Two New Chemical Constituents from the Stem Bark of Garcinia mangostana

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Abstract: A detailed chemical study on the ethyl acetate and methanol extracts of the stem bark of Garcinia mangostana resulted in the successful isolation of one new prenylated xanthone, mangaxanthone B (1), one new benzophenone, mangaphenone (2), and two known xanthones, mangostanin (3) and mangostenol (4). The structures of these compounds were elucidated through analysis of their spectroscopic data obtained using 1D and 2D NMR and MS techniques.

Keywords: Garcinia mangostana; Clusiaceae; prenylated xanthone; benzophenone

1. Introduction

The Clusiaceae family consists of approximately 40 genera [1], including Garcinia, Mesua and Cratoxylum [2]. Some Clusiaceae plants are used in traditional medicine to treat various illnesses. For example, Garcinia schomburgkiana Pierre is used to treat coughs and menstrual problems [3] while Mesua ferrea is used in the treatment for dyspepsis and renal disease [4]. Clusiaceae plants contain numerous biologically active secondary metabolites, such as benzophenones, xanthones, coumarins...
and flavonoids. The pharmacological properties of these secondary metabolites include antifungal activity in *Calophyllum thwaitesii* [5], antioxidant activity in *Cratoxylum cochinchinense* [6], antimicrobial and antibacterial activities in *Garcinia cowa* [7] and anticancer activity in *Garcinia paucinervis* [8]. *Garcinia* plants are mainly found in tropical countries such as Malaysia, Thailand and Brazil [1]. *Garcinia* plants are currently being more avidly studied due to their abilities to treat dysentery, pain, tapeworm infestations and many more ailments [9]. Being a member of the *Garcinia* genus, the mangosteen is also well known to be a rich source of xanthones and benzophenones, especially polyprenylated xanthones and oxygenated xanthones [10]. These secondary metabolites have been reported to possess biological properties against fungi [11], bacteria [12,13] and also cancer [14,15]. Ryu and co-workers also reported that some oxygenated xanthones from the seedcases of this plant possess neuraminidase inhibitory activity [16]. The discoveries of these beneficial secondary metabolites have revitalized our interest to investigate more extensively on the stem bark of *Garcinia mangostana*. Herein, we describe the isolation as well as the characterization of a new prenylated xanthone, mangaxanthone B (1) and a new benzophenone, mangaphenone (2), along with two other known xanthones mangostanin (3) and mangostenol (4).

2. Results and Discussion

The stem bark of *Garcinia mangostana* was extracted with ethyl acetate (EtOAc) and methanol (MeOH) followed by fractionation of these extracts to obtain a new prenylated xanthone, mangaxanthone B (1), a new benzophenone, mangaphenone (2) and two other known compounds mangostanin (3) and mangostenol (4). Structural elucidation of these compounds were performed by analysing their spectroscopic data. The structures of compounds 3 and 4 were confirmed by comparing their spectroscopic data with data available from the literature. The structures of compounds 1–4 are illustrated in Figure 1.

**Figure 1.** Structures of compounds 1–4.
Compound 1 was isolated as a yellow solid (m.p. = 194–195 °C) and found to have a molecular formula of C\textsubscript{25}H\textsubscript{30}O\textsubscript{7} through the EIMS spectrum, which showed a molecular ion peak at \textit{m/z} 442. The FTIR absorption indicated the existence of OH (3447 cm\textsuperscript{-1}), alkane side chain CH (2938 cm\textsuperscript{-1}), aromatic moiety C=C (1457 cm\textsuperscript{-1}), CO (1601 cm\textsuperscript{-1}) and alkene moiety CH (826 cm\textsuperscript{-1}) bands. Besides, the \(\lambda_{\text{max}}\) at 209, 245, 262, 316 and 353 nm in the UV-Visible spectrum are the characteristic absorption bands of an aromatic benzene chromophore, which indicated the presence of a xanthone nucleus.

