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A NEW DEPSIDONE FROM THE TWIGS OF *GARCINIA COWA*

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Abstract – A new depsidone, cowadepsidone (**7**), along with six known xanthones (**1**-**6**) were isolated from the twigs of *Garcinia cowa*. Their structures were determined on the basis of spectroscopic methods. The cytotoxicity against KB, MCF-7 and NCI-H187 cancer cell lines of compounds **2**-**7** were also reported.

The tropical family Clusiaceae is well known to be a rich source of isoprenylated xanthones, depsidones and biflavonoids.[1-4] In particular, the genus *Garcinia* has also provided many bioactive isoprenylated and rearranged xanthonoids and biflavonoids.[3-7] In our continuing phytochemical study of Thai medicinal plants, we have found many polyoxygenated xanthones from the barks and dried fruits of *Cratoxylum cochinchinense*.[8,9] In this paper, we describe the isolation and structural elucidation of a new depsidone together with six known xanthones (Figure 1) as well as their cytotoxic activity.

Compound **7** was isolated as a red gum. Its molecular formula was established as $C_{19}H_{18}O_7$ by ESITOFMS at *m/z* 359.1133 $[M+H]^+$, suggesting the presence of 11 degrees of unsaturations and supported by NMR data (Table 1). The IR spectrum showed absorption bands for hydroxyl group (3363 cm^{-1}) and a lactone carbonyl group chelated to an *ortho*-hydroxyl group (1658 cm^{-1}). The presence of the latter functionality was confirmed by resonances at δ 168.0 (C-11) and δ 10.98 (OH-1). The ^{1}H NMR data of **7** displayed signals of two aromatic protons at δ 6.70 (1H, s, H-6) and 6.27 (2H, br s, H-2, H-4).

Furthermore, the proton signals at δ 5.17 (1H, br s, H-13), 3.47 (2H, d, J = 6.8 Hz, H_2-12), 1.80 (3H, s, H_3-15), and 1.67 (3H, s, H_3-16) suggested the presence of a prenyl moiety in the structure.[9] In addition, a methoxyl signal at δ 3.77 (3H, s) was also observed in the ^{1}H NMR spectrum. Analyzing the 2D NMR spectra using HMQC and HMBC techniques enabled the assignment of ^{1}H and ^{13}C NMR signals. By comparing the NMR data of **7** with those of the known compound, garcinisidone-A,[4] the possible structure of **7** was established suggesting the same core structure for both compounds.

