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Cytotoxic xanthenes from the stem bark of *Garcinia cowa* Roxb

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ABSTRACT

The aims of this study was to isolate compounds from the methanol extract of stem bark of *Garcinia cowa* and to evaluated their cytotoxic activity against breast (MCF-7) and lung (H-460) cancer cell lines. The ethyl acetate fraction was separated by successive silica gel column chromatography to give three compounds. Based on spectroscopic comparison with those of the literature, these compounds were elucidated as 6-hydroxy-calabaxanthone (2), 2-(3-methyl-2-butenyl)-1,5,6-trihydroxy-3-methoxy-4-(1,1-dimethyl-2-propenyl)-9H-xanthen-9-one (3), rubraxanthone (4), -mangostin (5), a new compound 1,3,6-trihydroxy-7-methoxy-4-(4-acetoxy-3-methyl-2-butenyl)-8-(3,7-dimethyl-2,6-octadienyl)xanthone (6) and cowanin (7). Complete NMR assignment of 6-hydroxy-calabaxanthone (2) and cowanin (7) were published for the first time. All isolated compounds were subjected to MTT assay against MCF-7, H-460 and DU-145 cancer cell lines. -mangostin (5) and cowanin (7) were found to be potent against MCF-7 while 6-hydroxy-calabaxanthone (2) potent against DU-145 cell lines. Conclusion: The results indicate that *G. cowa* stem bark could be one of important sources of natural cytotoxic compounds.

Keywords: Structure elucidation, NMR spectroscopy, xanthenes, *Garcinia cowa* Roxb., cytotoxic activity, MCF-7, H-460, DU-145

INTRODUCTION

Garcinia cowa Roxb known as asam kandis in West Sumatra is a medium-sized tree, which attains a height of ca. 30 m. It is widely distributed throughout tropical region and has been used in the folk medicine for many medicinal purposes. The fruit was used for treatment of dysentery [1] and the bark as an antipyretic agent [2]. The crude extract of the leaves of *G. cowa* has some pharmacological properties such as inhibition of human low-density lipoprotein peroxidation and anti-platelet activities [3] and antitumor-promoting [4]. Nine prenylated polyhydroxyxanthenes as well as pyrano-xanthenes were found in the stem bark of this plant [5]. (-)-Hydroxycitric acid [6] as well as cowaxanthenes A-E were obtained from the fruit of *G. cowa* [7]. Antibacterial xanthenes, norcowanin, cowanin, cowanol and cowaxanthone, 1,3,6-trihydroxy-7-methoxy-2,5-bis(3-methyl-2-butenyl)xanthone were isolated from the latex of *G. cowa* [2] as well as cowagarcinones A-E, mangostin and fuscaxanthone A [8]. Previously, a new tetraprenyltoluquinone, [2E,6E,10E]-(+)-4-hydroxy-1-methyl-5-(3,7,11,15-tetramethyl-2,6,10,14-hexadecatetraenyl-2-cyclohexen-1-one (1) and a new xanthone, 2-(3-methyl-2-butenyl)-1,5,6-trihydroxy-3-methoxy-4-(1,1-dimethyl-2-propenyl)-9H-xanthen-9-one (3) as well as rubraxanthone (4) were isolated from the stem bark of this plant [9].

