

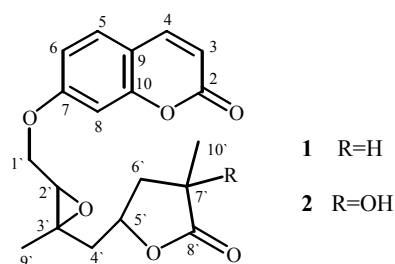
**O - T E R P E N O I D A L C O U M A R I N S F R O M  
*CLAUSENA EXCAVATA***

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**Abstract** - A new *O*-terpenoidal coumarin named excavacoumarin A (**1**) and a known one (**2**) were isolated from the leaves of *Clausena excavata* Burm. f. (Rutaceae) collected in Xishuangbanna, Yunnan. The structure of **1** was elucidated by spectroscopic analysis.

*Clausena excavata* Burm. f. (Rutaceae) is a bush widely distributed in Yunnan, China. The leaves and barks of this plant have been used as folk medicines for the treatment of dysentery, enteritis, and urethra infection.<sup>1</sup> *Clausena* species are known to be the rich sources of carbazole alkaloids and coumarins.<sup>2-3</sup> The constituents of *C. excavata* collected in Xishuangbanna have been studied<sup>4</sup> and this paper described the isolation and structure elucidation of a new *O*-terpenoidal coumarin, excavacoumarin A (**1**) and a known one (**2**)<sup>5</sup>.



**Figure 1.** Chemical structure of *O*-terpenoidal coumarins (**1-2**)

Excavacoumarin A (**1**), a white amorphous powder. HREIMS gave the formula as C<sub>19</sub>H<sub>20</sub>O<sub>6</sub> (at *m/z*

344.1261 [M]<sup>+</sup>, calcd 344.1260). The UV (240, 248.5, 290, 322.5 nm) and IR (1759, 1734, 1616 cm<sup>-1</sup>) spectrum suggested the presence of coumarin nucleus and two lactones.<sup>3</sup> The <sup>1</sup>H NMR spectra showed the characteristic signals of a typical AB system at δ<sub>H</sub> 6.33, 7.67 (1H each, d, J=9.5) assigned to H-3, H-4, respectively (**Table 1**). The <sup>1</sup>H and <sup>13</sup>C NMR spectra were similar to those of excavatin K<sup>5,6</sup> except for C-6', C-7' and C-10'. The difference in the <sup>13</sup>C NMR spectrum was the presence of two high field signals at δ<sub>C</sub> 35.8 (d) and 37.8 (t) (**Table 1**), and the absence of two double bond signals of excavatin K, indicating that **1** was the C-6'/C-7' saturated form of excavatin K.

**2**, a white amorphous powder. The molecular formula was obtained as C<sub>19</sub>H<sub>20</sub>O<sub>7</sub> based on HREIMS (at *m/z* 360.1212 [M]<sup>+</sup>, calcd 360.1209). The UV (239.5, 252, 292.5, 323 nm) and IR (1766, 1707, 1615 cm<sup>-1</sup>) spectrum suggested that the presence of coumarin nucleus and two lactones.<sup>3</sup> The <sup>1</sup>H and <sup>13</sup>C NMR spectra were similar to those of **1** except for C-6' and C-7' (**Table 1**). By means of HMBC experiment, the <sup>1</sup>H-<sup>13</sup>C long-range correlations between H-10' (δ<sub>H</sub> 1.66, s, 3H) and δ<sub>C</sub> 44.8t (C-6'), δ<sub>C</sub> 73.1s (C-7'), δ<sub>C</sub> 178.3s (C-8'); and between H-4' (δ<sub>H</sub> 2.05m, 1.96m, each 1H) and δ<sub>C</sub> 61.3d (C-2'), 58.5s (C-3'), 75.2d (C-5'), 44.8t (C-6'), 73.1s (C-7'), 17.1q (C-9') were observed, indicating that **2** was the C-7' hydroxylation of **1**.

The stereochemistry of those *O*-terpenoidal coumarins (**1-2**) remained to be determined. So far, the stereochemistry of this type of *O*-terpenoidal coumarins reported previously was not resolved.<sup>3-6</sup> Further structure elucidation on the stereochemistry pertaining to the C-2', C-3', C-5' and C-7' of **1**, **2**, and **3** is in the progress.

**Table 1.** The <sup>1</sup>H and <sup>13</sup>C NMR assignments for compounds (**1-2**) in C<sub>5</sub>D<sub>5</sub>N.

No.	<b>1</b> <sup>a</sup>		<b>2</b> <sup>b</sup>	
	<sup>1</sup> H <sup>b</sup>	<sup>13</sup> C	<sup>1</sup> H <sup>b</sup>	<sup>13</sup> C
2	/	160.8s	/	160.8s
3	6.33 (d, 9.5)	113.5d	6.33 (d, 9.5)	113.6d
4	7.67 (d, 9.5)	143.8d	7.68 (d, 9.5)	143.8d
5	7.42 (d, 8.6)	129.6d	7.42 (d, 8.6)	129.6d
6	6.99 (dd, 8.6, 2.5)	113.1d	6.99 (dd, 8.6, 2.4)	113.1d

