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Isolation, Purification and Structure Elucidation of a Xanthone from the Pericarp of Kamandiis *(Garcinia rubra Merr.)*

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ABSTRACT

The pericarp of *Garcinia rubra*, an endemic *Garcinia* species in the Philippines was extracted with dichloromethane. An isolate of the extract was acetylated giving monoacetyl and diacetyl derivatives. Analysis of these derivatives led to the elucidation of the xanthone 1,3,7-trihydroxy-2,4-bis(3-methylbut-2-enyl)-9H-xanthen-9-one. The compound was isolated and characterized through column chromatography, acetylation, FTIR, NMR and LCMS analyses.

Keywords: *Garcinia rubra; xanthones*

INTRODUCTION

Garcinia species have been known as ayurvedic plants in Southeast Asia to treat diseases such as dysentery, diarrhea and many others (Werayut and Wandee, 2008). Some of these species have been extensively studied for their biological activity. One of the well-known *Garcinia* species is mangosteen (*Garcinia mangostana*) which is recognized for its potential in treating cancer (Itoh et al., 2008) and other serious illnesses.

The *Garcinia* species found in the Philippines include *G. binucao* or *batuan* in Panay island, *G. mindanaensis* or *kariis* in Bukidnon, *G. venulosa* or *gatasan* in Luzon, *G. vidalii* or *piris* which is found in Benguet, *G. dulcis* or *takláng-anák* which is found from Luzon to Mindanao and the Malay Peninsula, *G. benthami* which is found in Palawan, *G. rubra* or *kamandiis*, and *G. mangostana* in some parts of the Philippines (Pelser et al., 2011). Among these *Garcinia* species, only few have been subjected to studies regarding isolation of bioactive compounds which include *G. mangostana* (Itoh et al., 2008), *G. dulcis* (Deachathai et al., 2005) and *G. benthami* (Nguyen et al., 2011).

This study reports the isolation, purification and structure elucidation of a xanthone from the pericarp of *G. rubra*. *G. rubra* is distributed in primary forests at low altitudes from northern Luzon to Mindanao (Brown, 1954) with fruits that are known as a souring agent in Palawan. Extensive examination of literature on *Garcinia* does not include reports on isolation of bioactive compounds from *G. rubra*. Structure elucidation of the xanthone was done using FTIR, ^{1}H-NMR, ^{13}C-NMR, 2D NMR and LCMS analyses.

RESULTS AND DISCUSSION

The fruit of *G. rubra* is characterized by the

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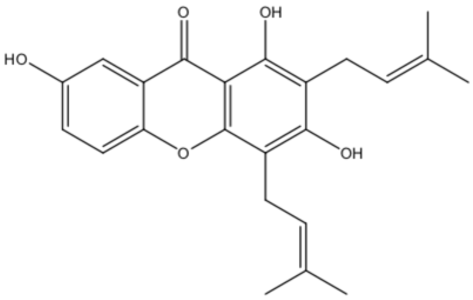
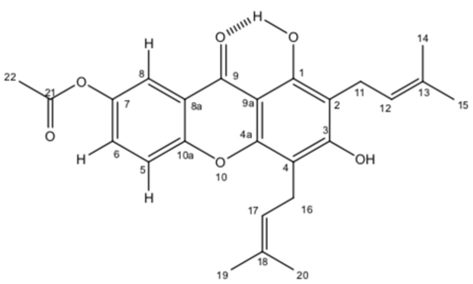
production of a prominent yellow sticky exudate upon slicing the pericarp. The dichloromethane extract of the oven-dried and powdered pericarp of *G. rubra* provided a major component 1,3,7-trihydroxy-2,4-bis(3-methylbut-2-enyl)-9H-xanthen-9-one **(1)**. The purification of **(1)** was simplified by acetylating the isolate containing **(1)**. Acetylation afforded compounds **(2)** and **(3)**. The structure of **(1)** was indirectly determined through the structure elucidation of its acetylation products.