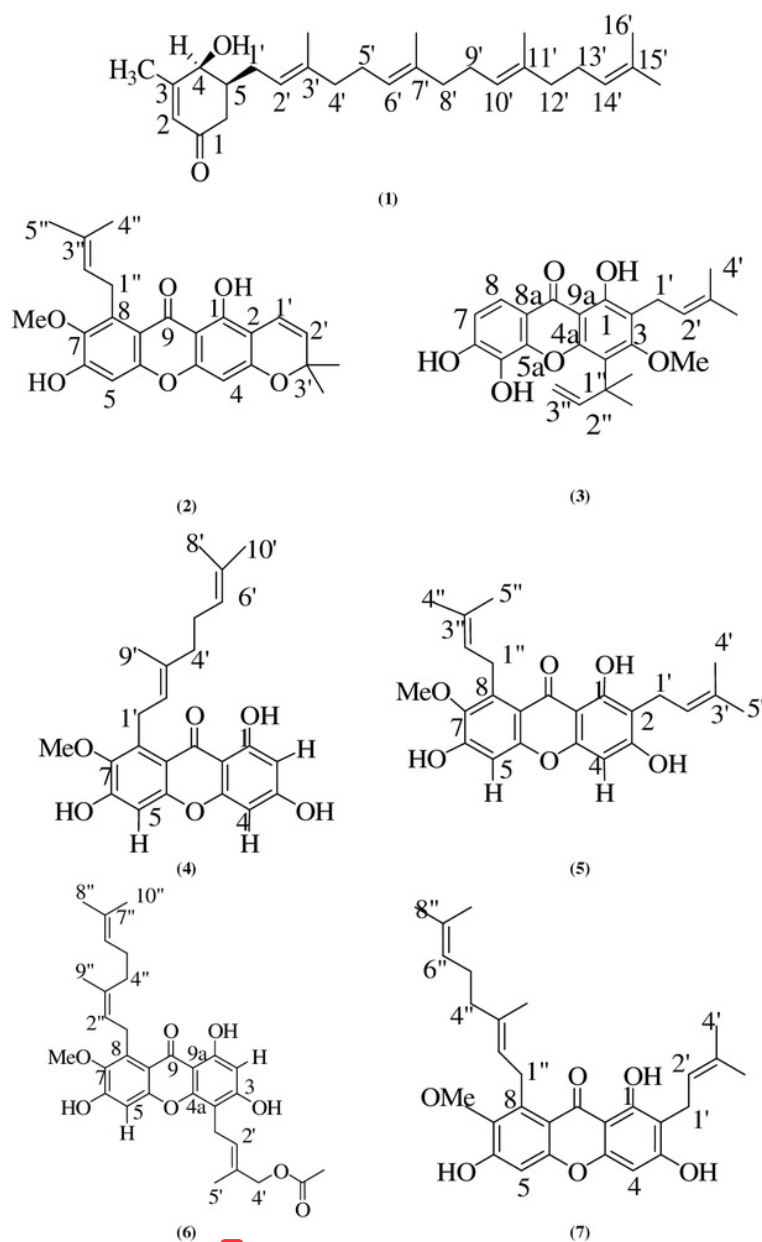


Fig. 1. Isolated compounds from the stem bark of *G. cowa*

In continuation of our study, the crude methanol extract of the stem bark of *G. cowa* was found to be active against several cell lines. Herein, we report the structure elucidation of five known xanthenes and one new xanthone as well as their cytotoxic against MCF-7, H-460 and DU-145 cancer cell-lines.

EXPERIMENTAL SECTION

Plant collection

G. cowa Roxb (stem bark) was collected at Sarasah Bonta, Harau Valley, and West Sumatra. The specimens (DR-180) was characterized by Dr Rusdi Tamin and kept at the herbarium of Andalas University, Padang, Indonesia.

Plant materials were chopped into small pieces and air-dried. The dried plant materials were ground into powder before extraction.

Isolation of xanthenes from the stem bark of *G. cowa*

The ground air-dried stem bark (2.2 kg) was percolated three times with methanol. The combined extracts were evaporated under reduced pressure to give a dark mass (113.54 g). The extract was successively partitioned between water and hexane followed by ethyl acetate and finally by butanol to give 5.7 g, 43.0 g and 45.0 g, respectively, of gums. All fractions were subjected to cytotoxic activity assay towards H460 cell line and based on the results, further work was continued on the ethyl acetate soluble fractions since it was found to be the active fraction.

A portion of ethyl acetate fraction (40 g) was subjected to CC on silica gel and successively eluted with hexane followed by hexane-EtOAc mixture, and finally washed with methanol to give five combined fractions (A-E). Each fraction was then tested for cytotoxic activity against H-460 cells. Except for fraction A, all fractions showed good activity with IC₅₀ value range of 6.3-26.9 µg/mL. Fraction B was subjected to repetitive RC (on 4 mm and 2 mm plates) to give 6-hydroxy-calabaxanthone (**2**) (11 mg) as yellow needles, after recrystallization from EtOAc-hexane (1:2). Fraction C was subjected to flash vacuum chromatography followed by repetitive CC on silica gel and purification on RC gave two semi-purified fractions, which were crystallized with EtOAc-hexane as 2-(3-methyl-2-butenyl)-1,5,6-trihydroxy-3-methoxy-4-(1,1-dimethyl-2-propenyl)-9H-xanthen-9-one (**3**) (5 mg), rubraxanthone (**4**) (93 mg) and α-mangostin (**5**) (47 mg). Fraction D (3.2 g) was subjected to silica gel CC using hexane as eluent followed by hexane-EtOAc mixture, EtOAc and finally with methanol to obtain four combined fractions (D_A-D_D). Further repetitive CC on sub-fraction D_D (1.3 g) and RC afforded 1,3,6-trihydroxy-7-methoxy-4-(4-acetoxy-3-methyl-2-butenyl)-8-(3,7-dimethyl-2,6-octadienyl)xanthone (**6**) (5.2 mg) and cowanin (**7**) (30 mg) as yellowish needles.

Cytotoxic assay:

The ability of the extracts and pure compounds to induce growth inhibition on several cell lines was determined using MTT assay [10]. Cells were attached by incubate suspension cells (180 l in each well except blank) for over night. Varying concentrations of the fractions and pure compounds were prepared from the stock solutions by serial dilution (100 µM, 10 µM, 1 µM, 0.1 µM) in RPMI-1640 to give a volume of 200 l in each well of microtiter plate (96-well). The assay for each concentration of extract was performed in quadruplicate and the culture plate incubated at 37 °C with 5 % (v/v) CO₂ for 96 hours. After 96 hours of incubation, 50 µl of 2 mg/mL MTT solution was added to each well and allowed to incubate for a further 4 hours. After 4 hours incubation, discard all supernatant. Subsequently, 100 l DMSO was added to each well and vigorously mixed to dissolve the formazan crystals. Absorbance values at 550 nm was measured with a microplate reader. Cytotoxicity was expressed as IC₅₀.

6-hydroxy-calabaxanthone (**2**) was isolated as yellowish needles, m.p. 152-154°C, UV MeOH λ_{max} (log ε) nm: 203 (4.00), 290 (4.12), 329 (3.84); IR ν_{max} (KBr) cm⁻¹: 3413, 2923, 1600, 1122; EIMS *m/z* (rel. int): 408,4 (M⁺, 24), 393 (100), 375 (23), 365 (57), 337 (9), 323 (14). ¹H NMR (500 MHz, d₆-Acetone): see Table 1. ¹³C NMR (125 MHz, d₆-Acetone): see Table 1

2-(3-methyl-2-butenyl)-1,5,6-trihydroxy-3-methoxy-4-(1,1-dimethyl-2-propenyl)-9H-xanthen-9-one (**3**) was isolated as yellow crystals; m.p. 115-117; requires 411.1788). UV EtOH λ_{max} (log ε) nm: 201 (4.50), 259 (4.46), 328 (4.02); IR ν_{max} (KBr) cm⁻¹: 3420, 2960, 2930, 1630, 1606, 1570, 1532, 1467, 1432, 1417, 1354, 1313, 1282, 1194, 1179, 1122, 1100, 1015, 985, 892, 794, 778. High-resolution FABMS gave [M+H]⁺ at 411.1808 (C₂₄H₂₇O₆ requires 411.1788). EIMS: *m/z* (rel. int): 411(M⁺, 14), 395(12), 368(23), 339(48), 355(74), 355(74), 367 (100).

