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Tetraoxygenated xanthones from the fruits of *Garcinia cowa*

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Abstract

Tetraoxygenated xanthones, cowaxanthones A–E, together with 10 previously reported tetraoxygenated xanthones, were isolated from the crude hexane extract of the fruits of *Garcinia cowa*. Cowaxanthone B has previously been reported as a synthetic xanthone. Their structures were elucidated by analysis of spectroscopic data, especially by 1D and 2D NMR. The antibacterial activities of the isolated compounds were also evaluated.

Keywords: *Garcinia cowa*; Guttiferae; Tetraoxygenated xanthones; Antibacterial activity

1. Introduction

Garcinia cowa Roxb. (Guttiferae, Cluciaceae), commonly known as "Cha muang", is a small to medium sized tree found scattered in lowland, undulating areas and peat swamp forests. The fruits and leaves are used for the improvement of blood circulation, as an expectorant for the treatment of coughs and indigestion and as a laxative, while the root is used for fever relief (Poomipamorn and Kumkong, 1997). The bark of *G. cowa* has been used in Thai folk medicine as an antipyresis agent (Na Pattalung et al., 1994). Previous investigation of the latex of *G. cowa* revealed the presence of antibacterial prenylated xanthones (Na Pattalung et al., 1994). Mahabusarakam et al. reported isolation of cowagarcinone A–E, mangostine and fuscaxanthone A from the latex of *G. cowa* (Mahabusarakum et al., 2005). Positive antibacterial activity from a preliminary screening of the crude hexane extract from the fruits of this plant prompted us to further investigate its chemical constituents. We report herein five new tetraoxygenated xanthones (**1**–**5**) and 10 known compounds (**6**–**15**). Their structures were elucidated from analyses of 1D and 2D NMR spectroscopic data. Antibacterial activity of **2**, **3**, **8**, **9**, **10**, **11**, **13** and **14** was investigated.

2. Results and discussion

The hexane extract of the fresh fruits of *G. cowa* was subjected to chromatographic purification to yield five new tetraoxygenated xanthones (cowaxanthones A–E: **1**, **2**, **3**, **4** and **5**), together with 10 known tetraoxygenated xanthones: 1,6-dihydroxy-3,7-dimethoxy-2-(3-methyl-2-butenyl)xanthone (**6**) (Nilar and Harrison, 2002), fuscaxanthone C (**7**) (Ito et al., 2003), 7-*O*-methylgarcinone E (**8**) (Likhitwitayawuid et al., 1997), β-mangostin (**9**) (Likhitwitayawuid et al., 1998), cowanol (**10**) (Na Pattalung et al., 1994), mangostanin (**11**) (Sen et al., 1981), 6-*O*-methylmangostanin (**12**) (Sen et al., 1980), cowanin (**13**) (Na Pattalung et al., 1994), α-mangostin (**14**), (Sen et al., 1982 and Mahabusarakum and Wiriyachitra, 1987) and cowaxanthone (**15**) (Na Pattalung et al., 1994). All structures were elucidated using 1D and 2D NMR spectroscopic data. The ^{13}C NMR signals were assigned from DEPT,

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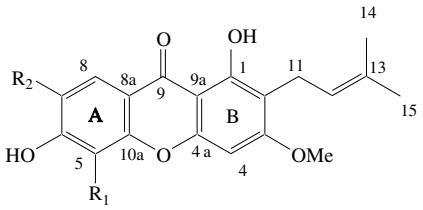
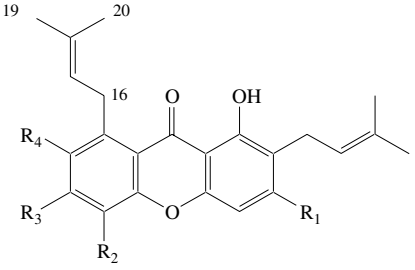
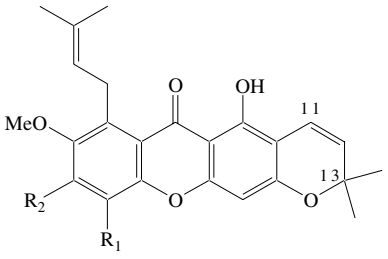
HMQC and HMBC spectra. The ^{1}H and/or ^{13}C spectroscopic data of known xanthones were also compared with those reported in the literatures.



