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SIX NEW *O*-TERPENOIDAL COUMARINS, EXCAVACOUMARINS B-G FROM *CLAUSENA EXCAVATA*

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Abstract -- Six new *O*-terpenoidal coumarins named excavacoumarins B-G (**1-6**) were isolated from the aerial part of *Clausena excavata* collected in Xishuangbanna, Yunnan.

Clausena excavata Burm. f. (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 this plant mainly contained alkaloids,[2-4] coumarins,[5-9] and flavonoid glycosides.[10] This paper described the isolation and structure elucidation of six new*O*-terpenoidal coumarins, excavacoumarins B-G (**1-6**) and one known coumarin (**7**).[5, 9]

The molecular formulae of excavacoumarins B-G (**1-6**) were established by high-resolution EIMS analyses. The inspection of NMR data (proton, carbon, DEPT) (**Table 1** and **2**) revealed that compounds (**1-6**) had the same coumarin moieties and different 10-carbon side chains. The ^{1}H and ^{13}C NMR data for the coumarin moieties of **1-6** were similar with those of excavacoumarin A,[8] indicating their side chains linked to C-7 *via* an oxygen. The ^{13}C NMR signals at δ_C 195.2 (s), 174.4 (s) and 161.1 (s), and the IR data (1728, 1750, 1755 cm^{-1}) revealed three carbonyls in **1**, suggesting a keto and lactone in the side chain. The proton signal at δ 5.61 (2H) appeared as a singlet, revealing the ketone (δ_C 195.2)

Table 1. The ^{1}H NMR data for compounds (**1-6**) (400 MHz, in C_5D_5N)[a]

^{1}H	**1**	**2**	**3**[b]	**4**[b]	**5**	**6**
3	6.36 (d, 9.5)	6.30 (d, 9.5)	6.34 (d, 9.5)	6.34 (d, 9.5)	6.31 (d, 9.5)	6.31 (d, 9.5)
4	7.76 (d, 9.5)	7.74 (d, 9.5)	7.74 (d, 9.5)	7.74 (d, 9.5)	7.66 (d, 9.5)	7.66 (d, 9.5)
5	7.50 (d, 8.4)	7.36 (d, 8.6)	7.44 (d, 8.5)	7.44 (d, 8.5)	7.39 (d, 8.6)	7.39 (d, 8.6)
6	7.07 (dd, 8.4, 2.7)	6.94 (dd, 8.6, 2.4)	6.98 (dd, 8.5, 2.5)	6.98 (dd, 8.5, 2.5)	6.95 (dd, 8.6, 2.6)	6.95 (dd, 8.6, 2.6)
8	7.11 (d, 2.7)	7.02 (d, 2.4)	7.03 (d, 2.5)	7.03 (d, 2.5)	7.04 (d, 2.6)	7.04 (d, 2.6)
1'	5.61 (s, 2H)	4.47 (dd, 14.0, 8.4)	4.36 (dd, 14.8, 5.6, 2H)	4.36 (dd, 10.8, 4.0, 2H)	4.56 (dd, 13.8, 8.5)	4.56 (dd, 13.8, 8.5)
		3.87 (dd, 14.0, 6.8)			4.41 (dd, 13.8, 6.9)	4.41 (dd, 13.8, 6.9)
2'	/	4.57 (dd, 8.4, 6.8)	4.95 (m)	4.10 (m)	4.50 (m)	4.50 (m)
4'	2.86 (dd, 5.0, 14.3)	2.14 (m)	2.84 (m)	2.81 (m)	2.18 (m)	2.34 (m)
	2.76 (dd, 3.3, 14.3)	1.59 (m)	2.72 (m)	2.56 (m)	1.63 (m)	1.53 (m)
5'	5.20 (m)	4.88 (m)	4.95 (m)	4.89 (m)	5.08 (m)	4.87 (m)
6'	7.09 (m)	2.79 (m)	7.26 (m)	7.26 (m)	2.72 (m)	7.34 (m)
		2.06 (m)			2.05 (m)	
7'	/	/	/	/	2.68 (m)	/
9'	6.51 (s)	1.62 (s, 3H)	5.65 (s)	5.62 (s)	1.62 (s, 3H)	1.64 (s, 3H)
	6.17 (s)		5.35 (s)	5.32 (s)		
10'	1.78 (s, 3H)	1.66 (s, 3H)	1.81 (s, 3H)	1.81 (s, 3H)	1.18 (d, 7.0, 3H)	1.80 (s, 3H)

[a] Coupling constants are presented in Hertz, unless otherwise indicated, all proton signals integrate to 1H.

