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NEW *O*-TERPENOIDAL COUMARINS FROM *CLAUSENA EXCAVATA*

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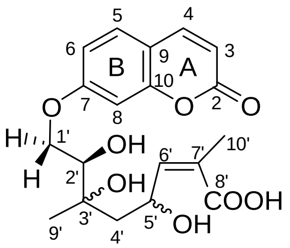
Abstract -- Chemical investigation on an ethanol extract from the aerial part of *Clausena excavata* resulted in the isolation of two new *O*-diterpenoidal coumarins, excavacoumarins H (**1**) and I (**2**).

Clausena excavata (Rutaceae) is a bush widely distributed in Yunnan, China. Leaves and barks of this plant have been used as folk medicine for the treatment of dysentery, enteritis, and urethra infection.[1] Previous researches revealed that the genus *Clausena* mainly contained carbazole alkaloids,[2-4] *O*-terpenoidal coumarins,[5-16] and tetranortriterpenoids,[17] and that *O*-terpenoidal coumarins, clauslactones A-J,[16] in particular, clauslactone C showed inhibitory effects on TPA-induced EBV-EA activation. Some coumarins isolated from this genus had antitumor activity[18] and antiplasmodial activity[19] as well. This paper describes the isolation and structure elucidation of two new *O*-terpenoidal coumarins, excavacoumarins H (**1**) and I (**2**) from the aerial part of *C. excavata* collected in Xishuangbanna, Yunnan Province.



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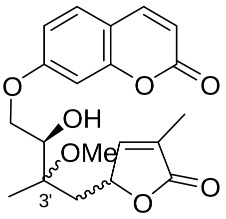

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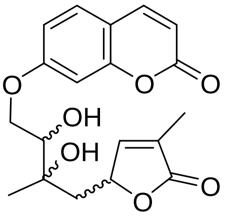

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Excavacoumarin H (**1**) was obtained as a white amorphous powder. The presence of a coumarin moiety was evident from the ^{1}H NMR spectra which showed the characteristic signals of a typical AB system at δ_H 6.21 and 7.90 (1H each, d, *J* = 9.5) assigned to H-3 and H-4, respectively.[13-16] The ^{1}H and ^{13}C NMR signals [δ_H 7.58 (d, 8.5), δ_C 131.6 (d); δ_H 6.97 (dd, *J* = 2.4, 8.5 Hz), δ_C 115.3 (d); δ_H 6.94 (d, 2.4), δ_C 104.1 (d)] of ring B indicated the presence of an oxygen-substitution at C-7.[13-16] The ^{13}C NMR spectra of **1** suggested the presence of a 10-carbon moiety besides the coumarin chromophore. The ^{1}H NMR spectra of **1** gave an olefinic methine at δ 7.40 (1H, m) corresponded to the H-6' of the α,β-unsaturated carbonyl in the side chain. The ^{1}H and ^{13}C NMR spectra of C-10 unit of terpenoidal side chain of **1** were similar to those of excavacoumarin G (**3**).[15] The coupling constant (*J* = 10.4 Hz) between H-1'β [δ 4.22 (1H, dd, *J* = 8.3, 10.4 Hz] and H-2'α [δ 4.95 (1H, dd, *J* = 2.0, 10.4 Hz)] suggested that H-2' was α-orientation. HREIMS gave the formula as $C_{19}H_{22}O_8$ (at *m/z* 378.1308 $[M]^+$, calcd 378.1315), which proved that excavacoumarin H (**1**) was the hydrolyzed product of excavacoumarin G (**3**) at both C-5' and C-8' position.

The molecular formula of **2** was determined to be $C_{20}H_{22}O_7$ based on HREIMS (at *m/z* 374.1368 $[M]^+$, calcd 374.1366). The IR (1730, 1700, 1610 cm^{-1}) spectra suggested that the presence of coumarin nucleus and one lactone.[16] The ^{13}C NMR spectra and DEPT of **2** showed 20 resonances for three methyl including one oxygen-substituted methyl (δ_H 3.22, δ_C 49.2), two methylene, eight methine, and seven quaternary carbons. The ^{1}H NMR spectra of **2** showed the characteristic signals of a typical AB system at δ_H 6.33, and 7.68 (1H each, d, *J* = 9.5 Hz) assigned to H-3, H-4, respectively.[13-16] The ^{1}H and ^{13}C NMR spectra of **2** were similar to those of excavacoumarin G (**3**)[15] except for the methoxyl moiety. Comparison of ^{13}C NMR of **2** and excavacoumarin G at C-3' position, the difference of chemical shift (about Δ 5 ppm) suggested that the methoxyl moiety (δ_H 3.22, δ_C 49.2) was linked at C-3' position. So, **2**, named excavacoumarin I, was identified to be the methoxylated compound of excavacoumarin G at C-3'.

