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NEW XANTHONES FROM THE BARKS AND FRUITS OF *CRATOXYLUM COCHINCHINENSE*

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Abstract – Four new xanthones named cochinxanthone D-G (**1**, **5**, **8**, and **9**) along with five known xanthones were isolated from the barks and the fruits of *Cratoxylum cochinchinense*. Extensive 1D, and 2D NMR and other spectroscopic studies were employed to determine the structure of all new compounds.

Cratoxylum cochinchinense (Clusiaceae) is a shrub tree distributed throughout Thailand. Several parts of this plant have been used by local Thai people in folk medicine for the treatment of diarrhea and as a diuretic effect. We have previously reported several phenolic compounds from the fruits and roots of this plant.[1,2] In the present work, we report the isolation and structure elucidation of four new xanthones (**1**, **5**, **8** and **9**) together with five known xanthones (**2-4**, **6**, and **7**) (Figure 1) from the barks and fruits of *C. cochinchinense*.

Fractionation of the dichloromethane extract of the barks of *C. cochinchinense* has resulted in the isolation of two new xanthones, cochinxanthone D (**1**) and cochinxanthone E (**5**) along with five known xanthones (**2-4**, **6**, and **7**) whereas two new oxygeranylated xanthones (**8** and **9**) were isolated from the minor fraction of EtOAc extracts of the dried fruits. The structures of all new xanthones were elucidated using spectroscopic data, especially 1D and 2D NMR techniques.

Cochinxanthone D (**1**) was a yellow solid mp 221–222 °C. The molecular formula ($C_{28}H_{32}O_6$) was in agreement with $[M–H]^-$ *m/z* 463.2126. The ^{1}H–NMR spectrum (Table 1) indicated the presence of a

hydrogen-bonded hydroxy group at δ 12.94 and two singlets aromatic protons at δ 6.64 and 7.42 which identified to H–5 and H–8, respectively, on the basis of HMBC correlations (Figure 2). In addition, one prenyl [δ 3.35 (*d*, *J* = 6.0 Hz, H–1′), 5.25 (*m*, H–2′), 1.72 (*s*, H– 4′) and 1.80 (*s*, H–5′)] and one geranyl group [δ 3.43 (*d*, *J* = 6.8 Hz, H–1″), 5.25 (*m*, H–2″), 2.05 (*m*, H– 4″), 2.05 (*m*, H–5″), 5.00 (*m*, H–6″), 1.55 (*s*, H–8″), 1.82 (*s*, H–9″) and 1.62 (*s*, H–10″)] were also observed in ^{1}H-NMR spectrum. The prenyl group was located on C–2 according to the HMBC correlations of H– 1′ (δ 3.35) to C–1 (δ 157.2), C–2 (δ 109.1) and C–3 (δ 160.5) whereas the geranyl group was placed on C–4 due to the correlation of H–1″ (δ 3.43) to C–3 (δ 160.5), C–4 (δ 104.9) and C–4a (δ 152.7). Therefore, the structure cochinxanthone D was indicated to be **1**.

