Cytotoxic and Antimalarial Prenylated Xanthones from *Cratoxylum cochinchinense*

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A new prenylated xanthone, 5-*O*-methylcelebixanthone (1), together with six known compounds; celebixanthone (2), 1,3,7-trihydroxy-2,4-di(3-methylbut-2-enyl)xanthone (3), cochinchinone A (4), α-mangostin (5), β-mangostin (6) and cochinchinone C (7) were isolated from roots of *Cratoxylum cochinchinense*. Their structures were elucidated by spectroscopic methods. Compounds 2 and 4—7 showed cytotoxic activity against the human lung cancer cell line (NCI-H187) with IC_{50} values ranging from 0.65 to 5.2 μg/ml. Compounds 1, 2, 6 and 7 also showed antimalarial activity against *Plasmodium falciparum* with IC_{50} values of 3.2, 4.9, 7.2 and 2.6 μg/ml, respectively.

Key words 5-*O*-methylcelebixanthone; antimalarial activity; cytotoxic activity; *Cratoxylum cochinchinense*

Cratoxylum cochinchinense (LOUR.) BLUME is a shrub tree belonging to the family Guttiferae. Some species of *Cratoxylum* have been used as traditional medicine.[1)] Previous chemical investigations of this genus have revealed the presence of xanthones, triterpenoids and flavonoids.[1—5)] In the course of our ongoing search for bioactive compounds from natural sources,[6,7)] hexane extract of the roots of *C. cochinchinense* exhibited cytotoxic activity against the NCI-H187 cell line and antimalarial activity against *Plasmodium falciparum*. Bioassay-guided investigation of this extract resulted in the identification of a new xanthone, 5-*O*-methylcelebixanthone (**1**), together with six known compounds (**2**—**7**). In this paper we report the isolation and structural elucidation of the new compound (**1**). The biological evaluation of all compounds is also reported. In addition, the full assignment of ^{1}H- and ^{13}C-NMR spectral data of **2** (Table 1) is also reported herein for the first time.

Compound **1** was deduced to have a formula of $C_{20}H_{20}O_6$ by HR-EI-MS, which showed a molecular ion peak at *m*/*z* 356.1229 (Calcd 356.1259). The IR spectrum showed absorption bands at 3357 cm^{-1} for hydroxyl group and 1646 cm^{-1} for conjugated carbonyl functionality which was also confirmed by the ^{13}C-NMR spectrum (Table 1) at δ 183.6 (C-9). This carbonyl moiety formed a hydrogen bond with a hydroxyl group, as evidenced by a proton signal at δ 13.10 (1-OH). The ^{1}H-NMR (Table 1) and COSY spectral data revealed two methoxyl groups at δ 4.10 (3H, s) and 3.82 (3H, s), one prenyl unit observed at δ 5.21 (1H, m, H-2′), 4.04 (2H, d, *J*=6.6 Hz, H-1′), 1.85 (3H, s, H-5′), and 1.69 (3H, d, *J*=1.5 Hz, H-4′) and a 1,2,3-trisubstituted benzene ring at δ 7.53 (1H, t, *J*=8.4 Hz, H-3), 6.91 (1H, dd, *J*=8.4, 0.9 Hz, H-4), and 6.78 (1H, dd, *J*=8.4, 0.9 Hz, H-2). In the