In the \textsuperscript{1}H-NMR spectrum, signals at \(\delta_{\text{H}} 6.38\) (s, 1H, H-4) and 6.90 (s, 1H, H-5) imply the presence of a xanthone nucleus. On the other hand, the prenyl moiety was evident from proton resonances at \(\delta_{\text{H}} 3.32\) (br d, 2H, H-1'), 5.25 (t, 1H, \(J = 6.9\) Hz, H-2'), 1.76 (s, 3H, H-4') and 1.62 (s, 3H, H-5') while the characteristic proton resonances of the 3-hydroxy-3-methylbutyl moiety were seen at \(\delta_{\text{H}} 3.42\) (m, 2H, H-1''), 1.70 (m, 2H, H-2'') and 1.28 (s, 6H, H-4'', H-5''). The \textsuperscript{1}H-NMR spectrum also showed characteristic resonances of a chelated hydroxyl group at \(\delta_{\text{H}} 13.74\) (s, 1H, 1-OH) and two methoxyl groups at \(\delta_{\text{H}} 4.00\) (s, 3H, 6-OCH\textsubscript{3}) and 3.79 (s, 3H, 7-OCH\textsubscript{3}) (see Table 1).

Table 1. \textsuperscript{1}H-NMR (500 MHz) and \textsuperscript{13}C-NMR (125 MHz) spectroscopic data for compound 1 (in Me\textsubscript{2}CO-\textit{d}_\textsubscript{6}) and 2 (in CD\textsubscript{3}OD).

| Position | 1  | 2  |
|----------|----|----|
|          | \(\delta_{\text{H}}\) | \(\delta_{\text{C}}\) | \(\delta_{\text{H}}\) | \(\delta_{\text{C}}\) |
| 1        | 160.8 | 160.8 | 160.8 | 160.8 |
| 2        | 110.3 | 110.3 | 110.3 | 110.3 |
| 3        | 162.2 | 5.96 (s) | 5.96 (s) | 91.1 |
| 4        | 6.38 (s) | 92.3 | 6.52 (d, \(J = 2.3\) Hz) | 106.5 |
| 4a       | 154.9 | 154.9 | 154.9 | 154.9 |
| 5        | 6.90 (s) | 98.5 | 5.96 (s) | 95.3 |
| 5a       | 155.4 | 155.4 | 155.4 | 155.4 |
| 6        | 158.6 | 158.6 | 158.6 | 158.6 |
| 7        | 144.1 | 144.1 | 144.1 | 144.1 |
| 8        | 138.4 | 138.4 | 138.4 | 138.4 |
| 8a       | 111.4 | 111.4 | 111.4 | 111.4 |
| 9        | 182.0 | 182.0 | 182.0 | 182.0 |
| 1'       | 3.32 (br d) | 21.2 | 142.7 | 142.7 |
| 2'       | 5.25 (t, \(J = 6.3\) Hz) | 122.6 | 6.52 (d, \(J = 2.3\) Hz) | 106.5 |
| 3'       | 130.6 | 130.6 | 130.6 | 130.6 |
| 4''      | 1.76 (s) | 17.1 | 6.39 (t, \(J = 2.3\) Hz) | 105.7 |
| 5''      | 1.62 (s) | 25.1 | 158.0 | 158.0 |
| 6''      | 6.52 (d, \(J = 2.3\) Hz) | 106.5 | 6.52 (d, \(J = 2.3\) Hz) | 106.5 |
| 1''      | 3.42 (m) | 22.2 | 28.5 | 28.5 |
| 2''      | 1.70 (m) | 44.9 | 69.7 | 69.7 |
| 3''      | 1.28 (s) | 28.5 | 28.5 | 28.5 |
| 1-OH     | 13.74 (s) | 3.54 (s) | 54.6 | 54.6 |
| 2-OCH\textsubscript{3} | | | 4.00 (s) | 55.8 |
| 6-OCH\textsubscript{3} | | | 3.79 (s) | 60.4 |
Meanwhile, the DEPT experiment indicated that this compound is composed of three methine (δ_C 92.3, 98.5 and 122.6), three methylene (δ_C 21.2, 22.2 and 44.9), four methyl (δ_C 17.1, 25.1, 28.5 × 2), two methoxyl (δ_C 55.8 and 60.4) and 13 quaternary carbons (δ_C 69.7, 102.9, 110.3, 111.4, 130.6, 138.4, 144.1, 154.9, 155.4, 158.6, 160.8, 162.2 and 182.0). These results are consistent with the 13C-NMR spectrum, which indicated the presence of 25 carbons. The presence of a xanthone skeleton was again obvious in the 13C-NMR spectrum, with the signal at δ_C 182.0, a characteristic signal for the carbonyl group in the xanthone skeleton. Moreover, six oxygenated aromatic carbons [C-7 (δ_C 144.1), C-4a (δ_C 154.9), C-5a (δ_C 155.4), C-6 (δ_C 158.6), C-1 (δ_C 160.8) and C-3 (δ_C 162.2)] were observed in compound 1 after further examination of the DEPT spectrum.

