



## TETRAOXYGENATED XANTHONES FROM THE LATEX OF *GARCINIA COWA* GROWING IN VIET NAM

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**Abstract.** Seven tetraoxygenated xanthenes, namely fuscaxanthone A, 7-*O*-methylgarcinone E, cowagarcinone A, cowaxanthone, rubraxanthone, cowanin and cowanol, were isolated from the dichloromethane extract of the latex of *Garcinia cowa* Roxb. ex Choisy. Their structures were elucidated on the basis of 1D, 2D NMR spectroscopic data and compared with reported data. This is the first time the chemical constituents of *Garcinia cowa* Roxb. ex Choisy have been investigated in Vietnam.

**Keywords:** tetraoxygenated xanthone, dichloromethane extract, latex, *Garcinia cowa*.

**Classification numbers:** 1.1.1; 1.1.6; 1.4.7.

### 1. INTRODUCTION

*Garcinia cowa* Roxb. ex Choisy (*G. cowa*), *Clusiaceae* family is widely distributed in Vietnam. The root and barks of *G. cowa* have been used in traditional medicine for treatment of fever or as antiseptic agent [1]. The fruits and young leaves of the tree are edible and are consumed popularly in Southeast Asian countries. Phytochemical studies and pharmacological activities of *G. cowa* from Thailand were reported recently [2 - 6]. According to previous reports, main chemical constituents of *G. cowa* are xanthenes which were demonstrated interesting bioactivities such as antimalarial [2], antimicrobial [3, 5, 7], anti-inflammatory [4, 8], antioxidant [3, 4, 9], antibacterial [5, 10, 11], antitumor-promoting activity [12] and cytotoxic activities [13 - 15].

As part of our research on this species growing in Vietnam, we report herein the isolation and structural elucidation of seven tetraoxygenated xanthenes (**1-7**), those are fuscaxanthone A (**1**), 7-*O*-methylgarcinone E (**2**), cowagarcinone A (**3**), cowaxanthone (**4**), rubraxanthone (**5**), cowanin (**6**), and cowanol (**7**) from the dichloromethane (DCM) extract of latex of *G. cowa* collected in Phu Quoc island.

## 2. MATERIALS AND METHODS

### 2.1. General

NMR spectra were recorded on a Bruker Advance 500 spectrometer at 500 and 125 MHz for  $^1\text{H}$  and  $^{13}\text{C}$ , respectively, at Institute of Chemistry - Vietnam Academy of Science and Technology. Chemical shifts are shown in  $\delta$  (ppm) in  $\text{CDCl}_3$  with tetramethylsilane (TMS) as an internal reference. Melting points were measured on Buchi B545 apparatus (no correction). Column chromatography (CC) were carried out on silica gel 60 (Merck, 5-40  $\mu\text{m}$ ), silica gel 100 (Merck, 63-200  $\mu\text{m}$ ), and/or sephadex LH-20 (GE Healthcare). Visualization of thin layer chromatography (TLC) plates was performed using UV light (254 and 365 nm), staining with  $\text{H}_2\text{SO}_4$  10 % solution. Commercial solvents were purified and dried, when necessary, by standard methods just prior to use.

### 2.2. Plant materials

The latex of *G. cowa* was collected in Phu Quoc island - Kien Giang province, in December 2015. The plant materials were identified by Dr. Nguyen Quoc Binh, Vietnam National Museum of Nature. The herbarium specimen has been deposited at Institute of Natural Products Chemistry - Vietnam Academy of Science and Technology with the plant specimen number GC2015128.

### 2.2. Extraction and isolation

The latex of *G. cowa* (3.0 kg) existing in a form of a brown solid, was crushed into small pieces and then was dried in the oven at the temperature of 45  $^\circ\text{C}$  in three days to achieve 2.8 kg dried latex. The dried latex was extracted with methanol (MeOH) (3 L  $\times$  3) at room temperature using conventional ultrasound-assisted technique. The solvent was evaporated under reduced pressure to give a dark brown residue (500.0 g). The residue was further extracted with DCM (500 mL  $\times$  3) to yield DCM extract (96.7 g). The left residue was then extracted with ethylacetate (EtOAc) (500 mL  $\times$  3) to afford EtOAc extract (145.1 g).

