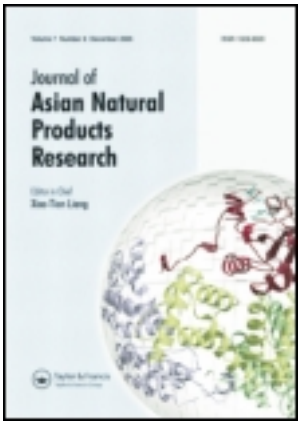

Journal of Asian Natural Products Research

Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/ganp20

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Wen-Wen Peng [a b], Yu-Qing Zheng [a], Yi-Shan Chen [a], Si-Meng Zhao [a b], Chang-Jiu Ji [a] & Ning-Hua Tan [a]

[a] State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming, 650201, Yunnan, China

[b] University of Chinese Academy of Sciences, Beijing, 100049, China

Published online: 17 Jan 2013.

To cite this article: Wen-Wen Peng, Yu-Qing Zheng, Yi-Shan Chen, Si-Meng Zhao, Chang-Jiu Ji & Ning-Hua Tan (2013) Coumarins from roots of Clausena excavata, Journal of Asian Natural Products Research, 15:3, 215-220, DOI: 10.1080/10286020.2012.758635

To link to this article: http://dx.doi.org/10.1080/10286020.2012.758635

Journal of Asian Natural Products Research, 2013
Vol. 15, No. 3, 215–220, http://dx.doi.org/10.1080/10286020.2012.758635

Coumarins from roots of *Clausena excavata*

Wen-Wen Peng[ab], Yu-Qing Zheng[a], Yi-Shan Chen[a], Si-Meng Zhao[ab], Chang-Jiu Ji[a] and Ning-Hua Tan[a]*

[a]*State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650201, Yunnan, China;* [b]*University of Chinese Academy of Sciences, Beijing 100049, China*

(*Received 29 September 2012; final version received 11 December 2012*)

Two new coumarins, clauexcavatins A (**1**) and B (**2**), along with seven known ones (**3**–**9**), were isolated from the roots of *Clausena excavata* Burm. f. (Rutaceae). Their structures were elucidated on the basis of spectral data.

Keywords: *Clausena excavata*; Rutaceae; coumarin; clauexcavatin A; clauexcavatin B

1. Introduction

Clausena excavata Burm. f. (Rutaceae) is a bush tree growing in Xishuangbanna, Yunnan province, China. Its leaves and barks are used as a traditional Chinese medicine to treat dysentery, enteritis, and urethra infection [1,2]. The previous investigations [3–5] on its stems, barks, and roots led to the isolation of carbazole alkaloids [6,7] and coumarins [3,8,9]. This chemical study on the roots of the plant led to the isolation of two new coumarins, clauexcavatins A (**1**) and B (**2**), along with seven known ones (**3**–**9**) (Figure 1).

2. Results and discussion

Clauexcavatin A (**1**) was obtained as pale yellow gum. Its molecular formula $C_{19}H_{20}O_5$ was determined by the HR-ESI-MS, due to the presence of a pseudomolecular ion peak at *m/z* 329.1382 $[M + H]^+$, $\Omega = 10$. The IR spectrum showed absorption bands at 3437, 1734, and 1603 cm^{-1}, indicative of the existence of hydroxyl and lactonic carbonyl groups, and aromatic ring, respectively. The ^{13}C NMR spectrum (Table 1) revealed the presence of one phenyl ring, two pairs of double bonds, four methyls, one oxymethylene (δ_C 61.3), one oxymethine (δ_C 95.5), and three quaternary carbons including one lactonic carbonyl group at δ_C 160.2 and one oxygenated quaternary carbon at δ_C 78.8. The ^1H NMR spectrum (Table 1) exhibited two pairs of AB-spin system in the olefinic region at δ_H 7.96 and 6.05 (each 1H, d, $J = 9.7$ Hz) and δ_H 6.45 and 5.69 (each 1H, d, $J = 9.9$ Hz). They were attributed to four protons of two double bonds. The absence of other proton signals in the downfield region, together with the results of the UV spectrum, suggested the presence of a 5,7-dioxygenated 6,8-disubstituted coumarin skeleton [10,11]. The HMBC correlations (Figure 2) of H-5″, H-6″/C-2″ and H-4″/C-3″, and the ^1H–^1H COSY correlation of H-2″/H-4″ indicated the presence of a [—$CH(CH_2OH)$—$C(CH_3)_2$—] moiety. The HMBC correlations of H-5′, H-6′/C-3′, and H-4′/C-2′, and the ^1H–^1H COSY correlation of H-3′/H-4′ revealed the existence of another [—CH═CH—$C(CH_3)_2$—] moiety. The HMBC correlations of H-3′/C-6 and H-4′/C-5 showed that the [—CH═CH—$C(CH_3)_2$—] moiety was fused at