¹H NMR (500 MHz, CdCl₃): 1.67(6H, s, 1''-Me₂), 1.72 (3H, d, J= 1.2 Hz, 3'-Me), 1.80 (3H, s, 4 - Me), 3.41 (2H, brd, J= 6.4 Hz, H-1'), 3.78 (3H, s, 6-OMe), 5.06 (1H, dd, J= 10.6 Hz & 1.4 Hz, H-3''), 5.26 (1H, dd, J= 17.7 & 1.4 Hz, H-3''), 5.30 (1H, m, H-2'), 6.01 (1H, brs, 6-OH), 6.15 (1H, s, 5-OH), 6.64, 1H, dd, J= 17.7 Hz & 10.6 Hz, H-2'', 6.96 (1H, d, J= 8.8 Hz, H-7), 7.73 (1H, d, J= 8.8 Hz, H-8), 13.34 (1H, s, H-1). ¹³C NMR (125 MHz, CdCl₃): 181.3, (C-9), 164.4 (C-3), 160.0 (C-1), 156.8 (C-2), 152.73 (C-4a), 149.2 (C-6), 144.8 (C-5a), 132.2 (C-3), 130.9 (C-5), 122.5 (C-2), 118.7 (C-4), 118.4 (C-2), 117.8 (C-8), 113.8 (C-8a), 112.9 (C-7), 105.6 (C-9a), 104.3 (C-3), 62.7 (3-OMe), 42.00 (C-1), 28.8 (Me₂-1), 25.7 (3'-Me), 22.9 (C-1), 18.0 (C-4).

Rubraxanthone (**4**) was isolated as yellow needles, m.p. 207-209. UV MeOH λ_{max} (log ε) nm: 203 (4.54), 241 (4.50), 312 (4.32), IR ν_{max} (KBr) cm⁻¹: 3425, 3240, 2965, 2855, 1641, 1162, 827; EIMS: *m/z* 410 [M]⁺ (23%), 341(100), 299(25), 69(33). High-resolution EIMS found: 410.1727 (calculated for C₂₄H₂₆O₆, *m/z* 410.1729).

¹H NMR (500 MHz, *d*₆-Acetone): 6.72 (1H, s, H-5), 6.21 (1H, *d*, *J* = 2.0 Hz, H-4), 6.18 (1H, *d*, *J* = 2.0 Hz, H-2), 5.20 (1H, *t*, *J* = 7.0 Hz, H-6'), 5.01 (1H, *t*, *J* = 7.0 Hz, H-2'), 4.07 (2H, *d*, *J* = 7.0 Hz, H-1'), 3.4 (3H, s, 7-OMe), 2.05 (2H, *t*, *J* = 7.0 Hz, H-5'), 1.98 (2H, *t*, *J* = 7.0 Hz, H-4'), 1.81 (3H, s, H-8'), 1.55 (3H, s, H-10'), 1.52 (3H, s, H-9').

¹³C NMR (125 MHz, *d*₆-Acetone): 182.00 (C-9), 164.8 (C-1), 163.6 (C-3), 157.3 (C-6), 156.9 (C-5a), 155.7 (C-4a), 143.8 (C-7), 137.5 (C-8), 134.3 (C-3'), 130.8 (C-7'), 124.3 (C-2'), 124.0 (C-6'), 111.0 (C-8a), 102.8 (C-9a), 101.7 (C-5), 97.6 (C-2), 92.9 (C-4), 60.2 (7-OMe), 39.6 (C-4'), 26.4 (C-5'), 25.8 (C-1'), 24.6 (C-9'), 16.5 (C-10'), 15.5 (C-8').

α -Mangostin (**5**) was isolated as yellowish needles, m.p. 181-183; UV (MeOH) λ_{\max} (log ϵ) nm: 203 (4.42), 244 (4.33), 317 (4.15); IR ν_{\max} (KBr) cm⁻¹: 3401, 2923, 1607, 1280. EIMS *m/z* (rel. int): 408.4 (M^+ , 24), 410 (100), 375 (23), 365 (57), 337 (9), 323 (14).

¹H-NMR (500 MHz, *d*₆-acetone): δ 13.81 (1H, s, 2'-OH), 6.84 (1H, s, H-5), 6.41 (1H, s, H-4), 5.31 (2H, *t*, *J* = 7.5 Hz, H-2', H-2''), 4.15 (2H, *d*, *J* = 7.5, H-1'), 3.82 (3H, s, 7-OMe), 3.38 (2H, *d*, *J* = 7.5, H-1'), 1.85 (3H, s, H-4'), 1.81 (3H, s, H-4'), 1.68 (3H, s, H-5'') and 1.67 (3H, s, H-5').

¹³C-NMR (125 MHz, *d*₆-acetone): δ 182.2 (C-9), 162.3 (C-1), 161.1 (C-3), 156.7 (C-6), 155.6 (C-5a), 155.1 (C-4a), 143.8 (C-7), 137.5 (C-8), 130.8 (C-3', C-3''), 124.1 (C-2'), 122.8 (C-2'), 111.4 (C-8a), 110.4 (C-9a), 103.0 (C-2), 102.1 (C-5), 92.5 (C-4), 60.7 (7-Me), 26.3 (C-1'), 25.3 (C-5'), 25.2 (C-5''), 21.3 (C-1'), 17.7 (C-4'), 17.3 (C-4').