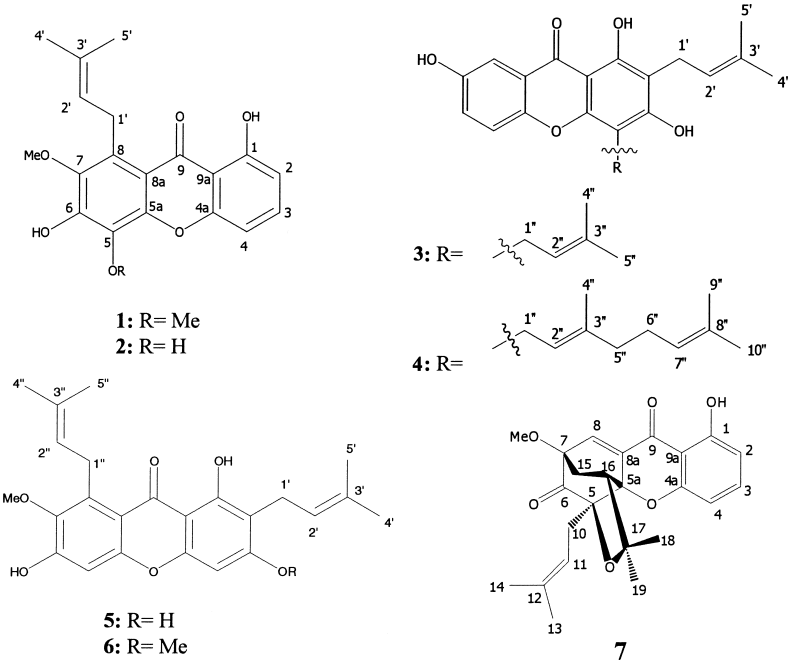

Fig. 1. Structures of Compounds **1**—**7**

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Table 1. ^{1}H-, ^{13}C- and DEPT Spectral Data of **1** and **2** ($CDCl_3$)

Position	**1**		**2**		DEPT[a]
	^{1}H	^{13}C	^{1}H	^{13}C	
1	—	162.2	—	161.8	C
2	6.78 (1H, dd, J=8.4, 0.9 Hz)	110.6	6.78 (1H, dd, J=8.1, 0.9 Hz)	111.2	CH
3	7.53 (1H, t, J=8.4 Hz)	136.1	7.52 (1H, t, J=8.1 Hz)	136.1	CH
4	6.91 (1H, dd, J=8.4, 0.9 Hz)	106.2	6.87 (1H, dd, J=8.1, 0.9 Hz)	106.8	CH
4a	—	155.2	—	154.6	C
5	—	145.4	—	132.1	C
5a	—	135.9	—	144.0	C
6	—	143.3	—	144.8	C
7	—	147.3	—	143.8	C
8	—	128.4	—	128.2	C
8a	—	114.5	—	111.5	C
9	—	183.6	—	184.1	C
9a	—	109.2	—	109.3	C
1′	4.04 (2H, d, J=6.6 Hz)	25.4	4.03 (2H, d, J=7.2 Hz)	25.8	CH_2
2′	5.21 (1H, m)	123.5	5.22 (1H, m)	123.7	CH
3′	—	131.7	—	133.0	C
4′	1.69 (3H, d, J=1.5 Hz)	25.9	1.69 (3H, d, J=1.2 Hz)	26.0	CH_3
5′	1.85 (3H, br s)	18.2	1.84 (3H, s)	18.1	CH_3
1-OH	13.10 (1H, s)	—	13.15 (1H, s)	—	—
5-OMe	4.10 (3H, s)	61.1	—	—	CH_3
7-OMe	3.82 (3H, s)	61.1	3.85 (3H, s)	63.1	CH_3

a) Analysis by DEPT 90° and 135°.

^{13}C-NMR spectrum, six oxygenated aromatic carbon signals appeared at δ 162.2 (C-1), 155.2 (C-4a), 147.3 (C-7), 145.4 (C-5), 143.3 (C-6), and 135.9 (C-5a). The above information indicated that compound **1** has a xanthone skeleton,[1,2,5] which was also supported by the UV spectrum (237, 256, 323, 380 nm).[1,2] The location of the prenyl side chain was assigned at C-8 (δ 114.5) based on the HMBC correlations of H-1′/C-8 (two-bond), H-1′/C-8a (three-bond), and H-1′/C-7 (three-bond) (Fig. 2). The position of two methoxyl groups (δ 4.10, 3.82) was established by the NOEDIFF and HMBC experiments. Only the 2H-1′ methylene protons at δ 4.04 gave NOE enhancement with the methoxyl protons at δ 3.82 (Fig. 2). In addition, the methoxyl protons at δ 3.82 and the 2H-1′ methylene protons at δ 4.04 showed correlation with C-7 (147.3) while the methoxyl protons at δ 4.10 showed correlation with C-5 (145.4) in the HMBC spectrum. The above observation concluded that the methoxyl groups at δ 3.82 and 4.10 should be located at C-7 and C-5, respectively. Therefore, the structure of **1** was characterized as 5-*O*-methylcelebixanthone.