[b] Data are exchangeable.

Figure 1. Chemical structures of coumarins (**1-7**)

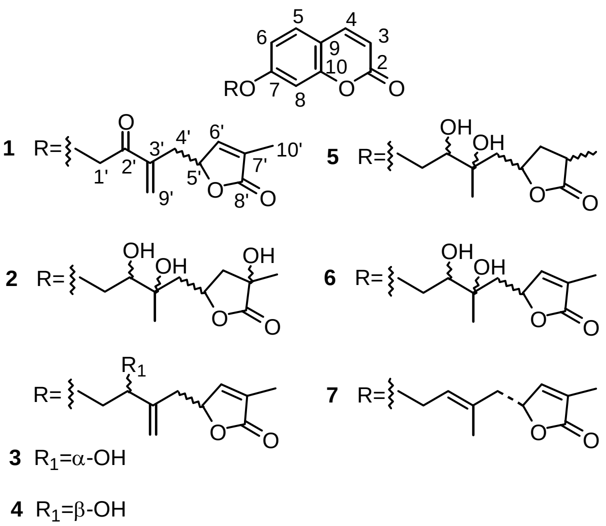

Table 2. The ^{13}C NMR data for **1-6** (100 MHz, in C_5D_5N)

^{13}C	**1**	**2**	**3**[a]	**4**[a]	**5**	**6**
2	161.1	161.0	161.2	161.2	160.9	160.9
3	113.7	113.2	113.4	113.4	113.2	113.2
4	144.1	143.9	144.1	144.1	143.8	143.8
5	129.9	129.5	129.7	129.7	129.5	129.5
6	113.1	113.1	113.3	113.4	113.1	113.1
7	162.0	163.2	162.8	162.8	163.0	163.1
8	102.5	102.1	102.3	102.3	102.0	102.0
9	113.2	113.0	114.9	114.9	113.0	113.0
10	156.3	156.5	155.6	155.6	156.4	156.4
1'	70.6	72.0	73.0	72.6	71.6	71.9
2'	195.2	75.6	80.4	80.8	78.8	79.0
3'	141.0	72.9	145.3	145.1	72.7	72.8
4'	34.8	46.4	37.0	37.0	46.1	44.3
5'	79.7	75.3	72.9	72.9	74.9	76.9
6'	149.9	46.0	149.9	149.9	39.4	151.7
7'	130.2	73.3	129.9	129.9	36.0	128.6
8'	174.4	178.8	174.4	174.4	179.6	174.4
9'	130.0	23.7	115.1	115.3	23.6	23.9
10'	10.7	24.0	10.7	10.7	15.2	10.6

[a] Data are exchangeable.

was at C-2'. The NMR signal at δ_C 130.0 attributed a methylene was assigned to the C-9' of **1**. Compound (**2**) was the hydration product of excavatin-M,[8, 9] having a hydroxyl at the C-2', C-3' and C-7', respectively. The ^{1}H and ^{13}C NMR of **3** were similar to those of **1** except for C-1', C-2' and C-3'. The major difference in their ^{13}C NMR spectra was the signal at δ_C 73.0 (d) in **3** (**Table 2**), suggesting that **3** was the C-2' hydrogenation product of **1**, further supported by its IR data (3447 cm^{-1}). However, the ^{13}C NMR data of C-1', C-2' and C-3' were in pairs (δ_C 73.0/72.6, 80.4/ 80.8, 145.3/145.1), indicating a mixture of **3** and its C-2' stereoisomer (**4**). Two analogues of **2**, **5** and **6** were obscure to direct isolation due to the slight difference in their polarity. But it was easy to distinguish their corresponding NMR signals in their mixture because of their different lactone moieties. The ^{13}C NMR signals of **5** at δ_C 36.0 (d) and 39.4 (t) attributed to the C-6' and C-7' suggested that the lactone of the side chain was saturated, same as that of excavacoumarin A.[8] Meanwhile, the signals at δ_C 128.6 (s) and 151.7 (d) revealed that **6** had an unsaturated 1actone residue in its side chain.[9] Compound (**7**) was previously reported by Bohlmann[5] and Adam,[9] respectively. The optical rotation ($[\alpha]_D^{14.7}$ –17.34) of **7** was consistent with the data of ref. 5. Therefore, **7** was the C-5' epimer of excavatin D.[9]