1,3,6-trihydroxy-7-methoxy-4-(4-acetoxy-3-methyl-2-butenyl)-8-(3,7-dimethyl-2,6-octadienyl)xanthone (**6**) was isolated as yellowish powder (5.2 mg); IR ν_{\max} (KBr) cm⁻¹: 3369, 1711, 1643; EIMS *m/z* (rel. int): 536 (M^+ , 4), 466 (38), 406.7 (100), 388 (27); ¹H NMR (500 MHz, *d*₆-Acetone): see Table 2. ¹³C NMR (125 MHz, *d*₆-Acetone): see Table 2

Cowanin (**7**) was isolated as yellowish needles, m.p. 137-139; UV λ_{\max} EtOH (log ϵ) nm: 243 (4.52), 315 (4.37); IR ν_{\max} (KBr) cm⁻¹: 3391, 2921, 1648, 1460, 1429, 1070, 1027; EIMS *m/z* (rel. int.): 410 (M^+ , 18), 409 (50), 353 (100), 335 (35); ¹H NMR (500 MHz, *d*₆-Acetone): see Table 3. ¹³C NMR (125 MHz, *d*₆-Acetone): see Table 3

RESULTS AND DISCUSSION

Five known and one new xanthones were isolated from the stem bark of *G. cowa*. Those are 6-hydroxy-calabaxanthone (**2**), 2-(3-methyl-2-butenyl)-1,5,6-trihydroxy-3-methoxy-4-(1,1-dimethyl-2-propenyl)-9H-xanthene-9-one (**3**), rubraxanthone (**4**), α -mangostin (**5**), a new 1,3,6-trihydroxy-7-methoxy-4-(4-acetoxy-3-methyl-2-butenyl)-8-(3,7-dimethyl-2,6-octadienyl)xanthone (**6**) and cowanin (**7**) were isolated from the stems of *G. cowa*. Their structures were elucidated with extensive by spectroscopic data.

6-Hydroxy-calabaxanthone (**2**), isolated as yellow amorphous powder, which gave molecular ion on the basis of its EIMS spectra at *m/z* 408.4 corresponding to molecular formula C₂₄H₂₄O₆. The evidence of xanthone chromophore was found from its UV absorption at λ_{\max} 203 nm (4.00), 290 nm (4.12) and 329 (3.84). Its IR spectrum showed the presence of hydroxyl and carbonyl groups at 3413 cm⁻¹, 1600 cm⁻¹, respectively [11]. The EI-MS spectrum showed significant fragment ion peaks at *m/z* 392, 374 and 336 arising from the loss of CH₃, OCH₃ and C₅H₉ from the M^+ ion, respectively. The ¹H NMR spectrum, the signals at δ 5.74 (1H, *d*, *J* = 10.0 Hz) and 1.48 (6H, s) together with a dimethyl proton signal at 1.48 (6H, s, H-4') indicated the present of a 2,2-dimethyl on the pyran ring system [12]. The ¹H NMR spectrum also contained signals of the hydrogen bonded hydroxyl group at δ 13.98 (1H, s), two aromatic proton signals at δ 6.27 (1H, s) and δ 6.88 (1H, s). The present of 3-methyl but-2-enyl group was noted due to the appearance of proton signals at δ 1.68 (3H, s), δ 1.85 (3H, s), an olefinic proton signals at δ 5.29 (1H, *t*, *J* = 6.5 Hz), a doublet of methylene proton at δ 4.14 (2H, *d*, *J* = 6.5).

The ¹³C NMR spectrum showed 23 carbon signals containing six oxygen-bearing aromatic carbon signals at δ 160.1 (C-3), δ 158.2 (C-1), δ 157.7 (C-5a), δ 156.5 (C-4a), δ 155.7 (C-6) and 144.2 (C-7) in low field region (δ 130 - δ 200). One carbonyl carbon and one allylic carbon signals also present at δ 182.3 (C-9) and δ 124.0 (C-2''), respectively. In the middle field region (δ 90 - δ 130), there are five aromatic carbon signals (non-oxygenated) present at 110.9 (C-8a), 104.4 (C-2), 103.5 (C-9a), 102.2 (C-5) and 94.0 (C-4). Two sp² carbon signals from chromene ring present at 127.8 (C-2') and 115.4 (C-1'). In high field region there is one oxygenated quaternary carbon, one dimethyl carbon and two methyl carbon signals present at δ 78.2 (C-3'), δ 27.8 (C-4'), δ 25.3 (C-5'') and δ 17.7 (C-4'), respectively. The HMBC spectrum showed that the chelated OH proton (δ 13.98) correlated to δ C-2 (δ 104.4) and C-4a (δ 156.5). Thus, the OH was assigned at C-1.

The connectivity of the chromene ring at C-2 was supported by the correlation between proton at C-1' (6.70) with C-1 (158.2), C-2 (104.4) and C-3 (160.1). The HMBC spectrum further exhibited the correlation between the vinylic proton at 5.74 (H-2') with the carbon at C-3' (78.2) as well as the aromatic carbon at C-2 (104.4) that revealing the connectivity between the chromene ring to main xanthone skeleton. An aromatic proton at δ 6.88 (H-5) showed long-range correlations with carbon signals at C-5a (157.7), C-6 (155.7) and C-7 (144.2) suggested the position of this proton at C-5. Another singlet proton at 6.27 was also assigned as H-4, based on its correlation with the carbon signals at C-2 (104.4), C-3 (160.1) and C-4a (156.5). The position of the methoxy was observed at C-7, based on its 2J correlation of its proton signal at 1.48 (H-4') with C-7 (144.2).