The other six known compounds were identified as celebixanthone (**2**),[8] 1,3,7-trihydroxy-2,4-di(3-methylbut-2-enyl)xanthone (**3**),[4] cochinchinone A (**4**),[5] α-mangostin (**5**),[9] β-mangostin (**6**)[9] and cochinchinone C (**7**)[5] by the analysis of 1D and 2D NMR information and by comparison with their reported physical and spectroscopic data.

As summarized in Table 2, all compounds were evaluated cytotoxic activity against the NCI-H187 (human lung cancer) cell line and for antimalarial activity against *P. falciparum*. Compounds **2** and **4**—**7** exhibited cytotoxic effect with IC_{50} values in the range of 0.65—5.2 μg/ml (Table 2). Xanthone **4** had the highest activity with IC_{50} 0.65 μg/ml. No cytotoxicity was observed with **1** and **3**. Compounds **1**, **2**, **6** and **7** also exhibited antimalarial activity (Table 2) with IC_{50} values of 3.2, 4.9, 7.2 and 2.6 μg/ml, respectively and no activity was observed in **3**—**5**. All compounds were found to be inactive with KB (human epidermoid carcinoma in the mouth) and BC (human breast cancer cells) cancer cell lines. It is interesting to note that the structural difference between 5-*O*-methylcelebixanthone (**1**) and celebixanthone (**2**) is only at C-5. Compound **1** possesses a methoxyl group at C-5 while **2** has a hydroxyl moiety which plays an important role in the

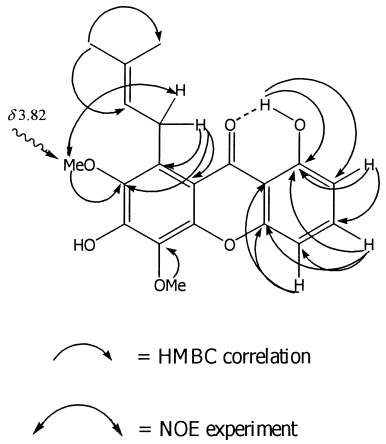

Fig. 2. HMBC Correlation and Selective NOE Experiment of **1**

Table 2. Biological Activities of **1**—**7**

Compound	Cytotoxicity[a] (IC_{50}, μg/ml)	Antimalaria[b] (IC_{50}, μg/ml)
1	Inactive[c]	3.2
2	5.2	4.9
3	Inactive[c]	Inactive[c]
4	0.65	Inactive[c]
5	2.4	Inactive[c]
6	1.7	7.2
7	2.3	2.6

a) Against NCI-H187 (human lung cancer) cell line. *b*) Against *Plasmodium falciparum*. *c*) Inactive at >20 μg/ml.

cytotoxicity against NCI-H187 cell line. In the case of **3** and **4** (**3** possesses a prenyl group at C-4, while **4** contains a geranyl group), the presence of a geranyl side chain is crucially important for the cytotoxic activity and both compounds are found to be inactive for antimalarial activity. Structural variation between α-mangostin (**5**) and β-mangostin (**6**) also corresponds to the remarkably different activity. The only difference between **5** and **6** is a substituent at C-3. Compound **5** contains a hydroxyl group but **6** has a methoxyl moiety. This methoxyl substituent appears to be particularly responsible for the antimalarial activity against *P. falciparum*.