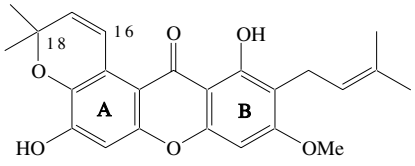
1:R_1 = OMe, R_2 = H
6:R_1 = H, R_2 = OMe



2: R_1 = OH, R_2 = H, R_3 = R_4 = OMe
7: R_1 = R_3 = R_4 = OMe, R_2 = H
8: R_1 = R_3 = OH, R_2 = prenyl, R_4 = OMe
9: R_1 = R_4 = OMe, R_2 = H, R_3 = OH
14: R_1 = R_3 = OH, R_2 = H, R_4 = OMe



3: R_1 = prenyl , R_2 = OH
11: R_1 = H, R_2 = OH
12: R_1 = H, R_2 = OMe



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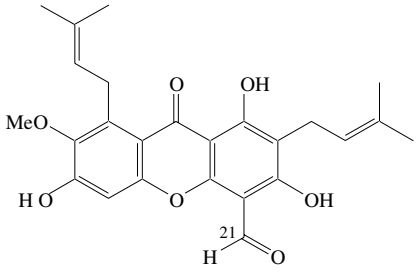

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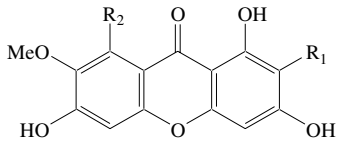

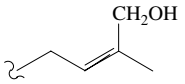

10: R_1 = , R_2 = geranyl
13: R_1 = prenyl, R_2 = geranyl
15: R_1= geranyl,R_2 = H

All new xanthones showed UV absorption bands of xanthone chromophores at λ_{max} 243–246 nm (strong), 258–268 nm (strong), 311–318 nm (medium) and 352–387 nm (weak) (Ito et al., 2003), while those with a chromene unit conjugated to the xanthone nucleus, i.e. **3** and **4**, exhibited bathochromic shifts of the same absorption bands. All compounds showed IR absorption bands at 3266–3412 and 1631–1649 cm^{-1} for hydroxyl and conjugated carbonyl groups, respectively. In the ^{1}H NMR spectra, a singlet proton at δ_H 12.98–14.52 revealed the presence of a hydroxyl group at C-1, chelated to a carbonyl group of the xanthone. The characteristic NMR signals of a prenyl group are given in Table 1. A signal of deshielded methylene protons of a prenyl side chain at δ_H 4.05–4.13 (except for **1** and **4**) due to the anisotropic effect of the carbonyl group (C-9), indicated the attachment of the prenyl moiety at C-8. These assignments were confirmed by HMBC correlations (Table 3). The 1-OH group showed cross-peaks with C-1, C-2 and C-9a, while the methylene protons (H-16) of the C-8 substituent showed cross-peaks with C-7, C-8 and C-8a.

Cowaxanthone A (**1**) was obtained as a yellow solid, m.p. 178.3–179.0 °C. The molecular formula was determined to be $C_{20}H_{20}O_6$ by HREIMS. Its ^{1}H NMR spectrum (Table 1) showed signals similar to those of **6**, but two singlet resonances of aromatic protons on ring A in **6** at δ_H 7.61 (1H, *s*) and 6.94 (1H, *s*) were replaced by two doublet signals of *ortho* aromatic protons [δ_H 7.93 (1H, *d*, $J = 8.7$ Hz) and 6.99 (1H, *d*, $J = 8.7$ Hz)]. The lowest-field aromatic proton (δ_H 7.93, H-8) showed cross-peaks with C-6 (δ_C 154.23), C-9 (δ_C 180.06) and C-10a (δ_C 149.57), suggesting its attachment at C-8, the deshielding zone of the carbonyl group.