The HMBC long range 3_J correlations between the chelated hydroxyl group with C-9a (δ_C 102.9) and C-2 (δ_C 110.3) and a 2_J correlation with C-1 (δ_C 160.8) were observed. This allows the assignment of the hydroxyl group to C-1 [δ_H 13.74 (s, 1H, 1-OH)]. The two prenyl moiety methyl groups resonating at δ_H 1.76 and 1.62 are correlated to the neighbouring C-3’ (δ_C 130.6) and C-2’ (δ_C 122.6) in the HMBC experiment. Moreover, the linkages between H-1’ [δ_H 3.32 (br d, 2H)] and C-2’ (δ_C 122.6) as well as C-3’ (δ_C 130.6) are seen. The linkages between H-2’ [δ_H 5.25 (t, 1H, J = 6.3 Hz)] and C-4’ (δ_C 17.1) and C-5’ (δ_C 25.1) are also clearly seen in the experiment (Figure 2). These correlations provide evidence for the presence of the prenyl side chain while the 2_J correlation between H-1’ [δ_H 3.32 (br d, 2H)] with C-2 (δ_C 110.3) and the 3_J correlations of H-1’ [δ_H 3.32 (br d, 2H)] with C-3 (δ_C 162.2) indicated that the prenyl moiety is positioned at C-2. In the COSY analysis, the coupling of H-1’ and H-2’ was very strong.

**Figure 2.** The HMBC correlation of 3-hydroxy-3-methylbutyl (I) and prenyl (II) moieties in compound 1.

A pair of overlapping aliphatic methyls [δ_H 1.28 (s, 6H, H-4”, H’’)] gave clear correlations with C-2” (δ_C 44.9) and C-3” (δ_C 69.7) indicating the methyls to be attached to C-3”. H-2” [δ_H 1.70 (m, 2H)] gave cross peaks to C-4”(δ_C 28.5), C-5” (δ_C 28.5), C-3” (δ_C 69.7) and C-1” (δ_C 22.2). Meanwhile H-1” [δ_H 3.42 (m, 2H)] showed a 2_J correlation with C-2” (δ_C 44.9) and a 3_J correlation with C-3” (δ_C 69.7). These long range correlation signals allow us to conclude a 3-hydroxy-3-methylbutyl moiety (Figure 2). The coupling of H-1” and H-2” was seen in the COSY experiment. Moreover, the cross peaks of H-1” [δ_H 3.42 (m, 2H)] to C-7 (δ_C 144.1), C-8 (δ_C 138.4), C-8a (δ_C 111.4) and the cross-peak of H-2” [δ_H 1.70 (m, 2H)] to C-8 (δ_C 138.4) in the HMBC analysis were suggestive of the 3-hydroxy-3-
methylbutyl moiety location at C-8. In addition, the two aromatic methoxyl groups [6-OCH₃ (δ_H 4.00) and 7-OCH₃ (δ_H 3.79)] are assigned at C-6 and C-7 because of their ³J correlations with δ_C 158.6 (C-6) and δ_C 144.1 (C-7). The two remaining aromatic methine protons of the xanthone skeleton were determined to be at the two remaining available carbons, C-4 and C-5. The long range correlations between H-4 (δ_H 6.38) and C-2 (δ_C 110.3), C-3 (δ_C 162.2), C-4a (δ_C 154.9), C-9 (δ_C 182.0) and C-9a (δ_C 102.9) places the δ_H 6.38 (H-4) singlet at C-4. The signal at δ_H 6.90 (H-5) was assigned to C-5 based on its HMBC correlations with C-5a (δ_C 155.4), C-6 (δ_C 158.6), C-7 (δ_C 144.1), C-8 (δ_C 138.4), C-8a (δ_C 111.4) and C-9 (δ_C 182.0). Compound 1 differs from a closely related compound, garcinone D [17] in the presence of a methoxyl group at C-6 where garcinone D has a hydroxyl group at that position. The methoxyl group in compound 1 was assigned from the observation of a HMBC correlation between 6-OCH₃ (δ_H 4.00) and C-6 (δ_C 158.6). The HMBC correlations are illustrated in Figure 3. Therefore, compound 1 was elucidated to be 1,3-dihydroxy-8-(3-hydroxy-3-methylbutyl)-6,7-dimethoxy-2-(3-methyl-2-buten-1-yl)-xanthone and it was named trivially as mangaxanthone B.