The crude DCM extract was subjected to column chromatography over silica gel, eluted with DCM-MeOH in a polarity gradient manner (v/v, 100:0 to 0:100) to afford five fractions (Frs. GCN1–GCN5). Fraction GCN1 (22.4 g) was fractionated by employing CC with hexane-EtOAc (v/v, 100:0 to 0:100) as an eluent to give ten subfractions GCN1.1–GCN1.10.

Subfraction GCN1.4 (6.4 g) was chromatographed over silica gel, eluting with 50 % DCM-hexane to afford five subfractions GCN1.4.1–GCN1.4.5. Further chromatography of subfraction GCN1.4.1 (0.8 g) over silica gel using hexane-acetone (v/v, 10:1) as the mobile phase to yield compound **1** as bright yellow oil (GCN141, 0.04 g). Crystallization of subfraction GCN1.4.2 (1.83 g) in hexane-DCM (v/v, 1:1) provided compound **2** as pale yellow needles (GCN142, 0.23 g). Subfraction GCN1.4.4 (0.71 g) was isolated by CC using eluent of 10 % acetone in hexane to give compound **3** which was crystalized in DCM to appear as pale yellow needles (GCN144, 0.12 g).

Subfraction GCN1.6 (3.5 g) was separated by repeated CC with DCM-hexane (v/v, 1:1) to yield compounds **6** (GCN162, 1.43 g) as a pale yellow solid.

Compound **4** (GCN182, 0.26 g), as a yellow solid, was obtained from subfraction GCN1.8 (3.12 g) by repeated purification on sephadex LH-20 chromatography with eluent of 5 % DCM-MeOH.

Fraction GCN2 (37.5 g) was fractionated by CC with a gradient of hexane-EtOAc (v/v, 100:0 to 0:100) to afford eleven subfractions GCN2.1-GCN2.11. Subfraction GCN2.2 (1.76 g) was further purified by chromatography over silica gel with eluent of hexane-ethylacetate (v/v, 16:1) to give compound **5** (GCN228, 0.02 g) as pale yellow solid. Subfraction GCN2.6 (2.2 g) was separated by Sephadex LH-20 chromatography with 100 % MeOH to give four subfractions. Compound **7** (GCN262, 0.85 g) was obtained from the second subfraction by crystallization in DCM to appear as yellow needles.

**Fuscaxanthone A (1)**: Bright yellow oil.  $^1\text{H-NMR}$  (500 MHz,  $\text{CDCl}_3$ )  $\delta$  (ppm): 13.72 (s, OH-1), 6.86 (1H, s, H-5), 6.75 (1H, d,  $J = 10.0$  Hz, H-10), 6.35 (1H, s, OH-6), 6.27 (1H, s, H-4), 3.83 (3H, s,  $\text{OCH}_3$ -7), 5.59 (1H, d,  $J = 10.0$  Hz, H-11), 5.29 (1H, t,  $J = 5.5$  Hz, H-2'), 5.05 (1H, br t, H-6'), 4.12 (2H, d,  $J = 6.0$  Hz, H-1'), 2.08 (2H, m, H-4', H-5'), 2.04 (2H, m, H-4', H-5'), 1.85 (3H, s, H-10'), 1.63 (3H, s, H-8'), 1.57 (3H, s, H-9'), 1.49 (6H, s, H-13,14).  $^{13}\text{C-NMR}$  (125 MHz,  $\text{CDCl}_3$ )  $\delta$  (ppm): 181.9 (C-9), 159.8 (C-3), 157.9 (C-1), 156.3 (C-4a), 155.7 (C-5a), 154.5 (C-6), 142.7 (C-7), 137.1 (C-8), 135.6 (C-3'), 131.3 (C-7'), 127.1 (C-11), 124.3 (C-6'), 124.3 (C-2'), 115.7 (C-10), 112.3 (C-8a), 104.5 (C-2), 103.8 (C-9a), 101.6 (C-5), 94.1 (C-4), 77.9 (C-12), 62.1 (7-OMe), 39.7 (C-4'), 28.3 (C-13, 14), 26.8 (C-1'), 26.5 (C-5'), 25.6 (C-8'), 17.7 (C-9'), 16.5 (C-10').