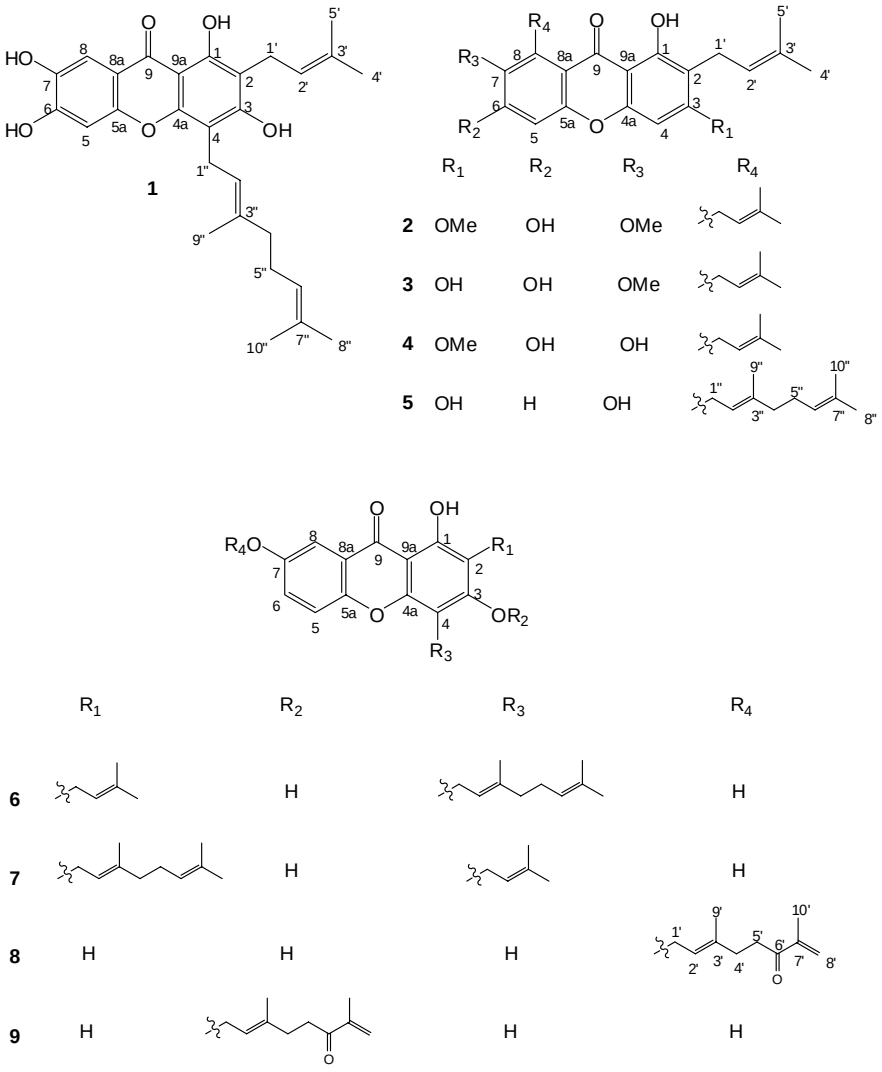

Figure 1. Compounds isolated from *C. cochinchinense*

Cochinxanthone E (**5**) was a yellow viscous oil. Its molecular formula of $C_{28}H_{32}O_5$ was in agreement with

[M–H]$^-$ *m/z* 447.2172. The ^{1}H–NMR spectrum (Table 1) showed the presence of a hydroxyl group (δ 13.63), two *ortho*-coupled aromatic protons (δ 7.22 (*d*, *J* = 9.2 Hz, H–5) and 7.19 (*d*, *J* = 9.2 Hz, H–6) and one singlet aromatic proton (δ 6.31, *s*, H–4). The assignment of H–4, H–5 and H–6 were confirmed by the HMBC correlations (Figure 2). Other signals could be attributed to a geranyl [δ 4.30 (*d*, *J* = 6.4 Hz, H–1″), 5.28 (*m*, H–2″), 2.05 (*m*, H–4″), 2.10 (*m*, H–5″), 5.03 (*m*, H–6″), 1.57 (*s*, H–8″), 1.86 (*s*, H–9″) and 1.65 (*s*, H–10″)] and prenyl [δ 3.45 (*d*, *J* = 6.0 Hz, H–1′), 5.25 (*m*, H–2′), 1.76 (*s*, H–4′) and 1.84 (*s*, H–5′)] moieties. The methylene protons of the geranyl group at δ 4.30 (H–1″) together with cross-peaks in the HMBC spectrum with C–7 (δ 151.8), C–8 (δ 127.1) and C–8a (δ 118.4) suggested that this side chain was located at C–8. The prenyl group was placed on C–2 because the methylene protons δ 3.45 (H–1′) of the prenyl group showed cross-peaks in the HMBC spectrum with C–1 (δ 160.6), C–2 (δ 108.4) and C–3 (δ 162.2). Therefore, cochinxanthone E was assigned to be **5**.

