit. The pericarp of mangosteen has been used in Thai indigenous medicine for the treatment of skin infections, wounds and diarrhea for many years. People in Southeast Asian nations like Indonesia, Malaysia, Sri Lanka, Philippines and Thailand where *Garcinia mangostana* is cultivated in the tropical rainforest, have used the pericarp (peel, rind, hull or ripe) of the fruit as a traditional medicine for the treatment of abdominal pain, diarrhea, dysentery, infected wound, suppuration and chronic ulcer.

Xanthones, a kind of polyphenolic compounds that contain a distinctive chemical structure with a tricyclic aromatic ring are well known for their interesting phytochemical properties, which make them attractive to the pharmaceutical and medicinal industry. Recent phytochemical studies had revealed that these xanthones exhibited a variety of biological activities such as anti-oxidant, anti-inflammatory, anti-bacterial and anti-cancer effects [2]. The biological activities of this class of compounds are associated with their tricyclic scaffold but vary depending on the nature and position of the different substituents [3].

Our investigation on the EtOAc fractions of the pericarp of *G. mangostana* has led to the isolation of three prenylated xanthones: α-mangostin (**1**), β-mangostin (**2**) and 1, 6-dihydroxy-7-methoxy-8-isoprenyl-6', 6'-dimethylpyrano (2',3':3,2) xanthone (**3**). Their structures were identified by comparison of their spectral data with those in the literature. In this paper we report on the isolation and structure elucidation of the three xanthones, as well as the cytotoxicity of α-mangostin.

MATERIALS AND METHODS

General Experimental Procedures: The ^{1}H-NMR and ^{13}C-NMR were recorded in chloroform-D on Bruker 300 Ultrashield NMR spectrometer measured at 300 and 75 MHz. Chemical shifts are reported in ppm (δ) and the coupling constants are given in Hz. The infrared (IR) was recorded on the Perkin Elmer spectrum one FT-IR spectrometer. The ultraviolet (UV) spectra were recorded on Shimadzu UV-Vis 160i. The mass spectra were measured on Perkin Elmer Clarus 600T spectrometer 70 eV. Vacuum liquid chromatography (VLC) used Silica gel 60, 70-230 mesh ASTM (Merk 1.07747), Aluminium supported

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silica gel 60 F_{254} was used for thin layer chromatography, while glass supported silica gel 60 F_{254} was used for preparative thin layer chromatography.

Extraction and Isolation: The fruit of *Garcinia mangostana* was collected in Shah Alam, Selangor. The pericarp was air-dried, ground (1 kg) and macerated in methanol, filtered and concentrated under reduced pressure. The crude extract (207 g) was dissolved in methanol and water (1: 2) to form aqueous solution and partitioned with hexane followed by ethyl acetate. The ethyl acetate extract (12.8g) was subjected to fractionation by using Vacuum Liquid Chromatography (VLC) with various compositions of solvent system [Hex:Ea (7:3, 6:4, 5:5, 3:7) and DCM:MeOH (10:0, 9:1, 8:2, 5:5, 0:10)] to yield 11 fractions. Fraction 3 was subjected to purification by multiple preparative thin layer chromatography (pTLC) using hexane:ethyl acetate 2:8 system (Ea:hex) to afford three compounds: compound **1** (12mg), compound **2** (3mg) and compound **3** (6mg).

MTT Assay: The cytotoxicity assay was determined by 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) assay. Cells were seeded in 96-well microplate at 3×10^5 cells/ml, incubated at 37°C in 5% CO_2 and treated with sample at 2 × MFC in serum free medium (SFM) for 240 and 480 min. The culture medium was aspirated, replaced with 0.5 µg/ml MTT solution and incubated for 30 min in a CO_2 incubator. The solution was aspirated and added with 1,000 µl DMSO to dissolve the formazan crystals. After 30 min of rotary agitation, the absorbance of the solution at 570 nm was measured using Genesis10 UV-Vis spectrophotometer (Thermo Spectronic, NY, USA). The viable cell number was calculated from the standard curve of cell number by plotting a scattergram of the absorbance value against the known number of cells. IC_{50} values represent the compound concentration that reduced the mean absorbance at 570 nm to 50% of those in the untreated control wells.