**7-O-methylgarcinone E (2)**: Pale yellow needles, m.p. 222-223°C.  $^1\text{H-NMR}$  (500 MHz,  $\text{CDCl}_3$ )  $\delta$  (ppm): 13.84 (1H, s, OH-1), 6.39 (1H, s, OH-6), 6.33 (1H, s, H-4), 6.10 (1H, s, OH-3), 5.27 (1H, m, H-2'''), 5.27 (1H, m, H-2'), 5.25 (1H, m, H-2''), 4.07 (2H, d,  $J = 7.0$  Hz, H-1''), 3.80 (3H, s,  $\text{OCH}_3$ -7), 3.56 (2H, d,  $J = 7.0$  Hz, H-1'''), 3.46 (2H, d,  $J = 7.0$  Hz, H-1'), 1.87 (3H, s, H-4'''), 1.85 (3H, s, H-4'), 1.82 (3H, s, H-4''), 1.77 (3H, s, H-5'), 1.69 (6H, s, H-5'', 5''').  $^{13}\text{C-NMR}$  (125 MHz,  $\text{CDCl}_3$ )  $\delta$  (ppm): 182.5 (C-9), 161.5 (C-3), 160.6 (C-1), 155.1 (C-4a), 153.6 (C-5a), 152.3 (C-6), 142.3 (C-7), 131.8 (C-8), 135.8 (C-3'), 133.9 (C-3''), 132.7 (C-3'''), 123.5 (C-2''), 121.5 (C-2'), 121.1 (C-2'''), 114.0 (C-5), 112.0 (C-8a), 108.3 (C-2), 103.6 (C-9a), 93.2 (C-4), 62.0 (7-OMe), 26.4 (C-1''), 25.8 (C-5', 5'', 5'''), 22.6 (C-1'''), 21.5 (C-1'), 18.2 (C-4''), 18.0 (C-4'''), 17.9 (C-4').

**Cowagarcinone A (3)**: Pale yellow needles, m.p. 258-259°C.  $^1\text{H-NMR}$  (500 MHz,  $\text{CDCl}_3$ )  $\delta$  (ppm): 13.85 (1H, s, OH-1), 6.41 (s, OH-6), 6.33 (1H, s, H-4), 6.13 (1H, s, OH-3), 5.31 (1H, m, H-2'), 5.28 (1H, m, H-2'''), 5.26 (1H, m, H-2''), 5.03 (1H, br t,  $J = 6.5$  Hz, H-6''), 4.07 (2H, d,  $J = 6.0$  Hz, H-1''), 3.80 (3H, s,  $\text{OCH}_3$ -7), 3.58 (2H, d,  $J = 7.5$  Hz, H-1'''), 3.46 (2H, d,  $J = 7.5$  Hz, H-1'), 2.01 (2H, m, H-5''), 1.99 (2H, m, H-4''), 1.88 (3H, s, H-4'''), 1.85 (3H, s, H-4'), 1.82 (3H, s, H-10''), 1.77 (3H, s, H-5'), 1.69 (3H, s, H-5'''), 1.60 (3H, s, H-8''), 1.55 (3H, s, H-9'').  $^{13}\text{C-NMR}$  (125 MHz,  $\text{CDCl}_3$ )  $\delta$  (ppm): 182.3 (C-9), 161.6 (C-3), 160.5 (C-1), 155.1 (C-4a), 153.8 (C-5a), 152.5 (C-6), 142.4 (C-7), 135.8 (C-3'), 135.6 (C-3''), 133.8 (C-8), 132.9 (C-3'''), 131.3 (C-7''), 124.4 (C-6''), 123.4 (C-2''), 121.5 (C-2'), 121.3 (C-2'''), 113.7 (C-5), 112.0 (C-8a), 108.4 (C-2), 103.7 (C-9a), 93.3 (C-4), 62.1 (7-OMe), 39.5 (C-4''), 26.5 (C-1''), 26.3 (C-5''), 25.8 (C-5'), 25.6 (C-5'''), 25.6 (C-9''), 22.5 (C-1'''), 22.5 (C-1'), 18.0 (C-4'''), 17.9 (C-4'), 17.8 (C-10''), 16.7 (C-8'').