Table 1
^{1}H NMR spectroscopic data for cowaxanthomes A–E (**1**–**5**)in $CDCl_3$ (300 MHz)

Position	δ_H (*mult*., J_{Hz})				
	1	**2**	**3**	**4**	**5**
1-OH	12.98 (*s*)	13.84 (*s*)	13.77 (*s*)	13.34 (*s*)	14.52 (*s*)
3-OMe	3.95 (*s*)			3.90 (*s*)	
3-OH		6.25 (*brs*)			13.00 (*s*)
4	6.48 (*s*)	6.29 (*s*)	6.28 (*s*)	6.36 (*s*)	
5		6.75 (*s*)		6.82 (*s*)	6.95 (*s*)
5-OMe	4.12 (*s*)				
6-OMe		3.96 (*s*)			
6-OH	6.58 (*brs*)		6.50 (*brs*)	6.20 (*s*)	10.37 (*s*)
7	6.99 (*d*, 8.7)				
7-OMe		3.80 (*s*)	3.80 (*s*)		3.83 (*s*)
8	7.93 (*d*, 8.7)				
11	3.37 (*d*, 6.9)	3.45 (*d*, 6.9)	6.73 (*d*, 9.9)	3.35 (*d*, 7.2)	3.35 (*d*, 7.2)
12	5.24 (*mt*, 6.9)	5.30 (*mt*, 7.2)	5.56 (*d*, 9.9)	5.23 (*m*)	5.23 (*m*)
14	1.82 (*s*)	1.85 (*s*)	1.47 (*s*)	1.80 (*s*)	1.81 (*s*)
15	1.70 (*s*)	1.77 (*s*)	1.47 (*s*)	1.68 (*s*)	1.69 (*s*)
16		4.13 (*d*, 6.3)	4.05 (*d*, 6.3)	8.03 (*d*, 10.2)	4.08 (*d*, 6.3)
17		5.25 (*mt*, 6.3)	5.25 (*m*)	5.82 (*d*, 10.2)	5.23 (*m*)
19		1.68 (*s*)	1.69 (*s*)	1.50 (*s*)	1.69 (*s*)
20		1.85 (*s*)	1.82 (*s*)	1.50 (*s*)	1.84 (*s*)
21			3.55 (*d*, 7.2)		10.37 (*s*)
22			5.25 (*m*)		
24			1.69 (*s*)		
25			1.87 (*s*)		

The other *ortho* aromatic proton (δ_H 6.99) was then attributed to H-7. Irradiation of H-8 in a NOED experiment enhanced the doublet signal of H-7, indicating that H-8 was an *ortho* aromatic proton to H-7. In addition, the correlations between H-7 and C-5 (δ_C 133.63), C-6 and C-8a (δ_C 115.14), supported the above assignment. The methoxy protons [δ_H 4.12], in the HMBC spectrum (Table 3), showed a cross-peak with C-5, indicating the attachment of the methoxyl group at C-5. Therefore, C-6 was substituted by a hydroxyl group. In addition, the substituents on ring B were located at the same positions as found in **6** by the HMBC data (Table 3). Hence, cowaxanthone A was identified as 1,6-dihydroxy-3,5-dimethoxy-2-(3-methyl-2-butenyl)xanthone (**1**). Consequently, **1** differed from **6** in the location of the methoxyl group on ring A.

Cowaxanthone B (**2**) was isolated as an orange gum. The molecular formula was determined as $C_{25}H_{28}O_6$ by HREIMS. The ^{1}H NMR spectrum (Table 1) was similar to that of **14** except for one additional methoxy singlet at δ_H 3.96. The presence of the methoxyl group was confirmed by the oxymethyl carbon signal at δ_C 56.04 in the ^{13}C NMR spectrum (Table 2). This methoxyl group was assigned to be at C-6 (δ_C 158.14) due to a HMBC correlation (Table 3) between the methoxy protons (6-OCH_3) and an oxyquaternary carbon (C-6). The enhancement of signal of H-5 after irradiation of the methoxy protons (δ_H 3.96), in the NOED spectrum, supported the above conclusion. Furthermore, the HMBC data (Table 3) established attachments of all remaining substituents identical to those of **14**. Cowaxanthone B was assigned as 1,3-dihydroxy-6,7-dimethoxy-2,8-bis(3-methyl-2-butenyl)xanthone (**2**), a new naturally occurring tetraoxygenated xanthone, which was previously obtained by synthesis (Lu et al., 1998).