**Figure 3.** Key HMBC correlations between ¹H and ¹³C in compound 1.

Compound 2 was isolated as yellow-brown crystals (m.p. = 245–246 °C) and was found to have a molecular formula of C₁₄H₁₂O₆ via the EIMS (m/z 276 [M⁺]) analysis. The FTIR spectrum exhibited a strong absorption at 1728 cm⁻¹, which suggested the presence of a carbonyl group, and another strong absorption at 2924 cm⁻¹ representing the aromatic C-H stretching band. An aromatic benzene chromophore which is of a benzophenone skeleton, was confirmed by the maximum absorption peaks of 211, 214 and 309 nm in the UV-Visible spectrum.

The ¹H-NMR spectrum of compound 2 showed five aromatic proton signals which resonated at δ_H 5.96 (s, 2H, H-3 and H-5), δ_H 6.52 (d, 2H, J = 2.3 Hz, H-2' and H-6') and δ_H 6.39 (t, 1H, J = 2.3 Hz, H-4'). After a detailed inspection on the ¹³C NMR and DEPT spectra, it was found that the signals in the ¹³C-NMR spectrum indicated 14 carbons which consisted of one methoxyl (δ_C 54.6), five methine (δ_C 91.1, 95.3, 105.7 and 106.5 × 2) and eight quaternary carbons (δ_C 106.4, 142.7, 158.0 × 2, 161.5, 163.3 × 2 and 198.4). The benzophenone skeleton of compound 2 was demonstrated by signals resonating at δ_C 198.4 for the carbonyl group, as well as signals at δ_C 158.0, 161.5 and 163.3 for the hydroxylated aromatic carbons (see Table 1).

The structure of compound 2 was deduced based on the HMBC spectrum (See Figure 4). The aromatic protons at H-2' and H-6' [δ_H 6.52 (d, 2H, J = 2.3 Hz)] showed three-bond connectivities with, C-4' (δ_C 105.7), and C-7 (δ_C 198.4). Protons H-2' and H-6' also gave two-bond connectivities with
C-3' and C-5' (δC 158.0 × 2) respectively. Meanwhile H-4' has 2J correlations with C-3' and C-5' (δC 158.0 × 2) and 3J correlations with C-2' and C-6' (δC 106.5 × 2). The aromatic proton of H-3 [δH 5.96 (s, 1H)] exhibited three-bond (3J) and two-bond (2J) connectivities with C-1 (δC 106.4) (3J), C-5 (δC 95.3) (3J) and C-4 (δC 163.3) (2J) respectively. H-5 [δH 5.96 (s, 1H)] also showed correlations with C-1 (δC 106.4) (3J), C-3 (δC 91.1) (3J), C-4 and C-6 (δC 163.3 × 2) (2J) in the HMBC spectrum. Moreover, the methoxyl group was assigned to C-2 because of the 3J HMBC correlation of the methoxyl proton [δH 3.54 (s, 3H, 2-OCH3)] with C-2 (δC 161.5). The observation of a 2J correlation between H-3 and C-4 implies position 3 to be occupied by H-3. Moreover, we also observed a 2J correlation between H-5 and C-6 as well as between H-5 and C-4. Therefore, another proton is situated at C-5. 4-OH and 6-OH carbons have the same chemical shift values, thus justifying that the molecule is symmetrical. Hence, the two OH groups are at C-6 and C-4 and not at C-2. Hence, the structure of this compound was elucidated as (4,6-dihydroxy-2-methoxyphenyl)-(3,5-dihydroxyphenyl)methanone and it was given the trivial name mangaphenone.

![Figure 4. 2J, 3J and 4J HMBC correlations between protons and carbons in compound 2.](image-url)

### 3. Experimental

#### 3.1. Plant Material

The stem bark of *Garcinia mangostana* was collected from Melaka, Malaysia. A herbarium specimen (RG221) was deposited at the Herbarium in the Biology Department of UPM.