**Cowaxanthone (4)**: Yellow solid, m.p. 196-197°C.  $^1\text{H-NMR}$  (500 MHz,  $\text{CDCl}_3$ )  $\delta$  (ppm): 7.53 (1H, s, H-8), 6.82 (1H, s, H-5), 6.31 (1H, s, H-4), 5.25 (2H, dd,  $J = 6.5$  Hz, 7.0 Hz, H-2'), 5.03 (2H, m, H-6'), 3.94 (3H, s,  $\text{OCH}_3$ -7), 3.36 (2H, d,  $J = 7.5$  Hz, H-1'), 2.04 (2H, m, H-5'), 1.96 (2H, m, H-4'), 1.77 (3H, s, H-10'), 1.60 (3H, s, H-9'), 1.53 (3H, s, H-8').  $^{13}\text{C-NMR}$  (125 MHz,  $\text{CDCl}_3$ )  $\delta$  (ppm): 179.9 (C-9), 162.2 (C-3), 160.0 (C-1), 156.0 (C-4a), 152.9 (C-5a), 152.6

(C-6), 144.8 (C-7), 136.9 (C-3'), 131.4 (C-7'), 124.2 (C-6'), 121.8 (C-2'), 113.3 (C-8a), 110.0 (C-2), 105.0 (C-5), 102.8 (C-9a), 102.6 (C-8), 93.6 (C-4), 56.4 (C7-OMe), 39.7 (C-4'), 26.6 (C-5'), 25.5 (C-9'), 21.3 (C-1'), 17.5 (C-9'), 16.1 (C-10').

**Rubraxanthone (5):** Pale yellow solid, m.p. 201-202°C. <sup>1</sup>H-NMR (500 MHz, CDCl<sub>3</sub>)  $\delta$  (ppm): 6.70 (1H, *s*, H-5), 6.19 (1H, *d*, *J* = 2.0 Hz, H-4), 6.12 (1H, *d*, *J* = 2.0 Hz, H-2), 5.18 (1H, *m*, H-2''), 4.97 (1H, *m*, H-6''), 4.03 (2H, *d*, *J* = 6.0 Hz, H-1''), 3.71 (3H, *s*, OCH<sub>3</sub>-7), 1.97 (2H, *m*, H-5''), 1.93 (2H, *m*, H-4''), 1.76 (3H, *s*, H-10''), 1.53 (3H, *s*, H-9''), 1.48 (3H, *s*, H-8''). <sup>13</sup>C-NMR (125 MHz, CDCl<sub>3</sub>)  $\delta$  (ppm): 181.9 (C-9), 164.0 (C-3), 163.1 (C-1), 157.1 (C-4a), 155.8 (C-5a), 155.6 (C-6), 143.3 (C-7), 137.4 (C-8), 135.2 (C-3''), 131.2 (C-7''), 124.3 (C-6''), 123.4 (C-2''), 111.6 (C-8a), 101.8 (C-5), 103.2 (C-9a), 97.8 (C-2), 93.4 (C-4), 61.1 (C7-OMe), 39.7 (C-4''), 26.5 (C-5''), 26.2 (C-1''), 25.5 (C-9''), 16.3 (C-10'').