Cowaxanthone C (**3**) was isolated as a yellow-red gum. Its molecular formula $C_{29}H_{32}O_6$ was deduced by HREMS. The ^{1}H NMR spectrum (Table 1) was almost identical to that of **11** except that an aromatic proton at δ_H 6.87 (H-5) of **11** was replaced, in **3**, by a prenyl group [δ_H 5.25 (2H, *m*), 3.55 (2H, *d*, $J = 7.2$ Hz), 1.87 (3H, *s*) and 1.69 (3H, *s*)]. The ^{13}C NMR spectrum (Table 2) was in agreement with the ^{1}H NMR spectrum. In the HMBC spectrum (Table 3), the methylene protons (δ_H 3.55) of the prenyl unit showed cross-peaks with C-5 (δ_C 114.11), C-6 (δ_C 152.40) and C-10a (δ_C 153.43), indicating the attachment of the prenyl group at C-5. In addition, the HMBC data (Table 3) established locations of the other substituents and the dimethylchromene ring identical to those found in **11**. Therefore, cowaxanthone C (**3**) is a new naturally occurring tetraoxygenated xanthone.

Cowaxanthone D (**4**) was obtained as a yellow solid, m.p. 210.0–210.7 °C. The molecular formula was determined as $C_{24}H_{24}O_6$ by HREIMS. Comparison of its ^{1}H NMR spectrum (Table 1) with that of **9** revealed similar spectroscopic data, except for the absence of one prenyl group and one methoxyl group. New signals were observed: two doublet signals [δ_H 8.03 (1H, *d*, $J = 10.2$ Hz), 5.82 (1H, *d*, $J = 10.2$ Hz)] and a singlet signal [δ_H 1.50 (6H, *s*)], which was characteristic signals of a dimethylchromene ring. The dimethylchromene ring contained a lower-field *cis* olefinic proton [δ_H 8.03, H-16], suggesting that this olefinic proton was located in the deshielding zone of a carbonyl group, In the HMBC correlation (Table 3), H-16 showed

Table 2
^{13}C NMR spectroscopic data for cowaxanthones A–E (**1**–**5**) in $CDCl_3$ (75 MHz)

Position	δ_C				
	1	**2**	**3**	**4**	**5**[a]
1	159.67	160.63	157.90	159.62	167.09
2	112.27	108.45	104.39	111.49	111.00
3	164.06	161.57	159.72	163.62	167.00
3-OMe	55.97			55.85	
4	89.80	93.16	94.07	88.94	102.07
4a	155.72	155.04	156.24	155.45	157.49
5	133.63	98.31	114.11	102.21	101.77
5-OMe	61.94				
6	154.23[b]	158.14	152.40	150.76[b]	154.89[b]
6-OMe		56.04			
7	112.35	144.20	142.35	136.77	143.45
7-OMe		60.96	62.00		62.15
8	121.92	137.29	133.79	119.72	137.48
8a	115.14	111.89	111.88	108.79	112.35
9	180.06	182.10	182.37	182.41	181.32
9a	103.17	103.75	103.68	104.00	102.30
10a	149.57[b]	155.46	153.43	153.05[b]	155.20[b]
11	21.32	21.46	115.77	21.35	20.51
12	122.03	121.48	127.02	122.31	121.08
13	131.93	135.78	77.91	131.71	132.62
14	17.79	17.94[b]	28.33	17.78	17.81
15	25.79	25.92	28.33	25.81	25.80[b]
16		26.18	26.37	121.03	26.60
17		123.16	123.50	132.20	122.57
18		131.89	131.87	77.21	132.63
19		25.87	25.79[b]	27.35	25.79[b]
20		18.15[b]	18.21	27.35	18.23
21			22.64		190.08
22			121.09		
23			132.67		
24			25.83[b]		
25			18.00		