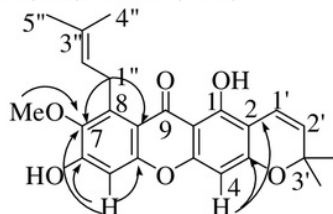


Fig. 2. The HMBC correlation for H-4, H-5 and H-1' of (2)

Furthermore, the location of prenyl unit was assigned at C-8 in the HMBC spectrum. This data was supported by long-range correlation between the methylene protons of prenyl unit at δ 4.14 (H-1''), with carbon signals at C-8 (δ 137.4), C-8a (δ 110.9) and C-7 (δ 144.2). Table 4.13 summarized all the spectrum of ^1H NMR, ^{13}C NMR, HSQC and HMBC. Based on the argument above, the structure of (2) was identified as 6-hydroxy-calabaxanthone [13].

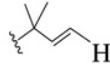
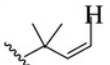
Table 1. ^1H -NMR, ^{13}C -NMR, HSQC and HMBC data of compound (2)

Position	δ_{C}	δ_{H}	HMBC
1	158.2		
2	104.4		
3	160.1		
4	94	6.27/ 1H/ s	C-1, C-2, C-3, C-2'
4a	156.5		
5	102.2	6.88/ 1H/ s	C-5a, C-6, C-7, C-8, C-8a
5a	157.7		
6	155.7		
7	144.2		
8	137.4		
8a	110.9		
9	182.3		
9a	103.5		
2'	78.15		
3'	127.8	5.74/ 1H/ d/ J=10.0	C-4', C-1, C-2, C-2'
4'	115.4	6.70/ 1H/ d/ J=10.0	C-2', C-3, C-2, C-1
1''	26.2	4.14/ 2H/ d/ J=6.5	C-8a, C-7, C-3''
2''	124	5.29/ 1H/ t/ J=6.5	C-4'', C-5''
3''	130.9		
4''	17.7	1.85/ 3H/ s	C-5'', C-2''
5''	25.3	1.68/ 3H/ s	C-4'', C-3'', C-2''
2'-CH ₃	27.8	1.48/ 6H/ s	C-2', C-3', C-4', C-3
7-OMe	60.6	3.85/ 3H/ s	C-7

2-(3-methyl-2-butenyl)-1,5,6-trihydroxy-3-methoxy-4-(1,1-dimethyl-2-propenyl)-9H-xanthen-9-one (3) was isolated from EtOAc fraction of stem bark of *G. cowa*, as a yellowish crystalline compound, m.p. 115-116 °C. The HRMS indicated a molecular formula of $\text{C}_{24}\text{H}_{26}\text{O}_6$. The UV spectrum showed three absorption bands, λ_{max} (log ϵ) at 201 nm (4.50), 259 nm (4.46) and 328 (4.02) which is typical for xanthone chromophore [14]. Its IR spectrum showed a characteristic hydroxyl absorption band at 3000 cm^{-1} , CH stretching at 2980 cm^{-1} and carbonyl stretching peak at 1620 cm^{-1} .

In the ^1H NMR spectrum, an *ortho* coupling was observed between the signal at δ 7.05 and δ 7.67, corresponding to two aromatic protons at H-7 and H-8, respectively. Furthermore, J correlation between the signal at δ 7.67 (H-8) and the carbonyl signal at 180.0 was observed in the HMBC spectrum. The assignment of the *ortho* coupled protons at C-7 and C-8 were accomplished by 2J and 3J correlation, which showed that H-7 correlated with δ 133.0 (C-5), δ 152.0 (C-6) and δ 116.6 (C-8) whereas H-8 was correlated to δ 152.0 (C-6), δ 147.0 (C-8a) and 182.0 (C-9). The chelated OH located at C-1 showed correlations with the carbon signals at δ 117.0 (C-2), δ 160.0 (C-1) and δ 105.7

(C-9a). The position of 3-methylbut-2-enyl group [2 methyl signals at δ 1.70 and δ 1.82; an olefinic proton signals at δ 5.32 (1H, *t*, J = 6.5 Hz), a doublet of methylene proton at δ 3.42 (2H, *d*, J = 6.5) at C-2 was confirmed by a HMBC correlation of proton signal at δ 3.42 (H-1') with the carbon signal at 160.0 (C-1) and 164.4 (C-3).

The presence of 1,1-dimethylallyl group [δ 6.64 (1H, *dd*, J =17.5, 10 Hz, , δ 4.84 (1H, *dd*, J = 10.0, 2.0 Hz, , (δ 5.06 (1H, *dd*, J = 17.5, 2.0 Hz, and two overlapping methyl peaks at δ 1.79] at position C-4 was confirmed by HMBC correlation between the methyl signals at δ 1.79 (1''-CH₃) and the carbon signal at 119.0 (C-4).

The position of 1,1-dimethylallyl group was further supported by the correlation of dimethyl proton 1''-CH₃ (δ 1.79) with the signal of C-4a (δ 151.0). The presence of OH group at C-5 and C-6 were confirmed by a 3J HMBC correlation of proton signal at δ 7.05 (H-7) with the carbon signal at δ 133.0 (C-5) and proton signal at δ 7.67 (H-8) with the carbon signal δ 152.0 (C-6), respectively. Therefore, based on the argument above, the structure of (3) was concluded to be 2-(3-methyl-2-butenyl)-1,5,6-trihydroxy-3-methoxy-4-(1,1-dimethyl-2-propenyl)-9H-xanthen-9-one [9].