(1)

The monoacetyl derivative **(2)** has an LCMS m/z value of 453.1 which corresponds to its methanol adduct $[M\text{-}H\text{+}MeOH]^-$. The FTIR data of **(2)** showed signals at 1650 cm^{-1} and 1740 cm^{-1} suggesting the presence of carbonyl groups. The signal observed at 1650 cm^{-1} is typical for carbonyl group in xanthones (Negi et al., 2013) while the additional signal at 1740 cm^{-1} is due to the carbonyl in the acetyl group. Also, the signal observed at 3500 cm^{-1} suggests the presence of hydroxyl group. The ^{1}H NMR spectrum (Table 1) of **(2)** suggests that the compound is a prenylated xanthone having three phenolic hydroxyl groups in which one is acetylated. The two free phenolic hydroxyl groups are at δ 13.08 (C1, H-bonded) and at δ 6.51.



(2)

One of the aromatic rings of the xanthone nucleus contains three aromatic hydrogens which are at δ 7.46 (aromatic H, dd, J = 9.1 Hz and 0.8 Hz, H-5), 7.45 (aromatic H, dd, J = 8.9 Hz and 2.4 Hz, H-6) and 7.95 (aromatic H, dd, J = 2.7 Hz and 0.7 Hz, H-8). The multiplicities and coupling constants suggests a trisubstituted benzene ring with protons at positions 1, 3 and 4. The signal at δ 7.95 being the most downfield among the aromatic hydrogens, is placed at position C-8 since it is near a carbonyl group. The xanthone also contains two prenyl groups. One prenyl group contains two allylic hydrogens at δ 3.48 (2H, d, J = 6.8 Hz, H-11), a vinylic hydrogen at δ 5.28 (1H, m, H-12), and two terminal methyl groups at δ 1.74 (3H, d, J = 1 Hz, H-14) and δ 1.78 (3H, d, J = 1 Hz, H-15). The other prenyl group contains two allylic hydrogens at δ 3.55 (2H, d, J = 7.1 Hz, H-16), a vinylic hydrogen at δ 5.28 (1H, m, H-17), and two terminal methyl groups at δ 1.86 (3H, d, J = 0.4 Hz, H-19) and 1.89 (3H, d, J = 0.5 Hz, H-20). Lastly, the acetyl group gave a singlet signal at δ 2.35 (3H, s, H-22).

The ^{1}H NMR data (Table 1) of the diacetyl derivative are the same as that of 2 except that the phenolic OH at C-3, which was at δ 6.51 in **(2)** is absent. Also, an additional acetyl group at δ 2.37 (3H, s) was observed. In the ^{13}C NMR results (Table 1) of **(3)**, the twelve aromatic carbon signals in the xanthone nucleus were assigned δ 158.48 (C-1), 116.85 (C-2), 154.23 (C-3), 112.69 (C-4), 152.47 (C-4a), 119.02 (C-5), 129.55 (C-6), 146.58 (C-7), 117.99 (C-8), 120.80 (C-8a), 106.94 (C-9a) and 153.58 (C-10a). The carbonyl carbon in the xanthone nucleus is found at δ 181.39 (C-9). The two prenyl side chains have their methylene carbons at δ 22.84 (C-11) and 23.19 (C-16). The two methine sp2 carbons in the side chain are located at δ 121.31 (C-12) and 121.22 (C-17). The two quaternary carbons are at δ 132.28 (C-13) and 132.39 (C-18). Lastly, the four terminal methyl carbons are at δ 17.89 (C-14), 25.73 (C-15), 25.73 (C-19) and 18.00 (C-20). The two acetyl groups gave the signals for the two carbonyl carbons at δ 169.34 (C-21) and 168.47 (C-23), and the two alpha methyl carbons at δ 21.03 (C-22) and 20.63 (C-24).

In the HMBC correlations of **(3)** (Figure 1), the methyl in the acetyl group correlated with C3 and methyl in the other acetyl group correlated with C7. One of the prenyl units has its vinylic hydrogen (H12) correlated with C2 and the other vinylic H (H17) correlated with C4. Also, correlations were observed from H5 to C7, C8a, C10a and C9, from H6 to C7, C8 and C10a, from H8 to C6, C7, C8a, C9 and C10a, from H11 to C1, and from H16 to C4, C4a and C9a. Correlation of H5 to C6 and H6 to C5 are not shown in the spectrum. This can happen since according to Reynolds and Mazzola (2015), it is common for 2-bond correlations in aromatic groups to be very weak or not observed. The other correlation using COSY, HSQC and HMBC analyses are shown in Table 2.