[a] ^{13}C NMR (125 MHz) of **5** in $CDCl_3$.
[b] Interchangeable.

cross-peaks with C-7 (δ_C 136.77) and C-18 (δ_C 77.21), while the other olefinic proton [δ_H 5.82, H-17] of this dimethylchromene ring showed cross-peaks with C-8 (δ_C 119.72) and C-18. These data supported fusion of the dimethylchromene ring at C-7 and C-8 of the xanthone nucleus with an ether linkage at C-7. The lower-field aromatic proton at δ_H 6.82 was attributed to H-5, since it showed correlations in the HMBC spectrum with C-6 (δ_C 150.76), C-7, C-8a (δ_C 108.79) and C-10a (δ_C 153.05). The chemical-shift value of C-6 indicated that the C-6 position was attached by a hydroxyl group. In addition, the HMBC data (Table 3) established the identical attachment of the substituents on ring B as in **9**. Thus, cowaxanthone D was elucidated as 1,6-dihydroxy-3-methoxy-2-(3-methyl-2-butenyl)-6′,6′-dimethylpyrano(2′,3′:7,8)xanthone (**4**). It was a new naturally occurring tetraoxygenated xanthone.

Cowaxanthone E (**5**) was isolated as a yellow gum. HREIMS established a molecular formula of $C_{25}H_{26}O_7$. The ^1H (Table 1) and ^{13}C NMR (Table 2) spectra were similar to those of **14** except for a high field aromatic proton [δ_H 6.29, (1H, *s*)] of **14** was replaced, in **5**, by a formyl group [δ_H 10.37, (1H, *s*)]. The ^{13}C NMR spectra (Table 2) confirmed the above conclusion by the presence of one additional carbonyl carbon of the formyl group at δ_C 190.08. The location of this formyl group at C-4 (δ_C 102.07) was confirmed by the HMBC correlations with C-3 (δ_C 167.00) and C-4 (δ_C 102.07) (Table 3). In addition, the substituents were assigned to the same positions as found in **14** by the HMBC data. Therefore, cowaxanthone E was identified as 1,3,6-trihydroxy-4-formyl-7-methoxy-2,8-bis(3-methyl-2-butenyl)xanthone (**5**). It was a new naturally occurring tetraoxygenated xanthone.

Table 3
Major HMBC correlations for cowaxanthones A–E (**1**–**5**) in $CDCl_3$

Proton	HMBC correlations				
	1	**2**	**3**	**4**	**5**
1-OH	1, 2, 9a	1, 2, 9a	1, 9a	1, 2, 9a	1, 2, 9a
3-OH					2, 3, 4
3-OMe	3			3	
4	2, 3, 4a, 9, 9a	2, 3, 4a, 9, 9a	3, 4a, 9, 9a	2, 3, 4a, 9a	
5		6, 7, 8a, 9, 10a		6, 7, 8a, 10a	6, 7, 8a, 9, 10a
5-OMe	5				
6-OMe		6			
6-OH			5, 6, 7		
7	5, 6, 8a				
7-OMe		7	7		7
8	6, 9, 10a				
11	1, 3, 12, 13	2, 3, 12, 13	1, 2, 3, 13	1, 2, 3, 12, 13	
12			2, 13		2, 3, 12, 13
14	12, 13	12, 13	11, 12, 13	12, 13	12, 13
15	12, 13	12, 13	11, 12, 13	12, 13	12, 13
16		7, 8, 8a, 17, 18	7, 8, 8a, 17, 18	7, 18	7, 8, 8a, 17, 18
17				8, 18	
19		17, 18	17, 18	17, 18	17, 18
20		17, 18	17, 18	17, 18	17, 18
21			5, 6, 10a, 22, 23		3, 4
24			22, 23		
25			22, 23		

The antibacterial activity of some of the compounds was shown in Table 4. Compound **11** showed the strongest inhibitory activity against *Staphylococcus aureus*, both penicillin-sensitive strain ATCC 25923 and methicillin-resistant strain MRSA SK1, with MIC values of 4 and