7	/	162.2s	/	162.2s
8	7.04 (d, 2.5)	102.2d	7.06 (d, 2.4)	102.3d
9	/	113.3s	/	113.3s
10	/	156.3s	/	156.4s
1'	4.48 (dd, 11.2, 3.1)	68.3t	4.49 (dd, 11.1, 5.3)	68.3t
	4.27 (dd, 11.2, 6.7)		4.23 (dd, 11.1, 2.6)	
2'	3.37 (dd, 6.7, 3.1)	61.3d	3.41 (dd, 5.3, 2.6)	61.3d
3'	/	58.5s	/	58.5s
4'	1.80-1.84 (m, 2H)	44.5t	1.96-2.05 (m, 2H)	44.4t
5'	5.00 (m)	75.6d	5.15 (m)	75.2d
6'	1.92 (m)	37.8t	2.64 (m)	44.8t
	1.60 (m)		1.95 (m)	
7'	2.38 (m)	35.8d	/	73.1s
8'	/	179.1s	/	178.3s
9'	1.44 (s, 3H)	17.1q	1.45 (s, 3H)	17.1q
10'	1.18 (d, 7.9, 3H)	15.1q	1.66 (s, 3H)	23.8q

<sup>a</sup> <sup>1</sup>H and <sup>13</sup>C NMR spectra were obtained at 400 and 100 MHz, respectively, and recorded at room temperature.

<sup>b</sup> Coupling constants were presented in Hertz, unless otherwise indicated, all proton signals integrate to 1H.

## EXPERIMENTAL

General Experimental Procedures – The mps were determined on an XRC-1 micromelting apparatus and were uncorrected. The UV spectra were obtained using an UV-210A spectrophotometer. The IR spectra were measured on a Perkin-Elmer-577 spectrophotometer. MS were performed on an Autospec-3000 spectrometer under 70 eV. 1D NMR spectra were recorded on a Bruker AM-400 spectrometer. 2D NMR spectra were recorded on a Bruker DRX-500 spectrometer.

Plant Material -- The aerial part of *Clausena excavata* Burm. f. was collected in Yunnan, China. A voucher specimen of this plant was deposited in Kunming Institute of Botany, Kunming, China.

Extraction and Isolation -- The powdered aerial part of *C. excavata* (6.0 kg) was extracted with 90% EtOH (12 L×3) under reflux for 24 h. The extract (620 g) was chromatographed over silica gel and eluted with CHCl<sub>3</sub>, CHCl<sub>3</sub> - EtOAc, EtOAc and MeOH, successively. The CHCl<sub>3</sub> - EtOAc elute (60 g) was subjected to silica gel column (petroleum - EtOAc 7:3, 6:4, 1:1, 4:6, 3:7) to give excavacoumarin A (**1**) (15 mg) and excavatin M (**2**) (150 mg).

Excavacoumarin A (**1**), amorphous powder, mp 87-89°C (from Me<sub>2</sub>CO),  $[\alpha]_{\text{D}}^{25.9} +22.96^{\circ}$  (c 3.92, CHCl<sub>3</sub>); UV  $\lambda_{\text{max}}^{\text{MeOH}}$  nm: 240, 248.5, 290, 322.5; IR  $\nu_{\text{max}}^{\text{KBr}}$  cm<sup>-1</sup>: 3445, 3086, 2974, 2935, 1759, 1734, 1616, 1510, 1282, 1164, 1126, 1021, 929, 838; EIMS (*m/z*, %): 344(M<sup>+</sup>, 10), 162 (25), 134 (18), 99 (100); HREIMS *m/z* 344.1261 (calcd for C<sub>19</sub>H<sub>20</sub>O<sub>6</sub> 344.1260); <sup>1</sup>H NMR and <sup>13</sup>C NMR see **Table 1**.

Compound (**2**), amorphous powder, mp 182-183.5°C (from Me<sub>2</sub>CO), lit<sup>5</sup>. 173-175°C,  $[\alpha]_{\text{D}}^{20.9} +29.41^{\circ}$  (c 3.06, C<sub>5</sub>H<sub>5</sub>N); UV  $\lambda_{\text{max}}^{\text{MeOH}}$  nm: 239.5, 252, 292.5, 323; IR  $\nu_{\text{max}}^{\text{KBr}}$  cm<sup>-1</sup>: 3487, 3084, 2961, 2940, 1766, 1707, 1615, 1353, 1284, 1208, 1128, 1103, 994, 859, 838; EIMS (*m/z*, %): 360 [M]<sup>+</sup> (65), 273 (16), 175 (25), 162 (100), 134 (94), 87 (99); HREIMS *m/z* 360.1212 (calcd for C<sub>19</sub>H<sub>20</sub>O<sub>7</sub> 360.1209); <sup>1</sup>H NMR and <sup>13</sup>C NMR see **Table 1**.

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## REFERENCES AND NOTES

1. Institutum Botanicum Kunmingense Academiae Sinicae, "*Flora Yunnanica*, Tomus 6 (Spermatophyta)", p. 759, ed. by, Institutum Botanicum Kunmingense Academiae Sinicae, Science Press, Beijing, 1995.
2. T. S. Wu, S. C. Huang, and P. L. Wu, *Heterocycles*, 1997, **45**, 969.
3. K. Nakamura, Y. Takemura, M. Ju-ichi, , C. Ito, and H. Furukawa, *Heterocycles*, 1998, **48**, 549.
4. L. J. Shang, G. Y. Wen, J. Zhou, and X. J. Hao, *Acta Botanica Yunnanica*, 1993, **15**, 299.
5. T. T. Thuy, H. Ripperger, A. Porzel, T. V. Sung, and G. Adam, *Phytochemistry*, 1999, **52**, 511.
6. F. Bohlmann and E. Clausen, *Chem. Ber.* , 1970, **103**, 3619.