Analysis of the acetyl derivatives led to the structure elucidation of the natural isolate, 1,3,7-trihyroxy-2,4-bis(3-methylbut-2-enyl)-9H-xanthen-9-one

Table 1. 400 MHz ^{1}H NMR spectral data of the monoacetyl derivative (2) and 500 MHz 1H NMR, 126 MHz ^{13}C NMR and HMBC correlation of the diacetyl derivative (3). The samples were dissolved in $CDCl_3$.

Monoacetyl derivative (2)	Diacetyl derivative (3)		Assignment
δ_H, ppm, multiplicity[a] (J = Hz)	δ_H, ppm, multiplicity[a] (J = Hz)	δ_C, ppm	
13.08, s (1H)	12.90, s (1H)	158.48	phenolic OH - C1
-	-	116.85	C2
6.51, s (1H)	-	154.23	phenolic OH - C3
-	-	112.69	C4
-	-	152.47	C5
7.46, dd (1H, J = 9.1, 0.8 Hz)	7.50, m (1H)	119.02	aromatic H - C5
7.45, dd (1H, J = 8.9, 2.4 Hz)	7.49, m (1H)	129.55	aromatic H - C6
-	-	146.58	C7
7.95, dd (1H, J = 2.7, 0.7 Hz)	7.97, d (1H, J = 1.47 Hz)	117.99	aromatic H - C8
-	-	120.80	C8a
-	-	181.39	C9
-	-	106.94	C9a
-	-	153.58	C10a
3.48, d (2H, J = 6.8 Hz)	3.31, m (2H)	22.84	allylic hydrogens - C11
5.28, m (1H)	5.16, t (1H, J = 6.85 Hz)	121.31	vinylic H - C12
-	-	132.28	C13
1.74, d (3H, J = 1 Hz)	1.86, d (3H, J = 0.49 Hz)	17.89	allylic hydrogens - C14
1.78, d (3H, J = 1 Hz)	1.69, d (3H, J = 0.49 Hz)	25.73	allylic hydrogens - C15
3.55, d (2H, J = 7.1 Hz)	3.42, m (2H)	23.19	allylic hydrogens - C16
5.28, m (1H)	5.15, t (1H, J = 6.36 Hz)	121.22	vinylic H - C17
-	-	132.39	C18
1.86, d (3H, J = 0.4 Hz)	1.70, d (3H, J = 0.49 Hz)	25.73	allylic hydrogens - C19
1.89, d (3H, J = 0.5 Hz)	1.78, d (3H, J = 0.49 Hz)	18.00	allylic hydrogens - C20
-	-	169.34	C21
2.35, s (3H)	2.36, s (3H)	21.03	OCH_3 hydrogens - C22
-	-	168.47	C23
-	2.37, s (3H)	20.63	OCH_3 hydrogens - C24
-	2.37, s (3H)	20.63	OCH_3 hydrogens - C24

[a] s-singlet; d-doublet; dd-doublet of doublets; m-multiplet

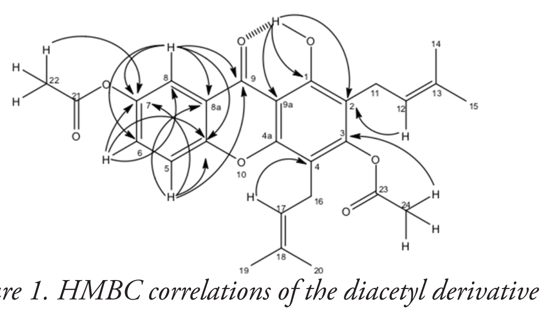


Figure 1. HMBC correlations of the diacetyl derivative (3).

(1) from the pericarp of *G. rubra*. This is the first xanthone isolated from *G. rubra* pericarp. This xanthone was reported to be present in the stem bark extracts of *Garcinia nitida* (Ee et al., 2005) and *Garcinia parvifolia* (Weng, 2016). Thus, *G. rubra* is another source of **(1)**. Other compounds from Philippine Garcinia are under investigation.