EXPERIMENTAL

General experimental procedures: These have been reported previously.[14]

Plant Material: Reported previously.[14]

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 on 2.0 kg silica gel and eluted with $CHCl_3$ (15 L), $CHCl_3$ - EtOAc (1:1, 25 L), EtOAc (15 L) and MeOH (10 L) to give four fractions (Frs. 1-4), successively. The Fr. 2 (60 g) was subjected column chromatography on silica gel (400 g) with petroleum ether - EtOAc 7:3 (2 L), 6:4 (3 L), 1:1 (3 L), 4:6 (3 L), 3:7 (3 L) to give 5 fractions (2A-2E). The fraction 2E (150 mg) was further purified through CC on Sephadex LH-20 (40 g) eluting with Me_2CO (250 mL) to give compound (**1**) (16 mg), and the fraction 2B (173 mg) over Sephadex LH-20 (40 g) eluting with Me_2CO (200 mL) to give compound (**2**) (10 mg).

Table 1. The ^1H- and ^{13}C-NMR spectral data of **1-3** (*J* in Hz, δ in ppm)

	1[a]		***2***[b]		***3***	
No	^1H	^{13}C	^1H	^{13}C	^1H	^{13}C
2	-	162.8 s		161.2 s	-	160.9 s
3	6.21 (1H, d, 9.5)	115.3 d	6.33 (1H, d, 9.5)	113.2 d	6.31 (d, 9.5)	113.2 d
4	7.90 (1H, d, 9.5)	146.2 d	7.68 (1H, d, 9.5)	143.3 d	7.66 (d, 9.5)	143.8 d
5	7.58 (1H, d, 8.5)	131.6 d	7.41 (1H, d, 8.5)	128.8 d	7.39 (d, 8.6)	129.5 d
6	6.97 (1H, dd, 2.4, 8.5)	115.3 d	6.95 (1H, dd, 2.3, 8.5)	113.1 d	6.95 (dd, 2.6, 8.6)	113.1 d
7	-	158.5 s	-	161.1 s	-	163.1 s
8	6.94 (1H, d, 2.4)	104.1 d	7.07 (1H, d, 2.3)	101.6 d	7.04 (d, 2.6)	102.0 d
9	-	115.3 s	-	112.8 s	-	113.0 s
10	-	153.3 s	-	155.9 s	-	156.4 s
1'	4.36 (1H, dd, 2.0, 8.3) 4.22 (1H, dd, 8.3, 10.4)	73.0 t	3.98 (1H, m) 4.30 (1H, m)	69.7 t	4.56 (dd, 13.8, 8.5) 4.41 (dd, 13.8, 6.9)	71.9 t
2'	4.95 (1H, dd, 2.0, 10.4)	80.8 d	4.17 (1H, m)	72.8 d	4.50 (m)	79.0 d
3'	-	74.4 s	-	76.7 s	-	72.8 s
4'	2.36 (1H, m) 2.25 (1H, m)	47.2 t	1.95-2.30 (2H, m)	38.3 t	2.34 (m) 1.53 (m)	44.3 t
5'	5.35 (1H, m)	76.9 d	5.51 (1H, m)	77.3 d	4.87 (m)	76.9 d
6'	7.40 (1H, m)	146.8 d	6.98 (1H, m)	149.5 d	7.34 (m)	151.7 d
7'	-	131.5 s	-	129.6 s	-	128.6 s
8'	-	176.1 s	-	173.8 s	-	174.4 s
9'	1.70 (3H, s)	27.8 q	1.89 (3H, s)	18.1 q	1.64 (s, 3H)	23.9 q
10'	1.12 (3H, s)	12.2 q	1.20 (3H, s)	10.5 q	1.80 (s, 3H)	10.6 q
OMe	-	-	3.22 (3H, s)	49.2 q	-	-

[a] Measured in CD_3COCD_3.

[b] Measured in $CDCl_3$.

Excavacoumarin H (**1**): mp 148-148.5 °C (Et_2O); $[\alpha]_D^{19}$ +44.3° (*c*, 0.50, EtOH); UV λ_{max}^{MeOH} nm: 205.6, 286.4, 315.4; IR ν_{max}^{KBr} : 3530, 3460, 1750, 1720, 1610, 1500, 1440 cm^{-1}; EIMS: *m/z* = 378 $[M]^+$, 360, 342, 316, 256, 232, 205, 175, 162; HREIMS: *m/z* = 378.1308 (calcd for $C_{19}H_{22}O_8$ 378.1315).

Excavacoumarin I (**2**): mp 105-105.5 °C (Et_2O); $[\alpha]_D^{19}$ +16.7° (*c*, 0.30, EtOH); UV λ_{max}^{MeOH} nm: 204.2, 220, 323.4; IR ν_{max}^{KBr} : 3450, 1730, 1700, 1610, 1500, 1450 cm^{-1}; EIMS: *m/z* = 374 $[M]^+$, 342, 330, 264, 205, 162; HREIMS: *m/z* = 374.1368 (calcd for $C_{20}H_{22}O_7$ 374.1366).

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