RESULTS AND DISCUSSION

Three diprenylated xanthones were isolated from the pericarp of *Garcinia mangostana.* All compounds have prenylated groups attached at C-2 and C-8. In compound **3** the prenyl group at C-2 formed a cyclized ring with OH at C-3 resulting in an additional ring. All xanthone has methoxy groups substituted either at C-3 or C-7.

Compound **1** was obtained as yellow powder exhibiting an M^+ at *m/z* 382, corresponding to molecular weight $C_{25}H_{34}O_3$. The ^1H NMR spectrum of 1 revealed two singlet at δ_H 6.84 and 6.32, a methoxy at δ_H 3.83 and two sets of prenyl signals at δ_H 4.11 and 3.47 (two methylenes), δ_H 5.31 (two vinyls) and δ_H 1.86, 1.79, 1.71 (four olefinic methyl) indicating that 1 is a trihydroxylated xanthone substituted with two prenyl groups. A downfield singlet at δ_H 13.80 showed the presence of a chelated hydroxyl that has to attach to C-1. The substitution pattern of ring A and B was deduced from the long range correlations observed in HMBC spectrum (Figure 1). Correlation of a methylene proton signaled at δ_H 3.47 with δ_H 108.5 (C-2), δ_H 135.7 (C-13) and δ_H 160.6 (C-1) and δ_H 121.5 (C-12) positioned the prenyl group at C-2. Another set of correlations were observed between the other methylene proton at δ_H 4.11 with δ_H (112.2 (C-9a), 123.2 (C-17), 142.6 (C-7), δ_H 137.1 (C-8) and δ_H 132.4 (C-18) thus confirming the location of another prenyl group at C-8. The methoxy proton at δ_H 3.83 showed correlation with δ_H 142.6 indicating its attachment at C-7. Analysis of the spectral data and comparison with literature [4] has confirmed that 1 is α-mangostin previously reported by Schmid, 1855 [5].

α-mangostin was previously reported from other *Garcinia* species such as *G. diocia* and *G. subelliptica* [6], the bark of *Garcinia speciosa* [7], the hexane extract of the fruit of *Garcinia cowa* [8] and the methanol extract of the twigs of *Garcinia studtii* [9]. A study of the methanol extract of the stem bark of *Allanblackia monticola* Staner L.C by Anatole *et al.* [10] also led to the isolation of α-mangostin. A study on anti-*Pseudomonas aeruginosa* xanthones [11], also revealed the isolation of this compound from the resin and green fruit of *Cratoxylum cochinchinense.*

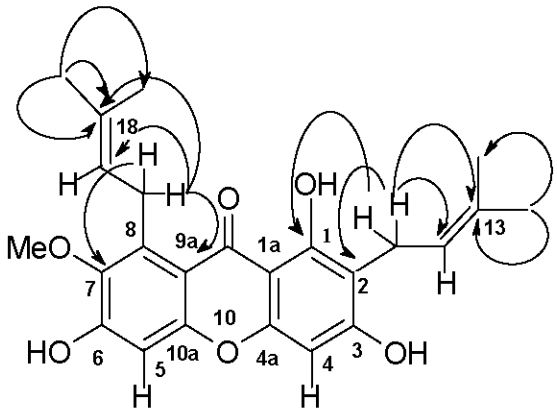
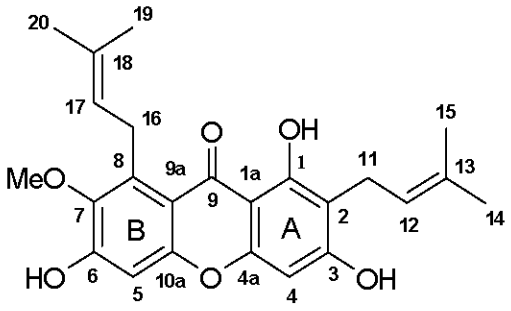