**Cowanin (6):** Pale yellow solid, m.p. 135-137°C. <sup>1</sup>H-NMR (500 MHz, CDCl<sub>3</sub>)  $\delta$  (ppm): 13.79 (1H, *s*, OH-1), 6.83 (1H, *s*, H-5), 6.32 (1H, *s*, OH-6), 6.29 (1H, *s*, H-4), 6.14 (1H, *s*, OH-3), 5.29 (1H, *m*, H-2'), 5.26 (2H, *m*, H-2''), 5.03 (2H, *t*, *J* = 7.0 Hz, H-6''), 4.10 (2H, *d*, *J* = 6.5 Hz, H-1''), 3.80 (3H, *s*, OCH<sub>3</sub>-7), 3.46 (2H, *d*, *J* = 7.0 Hz, H-1'), 2.06 (2H, *m*, H-5''), 2.01 (2H, *m*, H-4''), 1.84 (3H, *s*, H-4'), 1.83 (3H, *s*, H-10''), 1.77 (3H, *s*, H-5'), 1.59 (3H, *s*, H-9''), 1.55 (3H, *s*, H-8''). <sup>13</sup>C-NMR (125 MHz, CDCl<sub>3</sub>)  $\delta$  (ppm): 182.0 (C-9), 161.6 (C-3), 160.7 (C-1), 155.1 (C-5a), 155.8 (C-6), 154.5 (C-4a), 142.6 (C-7), 137.1 (C-8), 135.8 (C-3'), 135.6 (C-3''), 131.3 (C-7''), 124.3 (C-6''), 123.2 (C-2''), 121.5 (C-2'), 101.5 (C-5), 112.3 (C-8a), 108.4 (C-2), 103.7 (C-9a), 93.3 (C-4), 62.1 (7-OMe), 39.7 (C-5''), 26.6 (C-4''), 26.5 (C-1''), 25.8 (C-5'), 25.6 (C-9''), 21.5 (C-1'), 17.9 (C-4'), 17.7 (C-8''), 16.5 (C-10'').

**Cowanol (7):** Yellow needles, m.p. 123-124°C. <sup>1</sup>H-NMR (500 MHz, CDCl<sub>3</sub>)  $\delta$  (ppm): 13.83 (1H, *s*, OH-1), 6.77 (1H, *s*, H-5), 6.26 (1H, *s*, H-4), 5.49 (1H, *dt*, *J* = 8.0 Hz, 1.5 Hz, H-2'), 5.25 (1H, *dt*, *J* = 6.0 Hz, 1.0 Hz, H-2''), 5.03 (1H, *m*, H-6''), 4.35 (2H, *s*, H-4'), 4.08 (2H, *d*, *J* = 6.5 Hz, H-1''), 3.80 (3H, *s*, OCH<sub>3</sub>-7), 3.46 (2H, *d*, *J* = 7.0 Hz, H-1'), 2.04 (2H, *m*, H-5''), 1.99 (2H, *m*, H-4''), 1.83 (3H, *br s*, H-10''), 1.79 (3H, *s*, H-5'), 1.60 (3H, *d*, *J* = 1.0 Hz, H-9''), 1.54 (3H, *s*, H-8''). <sup>13</sup>C-NMR (125 MHz, CDCl<sub>3</sub>)  $\delta$  (ppm): 181.9 (C-9), 161.6 (C-3), 160.8 (C-1), 155.2 (C-4a), 155.8 (C-5a), 154.7 (C-6), 142.8 (C-7), 137.3 (C-8), 135.5 (C-3''), 133.5 (C-3'), 131.2 (C-7''), 126.9 (C-2'), 124.4 (C-6''), 123.4 (C-2''), 112.3 (C-8a), 108.4 (C-2), 103.5 (C-9a), 101.7 (C-5), 93.6 (C-4), 62.7 (C-4'), 61.9 (7-OMe), 39.7 (C-4''), 26.7 (C-5''), 26.5 (C-1''), 25.5 (C-9''), 22.6 (C-5'), 21.5 (C-1'), 17.6 (C-8''), 16.5 (C-10'').