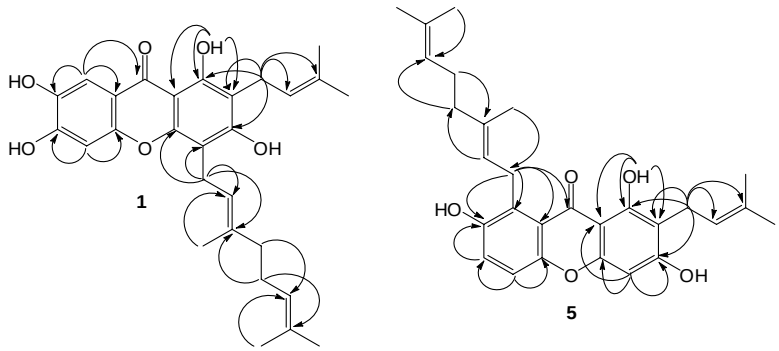

Figure 2. HMBC correlations of and **1** and **5**

Cochinxanthone F (**8**) was isolated as a yellow viscous oil with the molecular formula of $C_{23}H_{22}O_6$, on the basis of HR-MS. The ^{1}H–NMR spectral data of **8** (Table 1) showed a downfield hydroxyl proton (δ 12.94), a typical pattern of the 1,2,4-trisubstituted benzene ring [δ 7.57 (*d*, *J* = 3.0 Hz, H–8), 7.34 (*d*, *J* = 9.0 Hz, H–5) and δ 7.28 (*dd*, *J* = 3.0, 9.0 Hz, H–6)] and a *meta*-coupled aromatic proton [δ 6.37 (*d*, *J* = 2.0 Hz, H–2) and δ 6.27 (*d*, *J* = 2.0 Hz, H–4)]. The remaining ten carbons were accounted for as a *O*-geranyl side chain having two double bonds and a conjugated carbonyl carbon (Table 2). Two protons at δ 5.80 (*d*, *J* = 1.0 Hz, H_a–8′) and 6.00 (*s*, H_b–8′) attached to the carbon at δ 124.9 in HMQC indicated the presence of an exomethylene group, which was conjugated with the carbonyl group, as deduced from HMBC correlations (Figure 3). This conjugated system was determined to be at the end of the *O*-geranyl moiety because methylene protons at δ 5.80 and 6.00 (H–8′) showed a correlation to C–10′ (δ 17.6) in HMBC data. Therefore, the C10 substituent was deduced to be a 3,7-dimethyl-6-oxoocta-2,7-dienyl unit. This substituent was placed at C–7 by HMBC correlation of the methylene protons at δ 4.65 (H–1′) with C–7 (δ155.1). In addition, the irradiation of H–1′ (δ 4.65) enhanced signal intensity of H–8 (δ 7.57) in the

NOEDIFF experiment (Figure 3) was also supported the location of the *O*-geranyl moiety. Thus, cochinxanthone F was assigned to be **8**.

Cochinxanthone G (**9**) was also isolated as a yellow viscous oil. The molecular formula of **9**, $C_{23}H_{22}O_6$, was the same as **8**. The ^{1}H– and ^{13}C–NMR signals of **9** (Tables 1 and 2) were almost identical to those of **8**. However, NOE enhancements observed between the methylene protons at δ 4.60 (H–1′) and two aromatic protons at δ 6.39 (H–2) and 6.32 (H–4) suggested that the *O*-geranyl moiety was located at C–3 not C–7 as in **8**. Also, the HMBC correlation (Figure 3) of the methylene protons at δ 4.60 (H–1′) with C–3 (δ 165.9) supported the above information. In addition, both of compounds **8** and **9** showed the difference of R_f value on the TLC (R_f = 0.22 and 0.34 for compounds **9** and **8**, respectively, in 5% acetone-DCM). Therefore, cochinxanthone G was assigned as to be **9**, a structural isomer of **8**.