Table 4
Antibacterial activity of compounds from *G. cowa*

Compounds	Antibacterial activity (MIC, μg/mL)	
	S. aureus ATCC 25923	MRSA SK1
2	128	128
3	128	128
8	128	64
9	128	64
10	>128	>128
11	4	4
13	>128	>128
14	8	8
Vancomycin	0.5	1

4 μg/mL, respectively. Compound **14** exhibited moderate activity against both strains of *S. aureus* with MIC values of 8 and 8 μg/mL, respectively. Compounds **2**, **3**, **8**, **9**, **10** and **13** had weak antibacterial activity (MIC 64–128 μg/mL).

3. Conclusion

Fifteen tetraoxygenated xanthones were isolated from the fruits of *G. cowa*. Compound **11** exhibited strongest antibacterial activity while compound **14** only moderate activity but the others only weak activity. These results suggested that the crucial parts responsible for the antibacterial activity should be H-5, 6-OH and a C-8 prenyl group of ring A, together with a dimethylchromene ring attaching to ring B at C-2 and C-3 (an ether linkage at C-3).

4. Experimental

4.1. General

Melting points were determined on an Electrothermal 9100 melting point apparatus and are uncorrected. IR spectra were obtained using a FTS 165 FT-IR spectrometer (Perkin–Elmer 783). ^{1}H and ^{13}C NMR spectra were recorded on a Varian UNITY INOVA 500 MHz or a Bruker FTNMR Ultra Shield™ 300 MHz spectrometer using deuterochloroform solutions unless otherwise stated with TMS as internal standard. UV spectra were recorded using UV-160A spectrophotometer (SHIMADZU). EI and HREI mass spectra were measured on a Finnegan MAT 95 XL spectrometer. TLC and precoated TLC were performed on silica gel 60 GF_{254} (Merck). CC was performed on silica gel (Merck) type 100 (70–230 mesh ASTM) or on reversed phase silica gel C-18 with a mixture of MeOH–H_2O as eluent.

4.2. Plant material

The fruits of *G. cowa* Roxb. were collected from Sathingmoo district, amphur Singhanakhon, Songkhla province in the Southern part of Thailand, in June 2003. A voucher specimen (WIPAPAN01) has been deposited at the herbarium of the Department of Biology, Faculty of Science, Prince of Songkla University, Songkhla, Thailand.

4.3. Isolation

The crude hexane extract (25.20 g) was subjected to silica gel quick CC, eluting with hexane, EtOAc and MeOH in a polarity-gradient manner to afford 7 frs. Fr. 1 (1.33 g, eluted with pure hexane-5% EtOAc/hexane) was further purified by CC on silica gel to yield 3 subfrs. Subfr. 2 (182 mg, eluted with 5–7% EtOAc in hexane) was further purified by CC on silica gel and eluted with a gradient of hexane–acetone to afford **3** (62 mg) and **12** (9 mg). Fr. 2 (2.55 g, eluted with 5% EtOAc–hexane) was further separated by CC over silica gel to yield 3 subfrs. The first subfr. (97 mg, eluted with 4–8% EtOAc in hexane), upon standing at room temperature, yielded **7** (70 mg) as a yellow solid. The second subfr. (1.04 g, eluted with 8% EtOAc in hexane) was purified by silica gel CC to give 4 frs. The second fr. (69 mg, eluted with 10% acetone in hexane) was subjected to further silica gel CC with solvent mixtures of hexane, CH_2Cl_2 and MeOH in a polarity-gradient manner to afford **11** (22 mg) and **8** (2 mg). The third subfr. (835 mg, eluted with 8–20% EtOAc in hexane) was further purified by recrystallization from acetone–hexane to give **9** (475 mg) as a yellow solid. Fr. 3 (6.04 g, eluted with 5–20% EtOAc in hexane) was further purified by CC on silica gel to yield 4 subfrs. The fourth subfr. (3.15 g, eluted with 20–50% EtOAc in hexane) was further purified by CC on silica gel to afford 3 frs. The first fr. (42 mg, eluted with 2% MeOH in $CHCl_3$) was applied to a silica gel column eluted with solvent mixtures of hexane, CH_2Cl_2 and MeOH in a polarity-gradient manner and subsequent preparative TLC with 10% EtOAc in hexane (11 runs) to give **2** (38 mg) and **4** (8 mg). The second fr. (2.91 g, eluted with 2–4% MeOH in $CHCl_3$) was further purified by CC on silica gel with hexane, CH_2Cl_2 and MeOH in a polarity gradient manner to afford **1** (19 mg), **5** (2 mg), **6** (5 mg), **10** (105 mg), **13** (117 mg), **14** (79 mg) and **15** (2 mg).