### 3. RESULTS AND DISCUSSION

Compounds **1-7** were isolated from the DCM extract of the latex of *G. cowa* using repeated column chromatography on silica gel and sephadex LH-20 eluted with appropriate solvent mixtures. These compounds showed strong UV absorption band of xanthone chromophore at  $\lambda_{\max} = 254$  nm. Their spectral database (<sup>1</sup>H, <sup>13</sup>C-NMR) contain characteristic sp<sup>2</sup> protons, aromatic carbons and a carbonyl group for a xanthonoid skeleton with 1-3 prenyl or geranyl side chains. The structures of the isolated compounds are shown in Figure 1.

The <sup>1</sup>H, <sup>13</sup>C-NMR and HSQC spectra of compound **1** revealed 29 carbons including 5xCH<sub>3</sub>, 1xOMe, 6xCH sp<sup>2</sup>, 3xCH<sub>2</sub> and 14xCq (one C=O at  $\delta_C$  181.9). Furthermore, the COSY and HMBC correlations showed the presence of a tetraoxygenated xanthonoid skeleton with a geranyl group (C1'-C10') and a dimethylpyran ring (C10-C14) with a double bond C-10 ( $\delta_C$  115.7,  $\delta_H$  6.75)/ C-11 ( $\delta_C$  127.1,  $\delta_H$  5.59). Substituent positions were determined by C-H long-range correlations in the HMBC spectrum: proton of methoxy group to C-7; protons H-1' of

geranyl group to C-7, -8 and protons H-10, -11 to C-2. Two singlets at  $\delta_H$  6.27 and 6.86 were assigned to H-4 and H-5 by HMBC correlations of proton H-5 to C-6, -7 and the correlation of proton H-4 to C-3. By combination of all NMR spectral data and comparison with reported values [16], the structure of **1** was determined as fuscaxanthone A.

Compounds **2-7** also contain a tetraoxygenated xanthonoid skeleton with similar signals in their  $^1H$  and  $^{13}C$ -NMR spectra (12 aromatic carbons and a carbonyl group). All these compounds contain three phenolic hydroxyl groups at position C-1, -3, -6 and one methoxy group at C-7 on the xanthone frame. NMR data of compound **2** demonstrated the presence of three prenyl groups including  $6xCH_3$ ,  $3xCH=$ ,  $3xCH_2$  and  $3xCq\ sp^2$ . The position of each prenyl group was determined by HMBC correlations between C-2, -3/H-4', -1'; C-7, -8/H-1'' and C-6/H-1'''. All the NMR data of **2** were identical to reported values [6] of 7-O-methylgarcinone E.

The NMR spectrum of compound **3** exhibited  $2xCH_2$ ,  $1xCH_3$ ,  $1xCH=$  and  $1xCq\ sp^2$  more than compound **2**. Analysing the structure of **3** in comparison with reported value [9], **3** was determined to be cowagarcinone A.

Compound **4** or **5** contains only one geranyl group substituting on xanthonoid skeleton. The HMBC correlations of H-1' to C-1, -2, -3 in compound **4** and the correlation of H-1', -2' to C-8 in compound **5** proved that the geranyl group bonds to C-2 for **4** and to C-8 for **5**. Therefore, the structure of **4** and **5** were elucidated as cowaxanthone [5] and rubraxanthone [17,18], respectively.