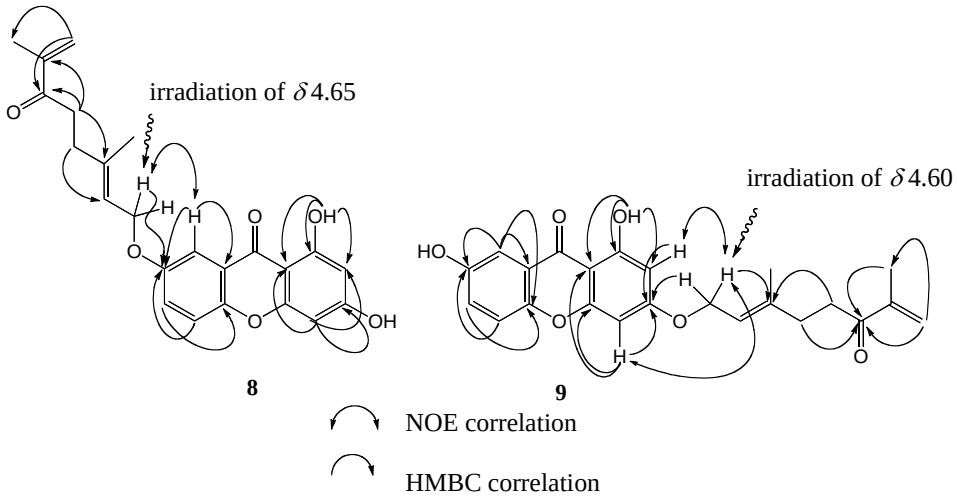

Figure 3. HMBC and NOE correlations of **8** and **9**

The remaining xanthones were characterized as cochinchinone A (**6**),[2] 2-geranyl-1,3,7-trihydroxy-4-(3-methylbut-2-enyl)xanthone (**7**),[3] β-magostin (**2**),[2] α-magostin (**3**),[2] and dulcisxanthone (**4**)[4] by analysis of 1D and 2D NMR information and comparison of their physical and spectral data with reported values.

Several xanthones have been isolated from the plants in the genus *Cratoxylum*, e.g. *C. cochinchinense*,[1,2,4] *C. formosum sp pruniflorum*,[5] *C. sumatranum*,[6] *C. maingayi*,[7] and *C. formosum*.[8] We now report four additional new polyoxygenated xanthones from the barks and dried fruits of *C. cochinchinense*. In addition, five known xanthones were also identified from the bark of this plant.

Table 1. ^{1}H–NMR Data of **1**, **5**, **8** and **9** in $CDCl_3$.

Position	1[a]	5[a]	8[b]	9[b]
2	–	–	6.37 (1H, d, *J* = 2.0 Hz)	6.39 (1H, d, *J* = 2.5 Hz)
4	–	6.31 (1H, s)	6.27 (1H, d, *J* = 2.0 Hz)	6.32 (1H, d, *J* = 2.5 Hz)
5	6.64 (1H, s)	7.22 (1H, dd, *J* = 9.2 Hz)	7.34 (1H, d, *J* = 9.0 Hz)	7.35 (1H, d, *J* = 9.5 Hz)
6	–	7.19 (1H, d, *J* = 9.2 Hz)	7.28 (1H, dd, *J* = 9.0, 3.0 Hz)	7.27 (1H, dd, *J* = 9.5, 3.0 Hz)
7	–	–	–	–
8	7.42 (1H, s)	–	7.57 (1H, d, *J* = 3.0 Hz)	7.59 (1H, d, *J* = 3.0 Hz)
1′	3.35 (2H, d, *J* = 6.0 Hz)	3.45 (2H, d, *J* = 6.0 Hz)	4.65 (2H, d, *J* = 6.0 Hz)	4.60 (1H, d, J = 6.0 Hz)
2′	5.25 (1H, m)	5.25 (1H, m)	5.51 (1H, m)	5.49 (1H, m)
4′	1.72 (3H, s)	1.76 (3H, s)	2.41 (2H, m)	2.33 (2H, m)
5′	1.80 (3H, s)	1. 84 (3H, s)	2.87 (2H, m)	2.87 (2H, m)
8′	–	–	5.80 (1H, d, *J* = 1.0 Hz); 6.00 (1H, s)	5.79 (1H, d, *J* = 1.0 Hz); 5.99 (1H, s)
9′	–	–	1.79 (3H, s)	1.78 (3H, s)
10′	–	–	1.88 (3H, s)	1.88 (3H, s)
1′′	3.43 (2H, d, *J* = 6.8 Hz)	4.30 (2H, d, *J* = 6.4 Hz)	–	–
2′′	5.25 (1H, m)	5.28 (1H, m)	–	–
4′′	2.05 (2H, m)	2.05 (2H, m)	–	–
5′′	2.05 (2H, m)	2.10 (2H, m)	–	–
6′′	5.00 (1H, m)	5.03 (1H, m)	–	–
8′′	1.55 (3H, s)	1.57 (3H, s)	–	–
9′′	1.82 (3H, s)	1.86 (3H, s)	–	–
10′′	1.62 (3H, s)	1.65 (3H, s)	–	–
1–OH	12.94 *s*	13.63 *s*	12.94 *s*	12.81 *s*