Rubraxanthone (4) was obtained as yellowish needles with melting point 201-203°C. The EI-MS spectrum showed that the molecular ion (M^+) was at m/z 410. The UV spectrum displayed the absorptions at λ_{\max} (log ϵ): 203 (4.54), 241 (4.50) and 312 (4.32) nm due to xanthone nucleus. The IR spectrum showed carbonyl absorption band at 1641 cm^{-1} and OH stretching absorption band at 3425 cm^{-1} , which was consistent with xanthone skeleton. The assignment of the methoxy group at C-7, *ortho* to the geranyl side-chain at C-8, was supported based on this data [15]. Based on HMBC correlation between aromatic proton at δ 6.11 with the carbon signals at δ 164.8 (C-1), 163.6 (C-3) and δ 102.8 (C-9a), the doublet protons at δ 6.11 and δ 6.21 were assigned as *meta* coupled aromatic proton (J =2.0 Hz) of H-2 and H-4, respectively. The H-4 showed correlations with carbon signals at δ 164.8 (C-1), δ 97.6 (C-2) and δ 155.7 (C-4a). Furthermore, correlations between aromatic proton at δ 6.72 with carbon signals at δ 181.9 (C-9), δ 156.9 (C-5a), δ 157.3 (C-6) and δ 111.0 (C-8a) were supported that this proton was assigned as H-5. Thus, this compound was characterized as rubraxanthone. This compound was previously isolated from stem barks of *G. pyrifera* [14].

-Mangostin (5) was isolated as yellowish needles, which melted at 181-183°C. The EI-MS spectrum showed the molecular ion peak at m/z 410 corresponding to the molecular formula C₂₄H₂₆O₆. The UV spectrum showed three absorption bands, λ_{\max} (log ϵ) at 203 (4.42), 244 (4.33) and 317 (4.15) nm, which were typical for xanthone chromophore. The IR spectrum showed carbonyl absorption band at 1628.27 cm^{-1} and OH stretching absorption band at 3435.61 cm^{-1} , which was consistent with xanthone skeleton. Based on this observation, the structure was determined as α -mangostin. The ^1H NMR and ^{13}C NMR spectrum were also compared with literature value and matched with the structure of α -mangostin [15].

1,3,6-trihydroxy-7-methoxy-4-(4-acetoxy-3-methyl-2-butenyl)-8-(3,7-dimethyl-2,6-octadienyl)xanthone (6) was isolated as yellow amorphous powder. The IR spectrum revealed the presence of hydroxyl group by absorption at 3300 cm^{-1} and conjugated ketone by absorption at 1652 cm^{-1} . The EI mass spectra of this compound showed a molecular ion peak at m/z 536, consistent with a molecular formula C₃₁H₃₆O₈.

The ^1H NMR spectrum showed a singlet signal at δ 13.47 of a chelated hydroxyl group 1-OH, two singlet signals of two aromatic proton signals at δ 6.32 (1H, *s*, H-2) and δ 6.93 (1H, *s*, H-5) and a singlet resonance of methoxyl protons at δ 3.83 (3H, *s*). Two side chains were detected: a geranyl side chain and a prenyl unit with an acetoxy group. The signals of the geranyl unit are represented by two olefinic protons δ 5.28 (H-2'') and δ 5.05 (H-6''), three sets of methylene groups at δ 4.22 (H-1''), δ 2.06 (H-4''), δ 2.03 (H-5'') and three vinylic methyl groups at δ 1.85 (H-9''), δ 1.57 (H-8'') and δ 1.62 (H-10''). Other signals were assigned to a 4-acetoxy-3-methyl-2-butenyl group, based on the signals δ 5.46 (H-2'), a doublet at δ 3.64 (H-1'), a singlet of two proton at δ 4.86 (H-4'), two sets a singlet of three proton at δ 2.17 (4'-OCOCH₃) and δ 1.78 (H-5'). The ^{13}C NMR spectrum of (6) showed 31 signals, including two carbonyl signals at δ 182.4 and 172.2.

The HMBC correlation of acetyl methyl protons and oxymethylene protons H-4' to the acetyl carbonyl group confirmed the position of the acetoxy group. The correlation of benzylic methylene protons H-1' (δ 3.64) to C-3 (δ 161.4), C-4a (δ 154.1) and C-9a (δ 104.1) confirmed the position of the 4-acetoxy-3-methyl-2-butenyl side chain to be C-4 and by comparison with the spectral data of cowagarcinone E [16]. The correlation of benzylic methylene protons H-1'' (δ 4.12) to C-7 (δ 143.0), C-8 (δ 137.5) and C-8a (δ 112.2) suggested that the geranyl side chain was

located at C-8. The position of aromatic protons at C-2 and C-5 were confirmed based on the correlation of proton signals at δ 6.32 with the carbon signals at δ 161.9 (C-1) and δ 104.1 (C-9a) whereas proton signal at δ 6.93 with the carbon signals at δ 155.9 (C-5a), δ 154.9 (C-6), (C-7) and 112.2 (C8a). The assignment of the protons and carbons of (6) are summarized in Table 2.

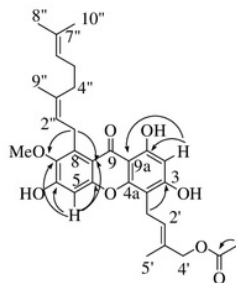


Fig.3. Selected HMBC of compound (6)

The ^1H NMR and ^{13}C NMR data were similar to covigarcinone E except in the position of 4-acetoxy-3-methyl-2-butenyl. Hence, compound (6) was established as a new xanthone 1,3,6-trihydroxy-7-methoxy-4-(4-acetoxy-3-methyl-2-butenyl)-8-(3,7-dimethyl-2,6-octadienyl)xanthone.