EXPERIMENTAL

General Experimental Procedures -- These have been reported previously.[8]

Plant Material -- The aerial part of *C. excavata* 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 three times with EtOH (12 L each) 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 rechromatographed over silica gel eluted with gradient petroleum-EtOAc (7:3, 6:4, 1:1, 4:6, 3:7) to give excavacoumarin B (**1**) (10 mg), excavacoumarin C (**2**) (12 mg), a mixture (11 mg) of excavacoumarin D (**3**) and excavacoumarin E (**4**), a mixture (8 mg) of excavacoumarin F (**5**) and excavacoumarin G (**6**), and **7** (450 mg).

Excavacoumarin B (**1**), $C_{19}H_{16}O_6$, colorless oil, $[\alpha]_D^{27.5}$ +2.92° (c 4.28, C_5H_5N); IR ν_{max}^{KBr} cm^{-1}: 3077, 2929, 1755, 1750, 1728, 1693, 1620, 1510, 1429, 1295, 1148, 1024, 929, 834; EIMS (*m/z*, %): 340 (M^+, 100), 243 (32), 162 (90), 134 (95), 97 (97), 89 (71); HREIMS *m/z* 340.0950 (calcd for $C_{19}H_{16}O_6$, 340.0947).

Excavacoumarin C (**2**), $C_{19}H_{22}O_8$, colorless oil, $[\alpha]_D^{27.3}$ +8.58° (c 6.70, C_5H_5N); IR ν_{max}^{KBr} cm^{-1}: 3460, 3089, 2931, 1764, 1712, 1615, 1511, 1432, 1385, 1231, 1129, 1100, 1016, 861; EIMS (*m/z*, %): 378 (M^+, 10), 204 (12), 162 (100), 134 (55), 95 (13), 69 (29); HREIMS *m/z* 378.1309 (calcd for $C_{19}H_{22}O_8$, 378.1315).

Excavacoumarin D (**3**) and E (**4**) (mixture), $C_{19}H_{18}O_6$, IR ν_{max}^{KBr} cm^{-1}: 3447, 3085, 2928, 1753, 1725, 1655, 1614, 1508, 1430, 1350, 1233, 1126, 1029, 916, 837; EIMS (*m/z*, %): 342 (M^+, 81), 243 (46), 176 (92), 162 (100), 147 (78), 134 (94), 97 (89); HREIMS *m/z* 342.1108 (calcd for $C_{19}H_{18}O_6$, 342.1103).

Excavacoumarin F (**5**) and G (**6**) (mixture), $C_{19}H_{22}O_7$ and $C_{19}H_{20}O_7$, IR ν_{max}^{KBr} cm^{-1}: 3448, 2936, 1750, 1617, 1510, 1430, 1402, 1283, 1024, 931, 838; EIMS (*m/z*, %): 362 (M^+ of **5**, 5), 360 (M^+ of **6**, 7); HREIMS *m/z* 362.1362 for **5** (calcd for $C_{19}H_{22}O_7$, 362.1366) and 360.1216 for **6** (calcd for $C_{19}H_{20}O_7$, 360.1209).

7, a stereoisomer of excavatin D, $[\alpha]_D^{14.7}$ -17.34° (c 3.46, CH_2Cl_2), lit.,[5, 9]: α_{578} -26° (CH_2Cl_2), α_D^{21} +34.0 ($CHCl_3$, c 2.00); $C_{19}H_{18}O_5$, EIMS (*m/z*, %): 326 (M^+, 20), 162 (84), 134 (25), 97 (100), 69 (287). The ^1H and ^{13}C NMR data were consistent with that of excavatin D.[9]

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