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O-TERPENOIDAL COUMARINS FROM *CLAUSENA EXCAVATA*

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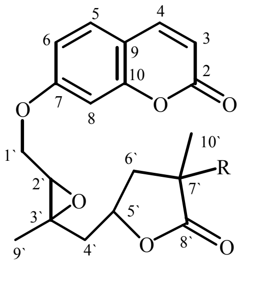
Abstract - A new *O*-terpenoidal coumarin named excavacoumarin A (**1**) and a known one (**2**) were isolated from the leaves of *Clausena excavata* Burm. f. (Rutaceae) collected in Xishuangbanna, Yunnan. The structure of **1** was elucidated by spectroscopic analysis.

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.[1] *Clausena* species are known to be the rich sources of carbazole alkaloids and coumarins.[2-3] The constituents of *C. excavata* collected in Xishuangbanna have been studied[4] and this paper described the isolation and structure elucidation of a new *O*-terpenoidal coumarin, excavacoumarin A (**1**) and a known one (**2**)[5].



1 R=H

2 R=OH

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

Excavacoumarin A (**1**), a white amorphous powder. HREIMS gave the formula as $C_{19}H_{20}O_6$ (at *m*/*z*

344.1261 $[M]^+$, calcd 344.1260). The UV (240, 248.5, 290, 322.5 nm) and IR (1759, 1734, 1616 cm^{-1}) spectrum suggested the presence of coumarin nucleus and two lactones.[3] The ^1H NMR spectra showed the characteristic signals of a typical AB system at δ_H 6.33, 7.67 (1H each, d, J=9.5) assigned to H-3, H-4, respectively (**Table 1**). The ^1H and ^{13}C NMR spectra were similar to those of excavatin K[5, 6] except for C-6', C-7' and C-10'. The difference in the ^{13}C NMR spectrum was the presence of two high field signals at δ_C 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 $C_{19}H_{20}O_7$ based on HREIMS (at *m*/*z* 360.1212 $[M]^+$, calcd 360.1209). The UV (239.5, 252, 292.5, 323 nm) and IR (1766, 1707, 1615 cm^{-1}) spectrum suggested that the presence of coumarin nucleus and two lactones.[3] The ^1H and ^{13}C NMR spectra were similar to those of **1** except for C-6' and C-7' (**Table 1**). By means of HMBC experiment, the ^1H-^{13}C long-range correlations between H-10' (δ_H 1.66, s, 3H) and δ_C 44.8t (C-6'), δ_C 73.1s (C-7'), δ_C 178.3s (C-8'); and between H-4' (δ_H 2.05m, 1.96m, each 1H) and δ_C 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.[3-6] 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 ^1H and ^{13}C NMR assignments for compounds (**1**-**2**) in C_5D_5N.

No.	**1**[a]		**2**[b]	
	^1H [b]	^{13}C	^1H [b]	^{13}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

[a] ^{1}H and ^{13}C NMR spectra were obtained at 400 and 100 MHz, respectively, and recorded at room temperature.

[b] 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 spectrophatometer. The IR spectra were measured on a Perkin-Elmer-577 spctrophotometer. MS were performed on an Autospec-3000 spectrometer under 70 eV. 1D NMR spectra were recorded on a Brucker AM-400 spectrometer. 2D NMR spectra were recorded on a Brucker DRX-500 spectrometer.

Plant Materal -- 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 $CHCl_3$, $CHCl_3$ - EtOAc, EtOAc and MeOH, successively. The $CHCl_3$ - 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_2CO), $[\alpha]_D^{25.9}$ +22.96° (c 3.92, $CHCl_3$); UV λ_{max}^{MeOH} nm: 240, 248.5, 290, 322.5; IR ν_{max}^{KBr} cm^{-1}: 3445, 3086, 2974, 2935, 1759, 1734, 1616, 1510, 1282, 1164, 1126, 1021, 929, 838; EIMS (*m/z*, %): 344(M^+, 10), 162 (25), 134 (18), 99 (100); HREIMS *m/z* 344.1261 (calcd for $C_{19}H_{20}O_6$ 344.1260); ^1H NMR and ^{13}C NMR see **Table 1**.

Compound (**2**), amorphous powder, mp 182-183.5°C (from Me_2CO), lit[5]. 173-175°C, $[\alpha]_D^{20.9}$ +29.41° (c 3.06, C_5H_5N); UV λ_{max}^{MeOH} nm: 239.5, 252, 292.5, 323; IR ν_{max}^{KBr} cm^{-1}: 3487, 3084, 2961, 2940,1766,1707, 1615, 1353, 1284, 1208, 1128, 1103, 994, 859, 838; EIMS (*m/z*, %): 360 $[M]^+$ (65), 273 (16), 175 (25), 162 (100), 134 (94), 87 (99); HREIMS *m/z* 360.1212 (calcd for $C_{19}H_{20}O_7$ 360.1209); ^1H NMR and ^{13}C NMR see **Table 1**.

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