Table 2. The ^1H -NMR, ^{13}C -NMR, HSQC and HMBC data of 1,3,6-trihydroxy-7-methoxy-4-(4-acetoxy-3-methyl-2-butenyl)-8-(3,7-dimethyl-2,6-octadienyl)xanthone (6)

Position	δC	δH (HSQC)	HMBC
1	161.9		
2	98.8	6.32/ 1H/s	C-1, C-9a
3	161.4		
4	103.8		
4a	154.1		
5	101.9	6.93/ 1H/ s	C-5a, C-6, C-7, C-8a,
5a	155.9		
6	154.9		
7	143		
8	137.5		
8a	112.2		
9	182.4		
9a	104.1		
1'	21.4	3.64/ 2H/ dl J= 7.0	C-3, C-4, C-4a, C-2', C-3'
2'	128.5	5.46/ 1H/ tl J= 7.0	C-4, C-1', C-4'
3'	130.7		
4'	64.1	4.86/ 2H/ s	C=O, C-5', C-1', C-2', C-3'
5'	21.3	1.78/3H/s	C-4'
1''	26.7	4.12/2H/dl J= 7.0	C-7, C-8, C-8a, C-2'', C-3''
2''	123.4	5.28/ 1H/ tl J=7.0	C-9'', C-8, C-4'', C-5''
3''	135.9		
4''	26.8	2.06/ 2H/ bt/ J= 7.0	C-5'', C-6'', C-7''
5''	39.9	2.03/ 2H/ bt/ J= 7.0	C-2'', C-3'', C-9'', C-6'', C-4''
6''	124.5	5.05/ 1H/ bt/ J=7.0	C-8'', C-7''
7''	131.6		
8''	17.9	1.57/ 3H/ s	C-6'', C-7'', C-10''
9''	16.8	1.85/ 3H/ s	C-2'', C-3'', C-5''
10''	25.9	1.62/ 3H/ s	C-8'', C-7'', C-6'', C-5''
1-OH		13.47/ bs	C-2, C-9a
7-OCH ₃	62.3	3.83/ 3H/ s	C-7
4'-C=O	172.6		
4'-OCOCH ₃	21.5	2.17/ 3H/ s	4'C=O

Proposed of Biosynthetic Pathway of 1,3,6-trihydroxy-7-methoxy-4-(4-acetoxy-3-methyl-2-butenyl)-8-(3,7-dimethyl-2,6-octadienyl)xanthone (6) as below.

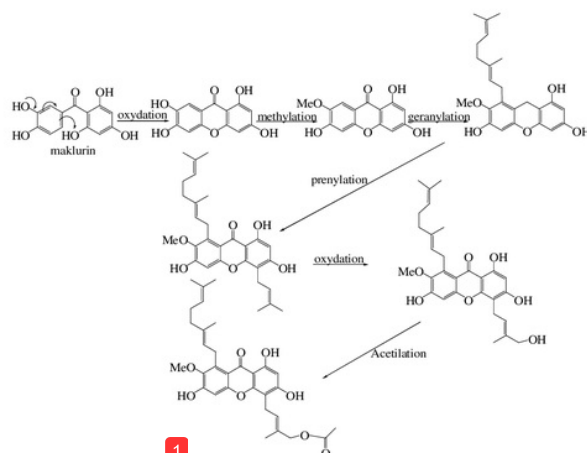


Fig.4. Proposed of Biosynthetic Pathway of 1,3,6-trihydroxy-7-methoxy-4-(4-acetoxy-3-methyl-2-butenyl)-8-(3,7-dimethyl-2,6-octadienyl)xanthone (6)

Cowanin (7) was isolated as a yellowish needles, m.p. 121-124 °C. It exhibited UV absorption bands at 243.60 nm (4.52) 315.80 nm (4.37), while hydroxyl and conjugated carbonyl absorption bands were observed at 3391 cm^{-1} and 1648 cm^{-1} , respectively, in the IR spectrum. The EI mass spectra of this compound showed a molecular ion peak at m/z 478, consistent with a molecular formula $\text{C}_{29}\text{H}_{34}\text{O}_6$.

The ^1H NMR spectrum displayed signals of one chelated hydroxyl group at δ 13.81 (s, 1-OH), two aromatic protons at δ 6.86 (1H, s) and δ 6.43 (1H, s) and one methoxy group at δ 3.83 (3H, s). The presence of the signal at δ 3.38 (2H, d, $J=7.0$), δ 5.31 (1H, m), δ 1.68 (3H, s) and 1.81 (3H, s) were suggestive of a prenyl moiety. The remaining signals appeared as the typical signals of a geranyl unit. These signals were a doublet of methylene protons H-1'' at δ 4.17, two broad triplets of the methine protons H-2'' and H-6'' at δ 5.27, two multiplets of the methylene protons H-4'' and H-5'' at δ 2.02 and δ 2.05, respectively, and three singlets of methyl groups H-8'', H-9'' and H-10'' at 1.56, 1.83 and 1.61, respectively. The ^{13}C NMR spectrum of (7) showed 29 signals, including, one carbonyl signal at δ 182.2.