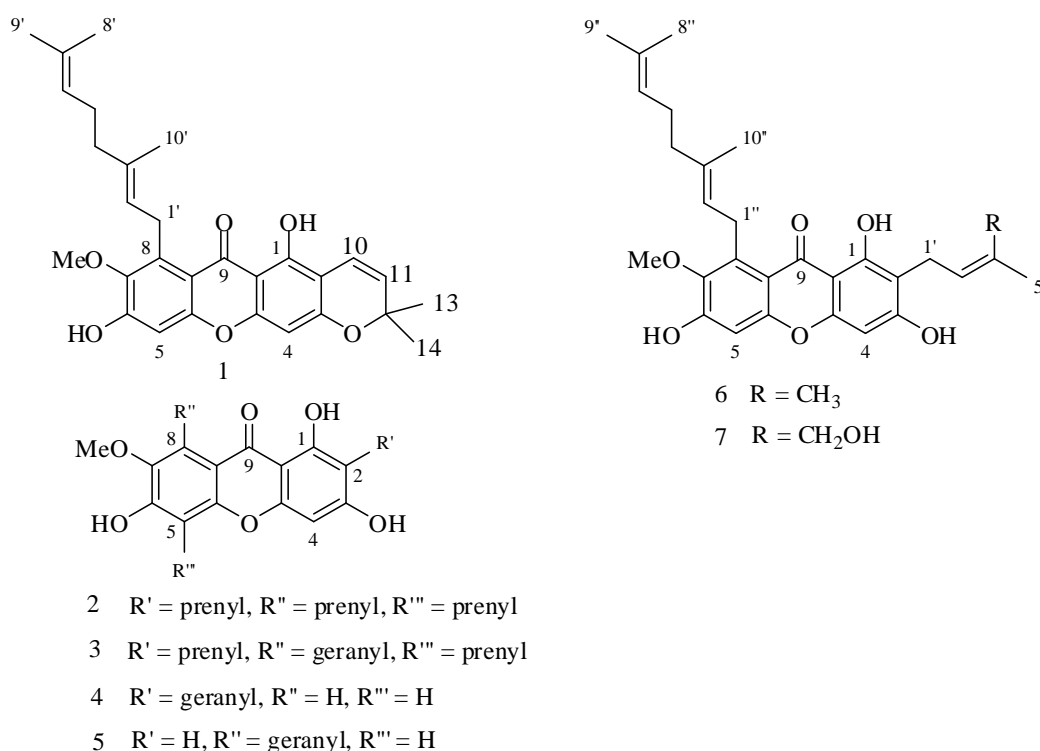


Figure 1. Structures of compound **1-7**.

The  $^1H$  and  $^{13}C$ -NMR spectrum of compound **6** exhibited the same resonance signals with those of compound **5** except for the replacement of aromatic proton H-2 by a prenyl group. The HMBC spectrum showed the correlation of H-1' to C-1, -2, -3 suggesting the appearance of one

prenyl group on the xanthone frame. In addition, all the NMR data of **6** were identical to those of reported values of cowanin [5].

The NMR spectral data and HMBC correlations of compound **7** were identical to those of cowanin (**6**) except for the absence of one methyl of the prenyl group and the appearance of an oxygenated methylenic group. The HMBC correlations of H-2' to the oxygenated methylenic carbon C-4' and the correlation of H-4' to C-2', -3', -5' proved that the hydroxyl group bonds to C-4'. Thus, compound **7** was determined as cowanol [5].

The antimalarial potential of 7-*O*-methylgarcinone E (**2**), cowaxanthone (**4**), cowanin (**6**) and cowanol (**7**) against *Plasmodium falciparum* was examined by Likhitwitayawuid K. et al. in 1998. All of these four compounds exhibited moderate activity against *Plasmodium falciparum* with their IC<sub>50</sub> values in the range of 1.50-3.00 µg/mL [2]. Evaluation of the antibacterial activity against Gram-positive bacteria: *B. cereus* TISTR 688, *B. subtilis* TISTR 008 and *M. luteus* TISTR 884 of compound rubraxanthone (**5**) and cowanin (**6**) showed that they both exhibited good activity with MICs of **6** in the range of 4–8 µg/mL while **5**, had better activity with MICs in the range of 1-2 µg/mL [11].

#### 4. CONCLUSIONS

The dichloromethane extract of the latex of *Garcinia cowa* Roxb. ex Choisy (Clusiaceae) collected from Phu Quoc island was separated by means of chromatography. Accordingly, seven tetraoxygenated xanthenes were isolated and elucidated as fuscaxanthone A (**1**), 7-*O*-methylgarcinone E (**2**), cowagarcinone A (**3**), cowaxanthone (**4**), rubraxanthone (**5**), cowanin (**6**) and cowanol (**7**). These compounds are first isolated and identified from *Garcinia cowa* Roxb. ex Choisy growing in Vietnam.

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