[a] Measured at 400 MHz NMR; [b] Measured at 500 MHz

Table 2. ^{13}C–NMR Data of **1, 5, 8** and **9** in $CDCl_3$.

Position	**1**[a]	**5**[a]	**8**[b]	**9**[b]
1	157.2	160.6	163.6	163.2
2	109.1	108.4	94.1	93.1
3	160.5	162.2	161.1	165.9
4	104.9	93.2	98.3	97.4
4a	152.7	156.1	157.8	157.7
5a	151.9	150.9	150.7	152.0
5	102.4	123.7	118.9	118.7
6	152.7	116.6	125.4	121.0
7	141.7	151.8	155.1	150.6
8	108.0	127.1	106.0	109.2
8a	112.4	118.4	122.3	123.3
9	180.0	183.5	180.5	180.4
9a	104.9	104.0	103.6	103.6
1′	21.2	21.5	65.4	65.3
2′	121.7	121.4	119.4	119.1
3′	134.6	134.9	140.6	141.7
4′	25.8	25.8	33.8	33.7
5′	17.6	17.9	35.6	35.5
6′	–	–	201.5	201.3
7′	–	–	144.4	144.4
8′	–	–	124.9	123.9
9′	–	–	16.9	17.0
10′	–	–	17.6	17.6
1″	21.2	25.8	–	–
2″	121.5	121.4	–	–
3″	137.9	137.9	–	–
4″	39.7	39.7	–	–
5″	26.4	26.4	–	–
6″	123.9	123.8	–	–
7″	131.8	131.5	–	–
8″	17.7	17.7	–	–
9″	16.2	16.3	–	–
10″	25.6	25.6	–	–

[a] Measured at 100 MHz NMR; [b] Measured at 125 MHz

EXPERIMENTAL

GENERAL

UV spectra were recorded with a Perkin-Elmer UV-Vis spectrophotometer. The IR spectra were recorded with a Perkin-Elmer FT-IR spectrophotometer. The ^{1}H– and ^{13}C–NMR spectra were recorded using 400 MHz Bruker FTNMR Ultra Shield and 500 MHz Varian UNITY INOVA spectrometers. Chemical shifts were recorded in parts per million (δ) in $CDCl_3$ with tetramethylsilane (TMS) as an internal reference. High resolution mass spectra were obtained using Bruker microTOF or MAT 95 XL mass spectrometers.

Quick column chromatography (QCC) and column chromatography (CC) were carried out on silica gel 60 H (Merck, 5–40 μm) and silica gel 100 (Merck, 63–200 μm), respectively. Precoated plates of silica gel 60 F_{254} were used for analytical purposes.

PLANT MATERIAL

The dried fruits and barks of *C. cochinchinense* were collected from Mae Fah Luang University, Tasud, Muang, Chiang Rai Province, northern part of Thailand in December 2006 and January 2008, respectively. Botanical identification was achieved through comparison with a voucher specimen No. SL–1 (PSU)[2] in the herbarium collection of the Department of Biology, Prince of Songkla University, Songkhla, Thailand.