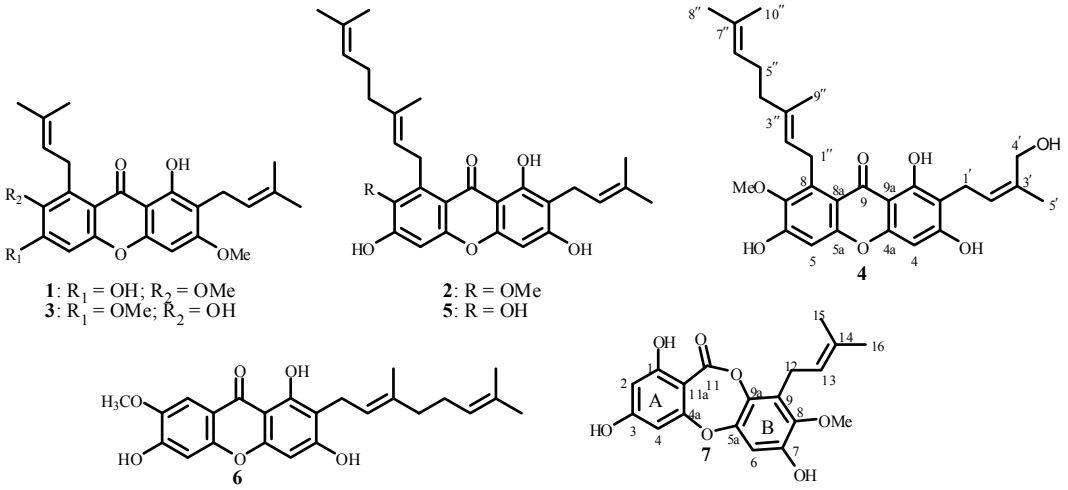

Figure 1. Structures **1**-**7**

Table 1. NMR spectral data (400 MHz, $CDCl_3$) of **7** in $CDCl_3$

No.	δ_H (mult., J in Hz)	δ_C	HMBC (^{1}H→^{13}C)
1	10.98 (s)	165.5 (s)	-
2	6.27 (br s)	100.6 (d)*	1, 3, 4, 11a
3	-	163.7 (s)	-
4	6.27 (br s)	100.5 (d)*	2, 3, 4a, 11a
4a	-	161.8 (s)	-
5a	-	146.5 (s)	-
6	6.70 (s)	105.5 (d)	5a, 8, 9a
7	-	147.0 (s)	-
8	-	142.7 (s)	-
9	-	128.3 (s)	-
9a	-	136.0 (s)	-
11	-	168.0 (s)	-
11a	-	98.8 (s)	-
12	3.47 (d, 6.8)	24.1 (t)	8, 9, 9a, 13, 14
13	5.17 (br s)	121.2 (d)	9, 15, 16
14	-	133.2 (s)	
15	1.80 (s)	18.0 (q)	13, 14, 16
16	1.67 (s)	25.7 (q)	13, 14, 15
OMe	3.77 (s)	61.8 (q)	8

* Interchangeable

The HMBC correlations of the methoxyl protons at δ 3.77 with the oxygenated carbon at δ 142.7 (C-8) and those of the methylene protons of a prenyl unit at δ 3.47 (H_2-12) with the carbons at δ 142.7 (C-8), 136.0 (C-9a), and 128.3 (C-9) established the attachment of the methoxyl group and the prenyl side chain at C-8 and C-9, respectively. The aromatic proton at δ 6.70 (H-6) showed HMBC connectivity to three aromatic carbons at δ 146.5 (C-5a), 142.7 (C-8) and 136.0 (C-9a), confirming the location of substituents on the B ring. Furthermore, the correlation of aromatic protons at δ 6.27 (H-2, H-4) with the aromatic carbons at δ 165.5 (C-1), 163.7 (C-3), 161.8 (C-4a), 100.6 (C-2), 100.5 (C-4) and 98.8 (C-11a) indicated the orientation of substituents on the A ring. The quaternary carbon signals of δ 165.5 (C-1), 163.7 (C-3), 147.0 (C-7) and its molecular formula $C_{19}H_{18}O_7$ indicated the presence of three hydroxy groups at C-1, C-3 and C-7, respectively. Thus, compound **7** was determined as cowadepsidone which reported for the first time as a metabolite of *G. cowa*. The remaining compounds were identified as β-mangostin (**1**),[9] cowanin (**2**),[10] 3,6-di-*O*-methyl-γ-mangostin (**3**),[11] cowanol (**4**),[10] norcowanin (**5**),[12] and cowaxanthone (**6**)[10] by the analysis of 1D and 2D NMR spectra and by comparison with their reported physical and spectroscopic data.

Table 2. Cytotoxicity of compounds **2-7**

Compounds	Cytotoxicity (μg/mL)		
	NCI-H187	KB	MFC-7
2	7.03	7.36	21.38
3	8.58	6.64	10.59
4	37.26	32.34	34.62
5	5.92	6.43	18.85
6	3.87	15.43	15.45
7	31.47	inactive	36.03
ellipticin	1.06	-	-
doxorubicin	-	9.61	9.17