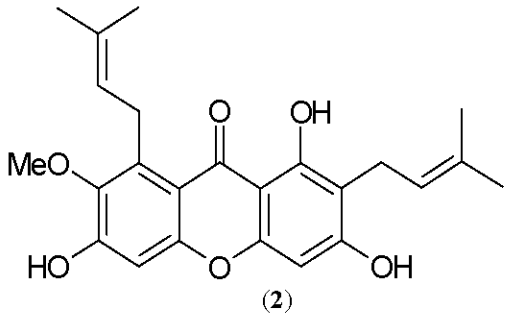
Fig. 1: Selected long range correlation of 1

Compound **2** was isolated as yellow amorphous with an M^+ value of *m/z* 396 corresponding to molecular weight $C_{26}H_{36}O_3$. The ^1H NMR of 2 displayed similar signal as **1** with an additional methoxy signal at δ_H 3.93. Two singlet at δ_H 6.39 and 6.86 were attributed to aromatic proton H-4 and H-5 respectively. The presence of two prenyl groups were obvious with the appearance of two vinyl (δ_H 5.29, H-12 & H-17), two methylene (δ_H 4.13, H-16 and 3.37, H-11) and four olefinic methyl protons at δ_H 1.69 (H-14, H-15), 1.85 (H-19) and 1.83 (H-20). Similar to **1** methoxy group at OMe-7 appeared at δ_H3.83. A downfield singlet at δ_H 13.44 (1H, s) confirms the presence of a chelated hydroxyl group substituted at C-1 of the xanthone ring. Signals for carbon NMR are given in the spectral data. Compound **2** was identified by comparison with the literature data [4] and deduced to be β-mangostin.

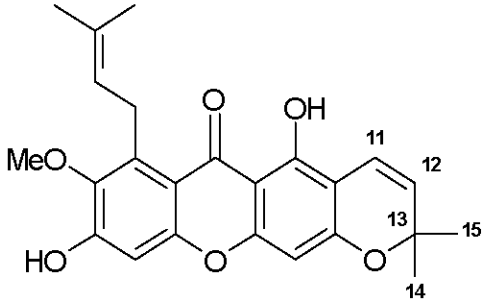
β-mangostin was also reported from the other parts of *Garcinia mangostana* apart from the pericarp. It can also be obtained from the stem bark, root bark and latex of the green fruit of *G. mangostana* [12]. Together with α-mangostin, β-mangostin was also isolated from the fruit of *Garcinia cowa* as reported in Panthong *et al.* [8]. This compound was reported by Lien *et al* [13] from the bark of *Cratoxylum cochinchinense.*



(**1**)



(**2**)



(**3**)

Compound **3**, as like the other two compounds, was also obtained as yellow amorphous displaying M^+ at *m/z* 378 corresponding to $C_{26}H_{34}O_2$. The ^1H NMR spectrum showed signals of two aromatic proton at δ_H 6.86 and 6.74, a methoxy group (δ_H 3.85) and a downfield signal at δ_H 13.71 attributed to chelated hydroxyl group, giving similar skeleton as in compound **1** and **2**. Signals for a set of prenyl group protons can be observed at δ_H 5.28 (vinyl), 4.10 (methylene) and 1.85 and 1.75 (methyl). Appearance of a pair of double bond signals at δ_H6.65 and 5.65 (*d*, *J*=9.5 Hz) and the remaining two olefin methyls at δ_H 1.49 gave indication that one of the prenyl groups has formed a cyclised ring with the hydroxyl group. Thus, based on the spectral data and comparison with literature data, compound **3** was assigned as 1, 6-dihydroxy-7-methoxy-8 isoprenyl-6',6'-dimethylpyrano(2',3',3,2) xanthone.