EXTRACTION AND ISOLATION

The barks of *C. cochinchinense* (1 kg) were extracted with MeOH, over a period of 3 days at rt and evaporated under reduced pressure to provide crude MeOH (200 g) extracts. This crude extract was partitioned with CH_2Cl_2 (2000 mL) and concentrated under reduced pressure to give CH_2Cl_2 extract (58.31 g). The CH_2Cl_2 extract was chromatographed by QCC over silica gel and eluted with a gradient of hexane-EtOAc to afford twenty fractions (CCD2A–CCD2T). Fraction CCD2D (10 g) was further purified by CC with a gradient of CH_2Cl_2–MeOH to give compounds **2** (50.1 mg), **3** (35.2 mg), **4** (20.5 mg), **5** (10 mg), **6** (1.5 g) and **7** (1.7 g). Fractions CCD2G (2.35 g) was subjected to CC (CH_2Cl_2–acetone, 95:5, v/v) to give compounds **1** (800 mg), **6** (150 mg) and **7** (200 mg).

The dried fruits of *C. cochinchinense* (1 kg) were extracted with hexane and EtOAc, respectively, over a period of 3 days each at rt and evaporated under reduced pressure to provide crude hexane (16 g) and EtOAc extracts (66 g). The EtOAc extract (66 g) was chromatographed by QCC over silica gel and eluted with a gradient of hexane-EtOAc (100% hexane and 100% EtOAc) to afford twenty fractions (E1–E20). Fraction E14 (3 g) was purified by CC with acetone-hexane (2:8, v/v) yielding seventeen fractions (E14A–E14Q). Fraction E14K (72.3 mg) was further purified by CC with acetone-hexane (3:7, v/v) to yield four subfractions (E14K1–E14K4). Compound **8** (4.0 mg) and **9** (3.6 mg) was obtained from fraction E14K2 (35.6 mg) by prep. TLC with 100% $CHCl_3$.

Cochinxanthone D **1**: Yellow solid. mp 221-222 °C. UV λ_{max} (MeOH) (log ε): 373 (3.68), 320 (3.95), 262 (4.23), 235 (4.15), 203 (4.35) nm. IR (neat) ν_{max}: 3438 (OH), 1637 (C=O) cm^{-1}. ^1H–NMR (400 MHz,

$CDCl_3$) and ^{13}C–NMR (100 MHz, $CDCl_3$) see Tables 1 and 2. HR–MS (APCI, –ve) *m/z* 463.2126 $[M–H]^-$ (calcd. for $C_{28}H_{31}O_6$, 463.2121).

Cochinxanthone E **5**: Yellow viscous oil. UV λ_{max} (MeOH) (log ε): 382 (3.43), 314 (3.92), 265 (4.18), 241 (4.20) nm. IR (neat) ν_{max}: 3437 (OH), 1638 (C=O) cm^{-1}. ^1H–NMR (400 MHz, $CDCl_3$) and ^{13}C–NMR (100 MHz, $CDCl_3$) see Tables 1 and 2. HR–MS (APCI, –ve) *m/z* 447.2172 $[M–H]^-$ (calcd. for $C_{28}H_{31}O_5$, 447.2171).

Cochinxanthone F **8**: Yellow viscous oil. UV λ_{max} (MeOH)(log ε): 368 (3.37), 308 (3.74), 259 (4.20), 236 (4.08) nm. IR (neat) ν_{max}: 3438, 1642 cm^{-1}. ^1H–NMR (500 MHz, $CDCl_3$) and ^{13}C–NMR (125 MHz, $CDCl_3$) see Tables 1 and 2. HR–MS (APCI, –ve) *m/z* 393.1337 $[M–H]^-$ (calcd. for $C_{23}H_{21}O_6$, 393.1338).

Cochinxanthone G **9**: Yellow viscous oil. UV λ_{max} (MeOH)(log ε): 375 (3.37), 305 (3.75), 259 (4.22), 237 (4.08) nm. IR (neat) ν_{max}: 3373, 2974, 1650 cm^{-1}. ^1H–NMR (500 MHz, $CDCl_3$) and ^{13}C–NMR (125 MHz, $CDCl_3$) see Tables 1 and 2. HR–EIMS *m/z* 394.1419 $[M]^+$ (calcd. for $C_{23}H_{22}O_6$, 394.1416).

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