Table 2. COSY, HSQC and HMBC data of the diacetyl derivative (3).

hydrogen/carbon position	COSY	HSQC	HMBC
1-OH	-	-	C1, C2, C3, C9a
2	-	-	-
3	-	-	-
4	-	-	-
4a	-	-	-
5	H6	C5	C7, C8a, C10a, C9
6	H5 and H8	C6	C7, C8, C10a
7	-	-	-
8	H6	C8	C6, C7, C8a, C9, C10a
8a	-	-	-
9	-	-	-
9a	-	-	-
10a	-	-	-
11	H12	C11	C12, C13
12	H11, H14, H15	C12	C2, C11, C14, C15
13	-	-	-
14	H12	C14	C12, C13, C15
15	H12	C15	C12, C13, C14
16	H17	C16	C17, C18
17	H16, H19, H20	C17	C4, C16, C19, C20
18	-	-	-
19	H17	C19	C17, C18, C20
20	H17	C20	C17, C18, C19
21	-	-	-
22	-	C22	C21, C7
23	-	-	-
24	-	C24	C23, C3

EXPERIMENTAL

Materials. Gravity column chromatography was done using Sigma-Aldrich Vetec™ Silica gel (60-120 mesh). TLC was performed using silica gel Macherey-Nagel Silica Gel G / UV 254 with iodine vapor as visualizing agent. The dichloromethane used was distilled. The ethyl acetate was from J.T. Baker, USA, the toluene was AR Grade from RCI Labscan, Thailand.

Plant material. The *G. rubra* fruit samples were gathered from El Nido, Palawan last May 2015. The fruit specimen was identified by the UPLB Museum of Natural History as *Garcinia rubra Merr*.

Sample Preparation. The pericarp was removed from the entire fruit. The thin peel on the pericarp was excluded and the rest of the pericarp was chopped into small pieces and air-dried. The air-dried pericarp were then oven-dried at 105oC and was ground using a Wiley-Mill.

Extraction and Isolation. The powdered pericarp was extracted with dichloromethane. The extract was subjected to column chromatography using DCM:EtOAc (9.2:0.8, v/v). The eluents were subjected to TLC using toluene:EtOAc (9:1, v/v). A major component was obtained with some impurities.

Acetylation. The major component was acetylated using two methods.

Method 1. The method for acetylation described by Shriner et al. (1998) was used. Sufficient amount of

3N NaOH was added to the impure sample until the solution is basic. Around 15 g of crushed iced prepared from distilled water was then added to the solution. Excess acetic anhydride was then added until the solution was acidic. Yellow amorphous solids formed were collected by filtration. The TLC chromatogram of the solid revealed three spots. A bright yellow crystal derivative was then purified by column chromatography using hexane:EtOAc (1.5:0.1, v/v). The isolate (2) was then subjected to LCMS and ^{1}H NMR spectroscopy.

Method 2. About 20 mg of the major component was dissolved in one mL pyridine. Ten mL acetic anhydride was then added. The mixture was allowed to react for 24 hours. The reaction mixture was then extracted with DCM. The DCM extract was then washed with distilled water and 2% HCl then subjected to column chromatography using hexane:EtOAc (1.5:0.1, v/v). A yellow crystal derivative (3) was collected and subjected to 2D NMR spectroscopy.

Spectroscopic Studies. The infrared spectrum of **(2)** was obtained using a Thermo Nicolet Avatar 330 Fourier Transform Infrared spectrometer. The sample was analyzed using the KBr pellet method. The NMR spectra of **(2)** was recorded in $CDCl_3$ with the use of a JEOL LA 400 Spectrometer. The NMR spectra of **(3)** in $CDCl_3$ was recorded using a 500 MHz Varian Nuclear Magnetic Resonance Spectrometer. The LCMS data of **(2)** was obtained using the Shimadzu Liquid Chromatography Tandem Mass Spectrometer (LCMSMS) 8040. The stationary phase was a Shim-pack HR-ODS column. Isocratic elution using 95% acetonitrile: 0.1% formic acid in type 1 water mobile phase was done. ESI-MS was operated in negative ion mode.

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