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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 xanthenes (**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 xanthenes, depsidones and biflavonoids.<sup>1-4</sup> In particular, the genus *Garcinia* has also provided many bioactive isoprenylated and rearranged xanthonoids and biflavonoids.<sup>3-7</sup> In our continuing phytochemical study of Thai medicinal plants, we have found many polyoxygenated xanthenes from the barks and dried fruits of *Cratoxylum cochinchinense*.<sup>8,9</sup> In this paper, we describe the isolation and structural elucidation of a new depsidone together with six known xanthenes (Figure 1) as well as their cytotoxic activity.

Compound **7** was isolated as a red gum. Its molecular formula was established as C<sub>19</sub>H<sub>18</sub>O<sub>7</sub> by ESITOFMS at *m/z* 359.1133 [M+H]<sup>+</sup>, 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<sup>-1</sup>) and a lactone carbonyl group chelated to an *ortho*-hydroxyl group (1658 cm<sup>-1</sup>). The presence of the latter functionality was confirmed by resonances at  $\delta$  168.0 (C-11) and  $\delta$  10.98 (OH-1). The <sup>1</sup>H NMR data of **7** displayed signals of two aromatic protons at  $\delta$  6.70 (1H, s, H-6) and 6.27 (2H, br s, H-2, H-4).

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

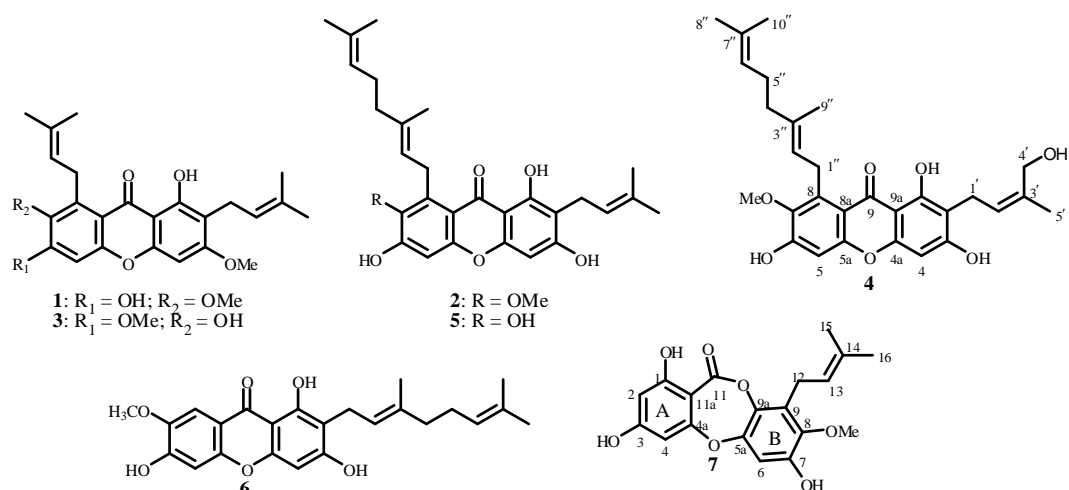


Figure 1. Structures 1-7

Table 1. NMR spectral data (400 MHz, CDCl<sub>3</sub>) of **7** in CDCl<sub>3</sub>

No.	$\delta_{\text{H}}$ (mult., $J$ in Hz)	$\delta_{\text{C}}$	HMBC ( <sup>1</sup> H→ <sup>13</sup> 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  $\delta$  3.77 with the oxygenated carbon at  $\delta$  142.7 (C-8) and those of the methylene protons of a prenyl unit at  $\delta$  3.47 (H<sub>2</sub>-12) with the carbons at  $\delta$  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  $\delta$  6.70 (H-6) showed HMBC connectivity to three aromatic carbons at  $\delta$  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  $\delta$  6.27 (H-2, H-4) with the aromatic carbons at  $\delta$  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  $\delta$  165.5 (C-1), 163.7 (C-3), 147.0 (C-7) and its molecular formula C<sub>19</sub>H<sub>18</sub>O<sub>7</sub> 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  $\beta$ -mangostin (**1**),<sup>9</sup> cowanin (**2**),<sup>10</sup> 3,6-di-*O*-methyl- $\gamma$ -mangostin (**3**),<sup>11</sup> cowanol (**4**),<sup>10</sup> norcowanin (**5**),<sup>12</sup> and cowaxanthone (**6**)<sup>10</sup> 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 ( $\mu\text{g/mL}$ )		
	NCI-H187	KB	MFC-7
<b>2</b>	7.03	7.36	21.38
<b>3</b>	8.58	6.64	10.59
<b>4</b>	37.26	32.34	34.62
<b>5</b>	5.92	6.43	18.85
<b>6</b>	3.87	15.43	15.45
<b>7</b>	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). Xanthenes **2**, **3** and **5** exhibited strong cytotoxicity against KB cancer cell line with the IC<sub>50</sub> value ranging from 6.43-7.36  $\mu\text{g/mL}$ , which were stronger than doxorubicin, a standard drug, (IC<sub>50</sub> 9.61  $\mu\text{g/mL}$ ) while **4** and **6** were found to be weakly activity. Xanthenes **2**, **3**, **5** and **6** also showed strong inhibitory effect against NCI-H187 cancer cell line with the range of IC<sub>50</sub> 3.87-8.58  $\mu\text{g/mL}$  and xanthone **6** had the highest cytotoxicity (IC<sub>50</sub> 3.87  $\mu\text{g/mL}$ ) 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<sub>50</sub> value of 10.59 µg/mL. All the rest of compounds were found to be moderately to weakly active with the IC<sub>50</sub> values ranging from 15.45-36.03 µg/mL.

It is interesting to note that the structural difference between xanthenes **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 xanthenes **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<sub>3</sub> 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<sub>3</sub>)  $\lambda_{\max}$  (log  $\epsilon$ ): 204 (4.63), 273 (4.10), 313 (3.51), 433 (2.77); IR (neat)  $\nu_{\max}$  cm<sup>-1</sup>: 3363 (OH), 1658 (C=O); <sup>1</sup>H NMR (400 MHz) and <sup>13</sup>C NMR (100 MHz) see Table 1; ESITOFMS (*m/z*): [M+H]<sup>+</sup> *m/z* 359.1133 (calcd for C<sub>19</sub>H<sub>19</sub>O<sub>7</sub>, 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.*<sup>13</sup> 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<sub>50</sub> values are summarized in Table 2.

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