As summarized in Table 2, compounds **2-7** were evaluated for their cytotoxicity against KB (oral human epidermal carcinoma), MCF-7 (breast cancer) and NCI-H187 (human, small cell lung cancer) cancer cell lines and all of them exhibited cytotoxic effect against all three cancer cell lines, except a new depsidone **7** was found to be inactive with KB cancer cell line (Table 2). Xanthones **2**, **3** and **5** exhibited strong cytotoxicity against KB cancer cell line with the IC_{50} value ranging from 6.43-7.36 μg/mL, which were stronger than doxorubicin, a standard drug, (IC_{50} 9.61 μg/mL) while **4** and **6** were found to be weakly activity. Xanthones **2**, **3**, **5** and **6** also showed strong inhibitory effect against NCI-H187 cancer cell line with the range of IC_{50} 3.87-8.58 μg/mL and xanthone **6** had the highest cytotoxicity (IC_{50} 3.87 μg/mL) whereas **4** and **7** exhibited weak activity. In case of cytotoxicity against MCF-7 cancer cell line, xanthone

3 was the best cytotoxicity with the IC_{50} value of 10.59 μg/mL. All the rest of compounds were found to be moderately to weakly active with the IC_{50} values ranging from 15.45-36.03 μg/mL.

It is interesting to note that the structural difference between xanthones **2** and **4** is only at C-4′. Xanthone **4** possesses a methylenehydroxyl moiety while **2** has a methyl group which plays an important role in the cytotoxicity against all three human cancer cell lines. In case of xanthones **2** and **5**, (**2** possesses a methoxyl group at C-7, while **5** contains a hydroxyl group), both of methoxyl and hydroxyl groups seemed to be no effective in the cytotoxicity.

EXPERIMENTAL

GENERAL

UV spectra were recorded with a Perkin-Elmer UV-Vis spectrophotometer. The IR spectra were recorded with a Perkin-Elmer FTS FT-IR spectrophotometer. The NMR spectra were recorded using 400 MHz Bruker spectrometer. Chemical shifts were recorded in parts per million (δ) in $CDCl_3$ with tetramethylsilane (TMS) as an internal reference. The ESITOFMS was obtained from a Micromass LTC mass spectrometer. 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 F254 were used for analytical purposes.

PLANT MATERIAL

The twigs of *G. cowa* were collected from Nong Khai Province in March 2010. The plant specimen (MFU-NPR 0014) has been deposited at Natural Products Chemistry Laboratory, Mae Fah Luang University, Chiang Rai, Thailand.

EXTRACTION AND ISOLATION

The air-dried twigs of *G. cowa* (3.34 kg) were successively extracted with *n*-hexane and acetone over a period of 3 days each at room temperature. The *n*-hexane extract (21.36 g) was subjected to QCC over silica gel eluted with a gradient of *n*-hexane- EtOAc (100% *n*-hexane to 100% EtOAc) to provide five fractions (A-E). Fraction B (145.9 mg) was further purified by CC with 10% acetone- *n*-hexane to give compound **3** (6.8 mg) whereas compounds **1** (17.1 mg) and **2** (6.1 mg) were obtained from fraction D (124.2 mg) by repeated CC with 15% acetone-*n*-hexane. Fraction E (1.63 g) was subjected to repeated CC with 20% acetone- *n*-hexane to afford compounds **4** (165.2 mg), **5** (40.1 mg), **6** (30.2 mg) and **7** (10.1 mg).

Cowadepsidone (**7**): Red gum; UV ($CHCl_3$) λ_{max} (log ε): 204 (4.63), 273 (4.10), 313 (3.51), 433 (2.77); IR (neat) ν_{max} cm^{-1}: 3363 (OH), 1658 (C=O); ^1H NMR (400 MHz) and ^{13}C NMR (100 MHz) see Table 1; ESITOFMS (*m/z*): $[M+H]^+$ *m/z* 359.1133 (calcd for $C_{19}H_{19}O_7$, 359.1131).

CYTOTOXIC ASSAY

The procedures for cytotoxic assay were performed by resazurin microplate assay (REMA) which was a modified method of fluorescent dye for the mammalian cell cytotoxicity according to Brien *et al*.[13] In this study, three cancer cell lines, KB (oral cavity cancer), MCF7 (breast cancer) and NCI-H187 (small cell lung cancer) were used. Ellipticin and doxorubicin were the reference substances in this study and the IC_{50} values are summarized in Table 2.

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