4.3.1. Cowaxanthone A (*1*)

Yellow solid, m.p. 178.3–179.0 °C; UV: λ_{max} MeOH nm (log ε): 246 (4.42), 282 (3.85), 318 (4.13), 361 (3.64); IR ν_{max}(KBr) cm^{-1}: 3301 (O–H), 1649 (C=O); MS *m*/*z* (rel. int): 356 [M]$^+$ (10), 355 (45), 340 (25), 312 (84), 300 (100), 297 (25), 177 (29), 148 (83), 140 (27), 97 (32), 95 (38), 85 (60), 83 (97), 69 (65), 57 (84); HREMS *m*/*z* 356.1250 [M]$^+$ (Calc. for $C_{20}H_{20}O_6$, 356.1260); for ^{1}H and ^{13}C NMR spectroscopic data, see Tables 1 and 2.

4.3.2. Cowaxanthone B (*2*)

Orange gum; UV: λ_{max} MeOH nm (log ε): 244 (4.33), 258 (4.26), 315 (4.15), 355 (3.71); IR ν_{max}(neat) cm^{-1}: 3365 (O–H), 1639 (C=O); MS *m*/*z* (rel. int): 424 [M]$^+$ (30), 423 (94), 380 (100), 368 (41), 352 (86), 336 (40), 324 (40); HREIMS *m*/*z* 424.1899 [M]$^+$ (Calc. for $C_{25}H_{28}O_6$, 424.1886); for ^{1}H and ^{13}C NMR spectroscopic data, see Tables 1 and 2.

4.3.3. Cowaxanthone C (*3*)

Yellow-red gum; UV: λ_{max} MeOH nm (log ε): 243 (3.95), 279 (4.16), 289 (4.19), 332 (3.90); IR ν_{max}(neat) cm^{-1}: 3348 (O–H), 1648 (C=O); MS *m*/*z* (rel. int): 476 [M]$^+$ (19), 475 (48), 460 (100), 432 (87); HREIMS *m*/*z* 476.2191 [M]$^+$ (Calc. for $C_{29}H_{32}O_6$, 476.2199); for ^{1}H and ^{13}C NMR spectroscopic data, see Tables 1 and 2.

4.3.4. Cowaxanthone D (*4*)

Yellow solid, m.p. 210.0–210.7 °C; UV: λ_{max} MeOH nm (log ε): 245 (4.44), 265 (4.42), 328 (4.30), 387 (3.81); IR ν_{max}(KBr) cm^{-1}: 3266 (O–H), 1631 (C=O); MS *m*/*z* (rel. int): 408 [M]$^+$ (15), 407 (46), 392 (47), 364 (60), 352

(100), 334 (16), 168 (21), 166 (25), 148 (86), 125 (21), 111 (32), 97 (51), 83 (65), 71 (66), 69 (70), 57 (99); HREIMS *m/z* 408.1555 $[M]^+$ (Calc. for $C_{24}H_{24}O_6$, 408.1573); for ^1H and ^{13}C NMR spectroscopic data, see Tables 1 and 2.