The assignments of the substituents were deduced from the HSQC and HMBC spectra (Table 3). The geranyl unit was located at C-8 according to the correlation of benzylic methylene proton H-1'' to C-7, C-8 and C-8a and the deshielding effect of carbonyl group on H-1''. The methylene protons H-1' correlated to C-1 and C-2 whereas the methylene protons H-1'' correlated to C-7, C-8 and C-8a, consequently, a prenyl unit was located at C-2. Aromatic proton at δ 6.84 showed two bond coupling correlation with C-6 and C-5a, and three bond coupling with C-7 and C-8a. It's confirmed the position of this proton at C-5. Another aromatic proton at δ 6.30 was attached in C-4 based on the two bond coupling correlation with C-4a and three bond coupling to C-2 and C-9a. The position of methoxyl group a C-7 was confirmed based on correlation between proton signals at δ 3.83 with carbon at δ 144.2 (C-7).

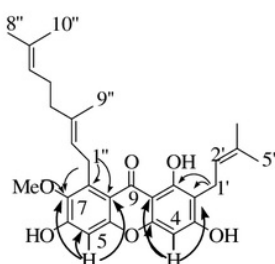


Fig.5. Selected HMBC of (7)

The mass spectral fragmentations supported the assigned structure. Typical fragmentations of the geranyl and prenyl substituents were being observed. This compound gave strong fragment ions at m/z = 409 $[\text{M}-69]^+$ and 353 $[\text{M}-69-$

56]⁺. A weak ion at $m/z = 367$ [M-111]⁺ could correspond to fragmentation involving the geranyl group and the *o*-methoxy group [16]. Thus, (7) was characterized as cowanin [17].

Table 3. The ¹H-NMR, ¹³C-NMR, HSQC and HMBC data of cowanin (7)

Position	δ _C	δ _H	HMBC
1	161.8		
2	108.7		
3	160.9		
4	93.5	6.30 / 1H / s	C-2, C-4a, C-9a
4a	154.8		
5	101.8	6.84 / 1H / s	C-6, C-7, C-8a
5a	155.3		
6	156		
7	142.8		
8	137.6		
8a	112.4		
9	182.2		
9a	103.8		
1'	21.7	3.46 / 2H / d / J= 7.0	C-2, C-1, C-2', C-3'
2'	121.7	5.27 / 1H / t / J= 7.0	C-4', C-5'
3'	135.9		
4'	26.08	1.78 / 3H / s	C-2', C-3', C-5'
5'	18.15	1.85 / 3H / s	C-2', C-3', C-4'
1''	26.7	4.10 / 2H / t / d / J=7.0	C-7, C-8, C-8a, C-3'', C-2''
2''	123.5	5.31 / 1H / t / J=7.0	C-9'', C-5''
3''	135.8		
4''	26.8	2.10 / 2H / bt / J= 7.0	C-3'', C-9'', C-2'', C-5'', C-6'', C-7''
5''	39.9	2.01 / 2H / bt / J=7.0	C-4'', C-5'', C-3'', C-2'', C-9''
6''	124.5	5.07 / 1H / bt / J=7.0	
7''	131.5		
8''	17.9	1.56 / s / 3H	C-10', C-7'', C-6''
9''	16.7	1.83 / s / 3H	C-5'', C-3'', C-2''
10''	25.9	1.61 / s / 3H	C-8'', C-7'', C-6''
7-CH ₃	62.3	3.81 / s / 3H	C-7

Cytotoxic activity of isolated compounds

The IC₅₀ values of less 10 μM, is considered as potent, while the IC₅₀ values of between 10 to 30 μM is considered as having good activity and IC₅₀ values of between 30 μM-100 μM is considered weak activity. All isolated compounds, had been evaluated for their cytotoxic activity against MCF-7, H-460 and DU-145 cell lines. The compounds (5) and (7) showed potent activities towards MF-7. Compound (5) also had potent activity against H-460, while compound (2) potent against DU-145 (Table 4).

Table 4. Cytotoxic activity of isolated compounds towards cancer cell lines

compounds	IC ₅₀ (μM)		
	MCF-7	H-460	DU-145
(2)	21.2 ± 8.4	11.4 ± 4.0	6.4 ± 2.5
(3)	11.5 ± 3.7	11.3 ± 2.9	10.9 ± 6.3
(4)	37.4 ± 36.3	17.5 ± 2.4	42.3 ± 13.7
(5)	4.1 ± 1.0	5.4 ± 3.3	11.3 ± 10.0
(6)	57.3 ± 36.3	28.8 ± 26.2	nd
(7)	5.3 ± 2.1	12.3 ± 3.4	nd

MCF-7 = Breast cancer cells

H-460 = Lung cancer cell cells

DU-145 = Prostate cancer cells

nd = not determined since limited amount of compound

CONCLUSION

The potential value of the stem bark of *G. cowa* from the West Sumatra region of Indonesia as a source of cytotoxic compounds has been reported for the first time here. Five known and one new xanthenes were isolated from the stem bark of *G. Cowa*. Those are 6-hydroxy-calabaxanthone (2), 2-(3-methyl-2-butenyl)-1,5,6-trihydroxy-3-methoxy-4-(1,1-dimethyl-2-propenyl)-9H-xanthen-9-one (3), rubraxanthone (4), α-mangostin (5), a new 1,3,6-trihydroxy-7-methoxy-4-(4-acetoxy-3-methyl-2-butenyl)-8-(3,7-dimethyl-2,6-octadienyl)xanthone (6) and cowanin (7) were isolated from the stem bark of *G. cowa*. Structure elucidations of these compounds were carried out by spectroscopic methods. All isolated compounds were active against MCF-7, H-460 and DU-145 cancer cell lines. α-mangostin (5) and cowanin (7) were found to be potent against MCF-7 while 6-hydroxy-calabaxanthone (2) potent against DU-145 cell lines.

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