Experimental

General Experimental Procedures Melting points were determined on an electrothermal melting point apparatus and are uncorrected. UV spectra were measured with a SPECORD S100 spectrophotometer (Analytikjena). The IR spectra were measured with a FTS FT-IR Perkin Elmer spectrophotometer, whereas ^{1}H- and ^{13}C-NMR spectra were recorded in $CDCl_3$ using Bruker Avance 300 MHz and/or Varian Unity Inova 500 MHz spectrometers, respectively. The EI-MS and HR-EI-MS mass spectra were obtained from a MAT 95 XL mass spectrometer. Quick column chromatography (QCC) and column chromatography (CC) were carried out on Si gel 60 F_{254} (Merck) and Si gel 100, respectively. Precoated plates of Si gel 60 GF_{254} were used for analytical purposes.

Plant Material The roots of *C. cochinchinense* were collected in October, 2003 at Thabo district, Nong Khai Province, northeastern part of Thailand. The plant was identified by Professor Puangpen Sirirugsa and the voucher specimen (SL-1 (PSU)) has been deposited at Department of Biology, Faculty of Science, Prince of Songkla University, Songkhla, Thailand.

Extraction and Isolation Air-dried roots of *C. cochinchinense* (2 kg) were extracted with hexane and methylene chloride, successively. The hexane extract (17 g) was subjected to quick column chromatography (QCC) over silica gel and eluted with a gradient of hexane–EtOAc to afford nine fractions (F1—F9). Fraction F1 (500.8 mg), upon standing at room temperature, gave compound **7**. Fraction F2 (10 g) was subjected to column chromatography (CC) using 20% EtOAc–hexane as eluent to give seven subfractions. F2-2 (1.2 g), upon standing at room temperature, yielded compound **7** (500.2 mg). The yellow solid of subfractions F2-3 (724 mg) was washed with hexane to afford a yellow solid (420 mg) which was subjected to CC with 20% EtOAc–hexane to yield **3** (7 mg), **4** (88.4 mg) and the mixture of **1** and **6** (51 mg). Separation of **1** and **6** (51 mg) by preparative TLC using 20% EtOAc–hexane as mobile phase afforded pure **1** (20 mg) and **6** (13 mg). Subfractions F2-7 (500 mg) and fraction F3 (400 mg), upon standing at room temperature, yielded **5** (20.2 mg) and **2** (15 mg), respectively.

Bioassay Procedures The cytotoxicity assay employed the colorimetric method.[10] Ellipticine, the reference substance, exhibited activity toward NCI-H187 cell line (IC_{50} 0.35±0.15 μg/ml). Antimalarial activity was evaluated against the parasite *Plasmodium falciparum* (K_1, mutidrug resistant), using the method of Trager and Jensen.[11] Quantitative assessment of *in vitro* malarial activity was determined by means of the microculture radioisotope technique based on the method described by Desjardins *et al.*[12] The inhibitory concentration (IC_{50}) represented the concentration that caused 50% reduction in parasite growth which was indicated by the *in vitro* uptake of [^{3}H]-hypoxanthine by *P. falciparum*. The standard compound was dihydroartemisnin (IC_{50} 4.5 nM).

5-*O*-Methylcelebixanthone (**1**): Yellow solid, mp 158—160 °C. IR (KBr) cm^{-1}: 3357, 1646, 1601, 1579. UV λ_{max} (MeOH) nm (log ε): 237 (4.24), 256 (4.17), 323 (3.78), 380 (3.41). EI-MS *m*/*z* 356 (28) (M^+). HR-EI-MS *m*/*z* $[M]^+$ 356.1229 (Calcd for $C_{20}H_{20}O_6$, 356.1259). δ_H ($CDCl_3$, 300 MHz) and δ_C ($CDCl_3$, 75 MHz), see Table 1.

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