4.3.5. Cowaxanthone E (**5**)

Yellow gum; UV: λ_{max} MeOH nm (log ε): 244 (4.33), 268 (4.07), 311 (4.16), 352 (3.83); IR ν_{max}(neat) cm^{-1}: 3412 (O–H), 1643 (C=O); MS *m/z* (rel. int): 438 $[M]^+$ (4), 437 (12), 381 (14), 366 (22), 177 (32), 160 (20), 148 (27), 132 (17), 118 (23), 111 (24), 97 (40), 84 (60), 82 (87), 69 (81); HREIMS *m/z* 438.1691 $[M]^+$ (Calc. for $C_{25}H_{26}O_7$,438.1679); for ^1H and ^{13}C NMR spectroscopic data, see Tables 1 and 2.

4.4. Antibacterial activity

Minimum inhibitory concentrations (MICs) were determined by the agar microdilution method (Lorian, 1996). Test samples were dissolved in dimethyl sulfoxide (DMSO, Merck). Serial 2-fold dilutions of the test samples were mixed with melted Mueller Hinton agar (Difco) in the ratio of 1:100 in microtiter plates (Nunc). Final concentration of the test samples in agar ranged from 128 to 0.25 μg/mL. *S. aureus* ATCC 25923 and MRSA SK1 isolated from Clinical specimen were used as test strains. Ten μL of inoculum suspensions (10^4 cfu) were dropped on agar surface. The inoculated plates were incubated at 35 °C for 16–18 h. MICs were recorded by reading the lowest concentration that inhibited visible growth. The test was performed in triplicates. Vancomycin was used as a positive control drug.

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References

Ito, C., Itoigawa, M., Takakura, T., Ruangrungsi, N., Enjo, F., Tokuda, H., Nishino, H., Furukawa, H., 2003. Chemical constituents of *Garcinia fusca*: structure elucidation of eight new xanthones and their cancer chemopreventive activity. J. Nat. Prod. 66, 200–205.

Likhitwitayawuid, K., Phadungcharoen, T., Manhidol, C., Ruchirawat, S., 1997. 7-*O*-Methylgarcinone E from *Garcinia cowa*. Phytochemistry 45 (6), 1299–1301.

Likhitwitayawuid, K., Phadungcharoen, T., Krungkrai, J., 1998. Antimalarial xanthones from *Garcinia cowa*. Planta Med. 64, 70–72.

Lorian, V., 1996. Antibiotics in Laboratory Medicines, fourth ed. William and Wilkins, Baltimore, pp. 28–32.

Lu, Z.X., Hasmeda, M., Mahabusarakam, W., Ternai, B., Ternai, P.C., Polya, G.M., 1998. Inhibition of eukaryote protein kinases and of a cyclic nucleotide-binding phosphatase by prenylated xanthones. Chemico-Biol. Interact. 114, 121–140.

Mahabusarakum, W., Wiriyachitra, P., 1987. Chemical constituents of *Garcinia mangostana*. J. Nat. Prod. 50 (3), 474–478.

Mahabusarakum, W., Chairerk, P., Taylor, W.C., 2005. Structure elucidation of xanthones from *Garcinia cowa* Roxb. latex. Phytochemistry 66, 1148–1153.

Na Pattalung, P., Thongtheeraparp, W., Wiriyachitra, P., Taylor, W.C., 1994. Xanthones of *Garcinia cowa*. Planta Med. 60, 365–368.

Nilar, L., Harrison, L.J., 2002. Xanthones from the heartwood of *Garcinia mangostana*. Phytochemistry 60, 541–548.

Poomipamorn, S., Kumkong, A., 1997. Edible Multipurpose Tree Species. FaungFa Printing (in Thai), Bangkok, p. 486.

Sen, A.K., Sarkar, K.K., Mazumder, P.C., Benerii, N., Uusvuori, R., Hase, T.A., 1980. A xanthones from *Garcinia mangostana*. Phytochemistry 19, 2223–2225.

Sen, A.K., Sarkar, K.K., Mazumder, P.C., Benerji, N., 1981. Minor xanthones of *Garcinia mangostana*. Phytochemistry 20, 183–185.

Sen, A.K., Sarkar, K.K., Mazumder, P.C., Benerji, N., Uusvuori, R., Hase, T.A., 1982. The structure of garcinones A, B and C: three new xanthones from *Garcinia mangostana*. Phytochemistry 21 (7), 1747–1750.