#### 3.2. General

The 1D (1H, 13C, DEPT) and 2D (COSY, HMQC and HMBC) NMR spectra were recorded on a Unity INOVA 500 MHz NMR instrument using tetramethylsilane (TMS) as the internal standard. EIMS spectra were obtained using a Shimadzu GCMS model QP2010 Plus spectrophotometer. The ultraviolet spectra were recorded on a Shimadzu UV-160A UV-Visible Recording Spectrophotometer. Infrared spectra were obtained using the universal attenuated total reflection (UATR) technique on a Perkin-Elmer 100 Series FT-IR spectrometer. Melting points were measured through Leica Galen III microscope which was equipped with Testo 720 temperature recorder.

#### 3.3. Extraction and Isolation

The air-dried powdered stem bark of *Garcinia mangostana* (2.0 kg) was first de-fatted using hexane followed by extraction with ethyl acetate (EtOAc, 3 × 5 L) for 72 h at room temperature then with 70% methanol (MeOH, 3 × 5 L) for another 72 h. The three extracts were concentrated to give
14.22 g of dark brown residue of EtOAc extract and 278.96 g of dark brown residue of MeOH extract. The EtOAc extract was subjected to vacuum column chromatography by eluting with a stepwise gradient system of hexane, chloroform (CHCl₃), ethyl acetate and methanol to afford 6 fractions. The fourth fraction was further fractionated through column chromatography using hexane–CHCl₃ and CHCl₃–MeOH to give 8 fractions. The last fraction was subjected to repeated chromatography by eluting with hexane–EtOAc (7:3) and CHCl₃–MeOH (9.8:0.2) to furnish compounds 1, 2 and 3. Meanwhile, the dry MeOH extract (278.96 g) was suspended in H₂O and then partitioned with n-butanol (n-BuOH, 400 mL). The n-BuOH soluble portion (2.56 g) was chromatographed in a polarity gradient manner (hexane, hexane–CHCl₃, CHCl₃, CHCl₃–EtOAc, EtOAc–MeOH and MeOH) and afforded eight fractions. Fraction 2 was further purified through column chromatography by using CHCl₃–MeOH (9:1) and compound 4 was thus obtained.

3.4. Spectral Data

Mangaxanthone B (1). Yellow crystals; m.p. 194–195 °C; UV (EtOH) \( \lambda_{\text{max}} \) (log ε): 209 (4.40), 245 (4.44), 262 (4.42), 316 (4.29) and 353 (3.79) nm; IR \( \nu \) (cm\(^{-1}\)): 3447, 3249, 2938, 1601, 1457, 1277 and 826; \(^1\)H-NMR (500 MHz, Me₂CO-\( \text{d}_6 \)) and \(^13\)C-NMR (125 MHz, Me₂CO-\( \text{d}_6 \)), see Table 1; EIMS \( m/z \) (rel. int.): 442(32), 424(28), 381(74), 369(43), 368(33), 354(23), 353(100), 327(53) and 325(33).

Mangaphenone (2). Brownish-yellow crystals; m.p. 245–246 °C; UV (EtOH) \( \lambda_{\text{max}} \) (log ε): 211 (4.11), 214 (4.12) and 309 (3.77) nm; IR \( \nu \) (cm\(^{-1}\)): 3599, 2924, 1728, 1261, 804 and 730; \(^1\)H-NMR (500 MHz, CD₃OD) and \(^13\)C-NMR (125 MHZ, CD₃OD), see Table 1; EIMS \( m/z \) (rel. int.): 276(67), 260(50), 168(70), 167(100), 153(22) and 69(44).

Mangostanin (3). Yellow amorphous powder. Spectral data are in agreement with the literature [18].

Mangostenol (4). Yellow solid; m.p. 159–160 °C. Spectral data are in agreement with the literature [19].

4. Conclusions

A new prenylated xanthone, mangaxanthone B and a new benzophenone, mangaphenone, were isolated along with two known xanthones, mangostatin and mangostenol, from the stem bark of *Garcinia mangostana*. Biological evaluation of these compounds is under way.

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Author Contributions

GCLE and AAK designed the research; IS performed research, IS, SST and SD analyzed the data; IS and GCLE wrote the paper. All authors read and approved the final manuscript.
Conflicts of Interest

The authors declare no conflict of interest.

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*Sample Availability*: Samples of the compounds 1–4 are available from the authors.

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