This compound was previously reported in Suksamrarn *et al.* 2003 from the MeOH extract of the pulverished fresh arils and seeds of *G. mangostana* and was tested for its antituberculosis potentials along with compound **1** and **2** [14]. Recently, Boonnak *et al.* [15] also isolated this compound from the roots of *Cratoxylum formosum* ssp. *Pruniflorum.*

α-mangostin (**1**) isolated as a major compound was assayed against three cancer cell lines which were HL-60 (Human T-promyelocytic leukaemia), MCF-7 (Human breast adenocarcinoma cancer) and HeLa (human cervical cancer) using MTT assay. Table 1 showed that α-mangostin exhibited *In vitro* cytotoxic effect against

Table 1: ^{1}H NMR data for compound **1**, **2** and **3**

Position	1 (δ_H)	2 (δ_H)	3 (δ_H)
1	-	-	-
2	-	-	-
3	-	-	-
4	6.32, *s*	6.39, *s*	6.27, *s*
5	6.84, *s*	6.86, *s*	6.86, *s*
6	-	-	-
7	-	-	-
8	-	-	-
9	-	-	-
10	-	-	-
11	3.47, *d* (*J*=7.5 Hz)	3.37, *d* (*J*=6.0 Hz)	6.74, *d* (*J*=9.9 Hz)
12	5.31, *m*	5.29, *m*	5.57, *d* (*J*=9.9 Hz)
13	-	-	-
14	1.79, *s*	1.69, *s*	1.49, *s*
15	1.86, *s*	1.69, *s*	1.49, *s*
16	4.11, *d* (*J*=6.3 Hz)	4.13, *d* (*J*=6.6 Hz)	4.10, *d* (*J*=6.9 Hz)
17	5.31, *m*	5.29, m	5.28, *m*
18	-	-	-
19	1.86, *s*	1.85, *s*	1.85, *s*
20	1.71, *s*	1.83, *s*	1.75, *s*
1-OH	13.80, *s*	13.44, *s*	13.71, *s*
3-OMe	-	3.93, *s*	-
7-OMe	3.83, *s*	3.83, *s*	3.85, *s*

Table 2: IC_{50} value of α-mangostin (**1**) tested against HL-60, MCF-7 and HeLa cancer cell lines

Cancer cell line	IC_{50} (μg/ml)
HL-60	7.7
MCF-7	10.5
HeLa	6.9

the three human cancer cell lines. It significantly inhibited the growth of HeLa and HL-60 cell lines with IC_{50} value of 6.9 and 7.7 μg/ml respectively. Compound **1** also showed significant cytotoxic effect against MCF-7 at slightly higher value, IC_{50} 10.5 μg/ml.

Apoptosis of tumor cells can be triggered by a diversity of extracellular and intracellular factors including cytokines, tumor suppressor genes, oncogenes, radiation andanticancer drugs. The growth inhibition by α-mangostin in the human cell lines was due to apoptosis as nuclear condensation and fragmentation and DNA ladder formation was observed in all of the cell lines. Like any other xanthones, α-mangostin possesses a six carbon conjugated ring structure with multiple carbon double bonds. The prenyl group in á-mangostin is considered to be implicated in the internalization into the cell, which in turn leads to interaction with the signal transduction molecules and the proteins involved in mitochondria permeability transition. α-mangostin has shown to possess diverse pharmacological activities such as antioxidant, antibacterial, anti-inflammatory activities and inhibition of oxidative damage [16-20]. β-mangostin and 1, 6-dihydroxy-7-methoxy-8-isoprenyl-6', 6'-dimethylpyrano (2', 3', 3, 2) xanthone are expected to display good cytotoxic effect as shown in α-mangostin considering both compounds have prenyl group as substituents similar to α-mangostin.

CONCLUSION

In summary, the phytochemical study on *G. mangostana* has led to the isolation of three prenylated xanthones; α-mangostin (**1**), β-mangostin (**2**) and 1, 6-dihydroxy-7-methoxy-8-isoprenyl-6',6'-dimethylpyrano (2',3',3,2) xanthone (**3**). The results from MTT assay showed that α-mangostin, a xanthone exhibits antiproliferative activity against human leukemia HL60 cells, human breast adenocarcinoma MCF-7 cells and human cervical cancer HeLa cells. α-mangostin had been shown to induce apoptosis in leukemia cell lines.

ACKNOWLEDGEMENTS

The authors would like to thank Faculty of Applied Sciences, Universiti Teknologi MARA (UiTM) for financial support.

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