*Corresponding author. Email: nhtan@mail.kib.ac.cn

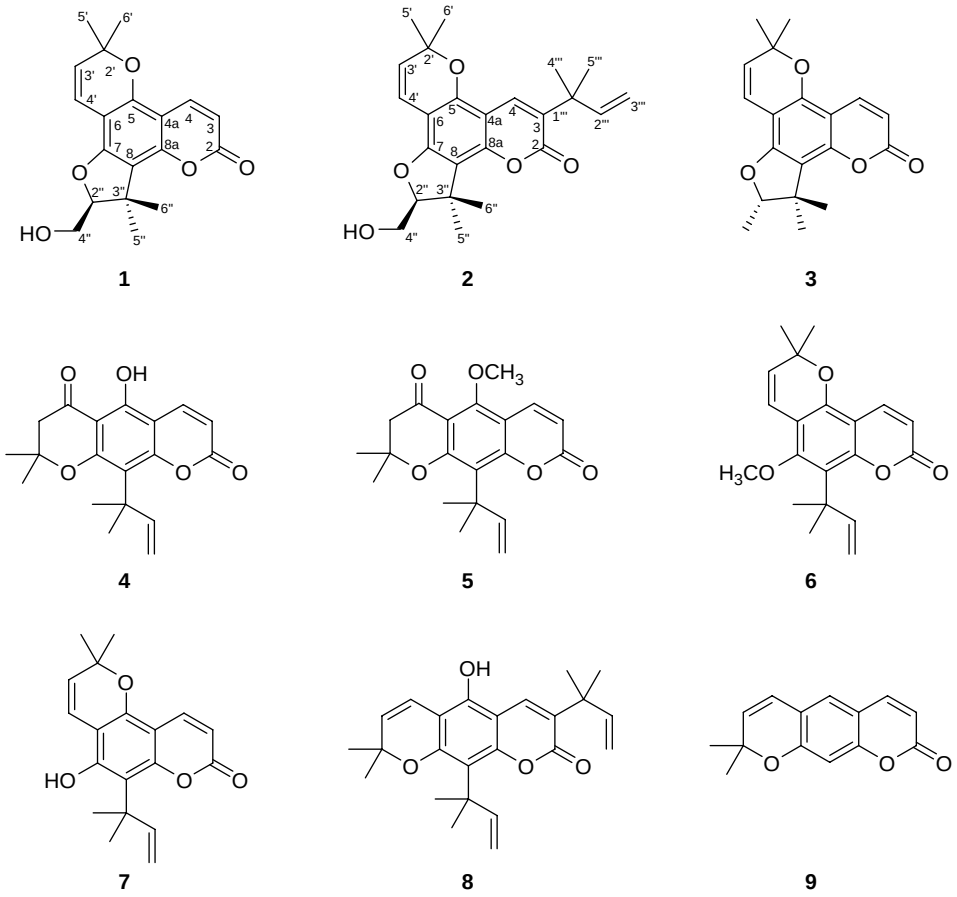


Figure 1. Structures of compounds **1**–**9**.

C-6 through an oxygen at C-5 position. In addition, the HMBC correlations of H-2″, H-5″, H-6″/C-8, and H-2″/C-7 indicated that the [—CH(CH$_2$OH)—C(CH$_3$)$_2$—] moiety was clearly located at C-8 through an oxygen at C-7 position. The optical rotation ($[\alpha]_D^{22.8}$ − 8.9 [*c* 0.38, CHCl$_3$]) was opposite to the optical rotation of the known compound pruniflorone M ($[\alpha]_D^{25}$ + 64.6 [*c* 0.04, CHCl$_3$]) [12,13], this implied that the absolute configurations of **1** at C-2″ and pruniflorone M at C-2′ are different, for they have only one similar chiral carbon. From the absolute configuration of pruniflorone M (2′*R*), that of **1** was found to be 2″*S*, which was further confirmed by calculation with the Gaussian 03 program [14] with the calculated value of −9.80°. Thus, the structure of **1** was established as shown in Figure 1.

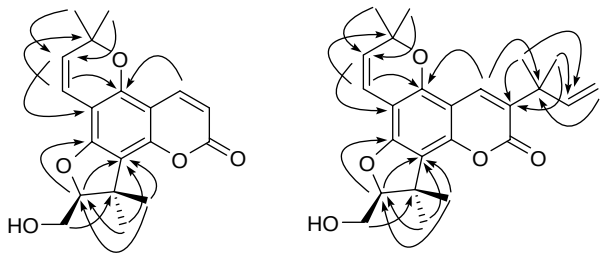
Clauexcavatin B (**2**) was isolated as pale yellow gum. The HR-EI-MS gave a molecular formula of $C_{24}H_{28}O_5$ from the ion peak at *m*/*z* 396.1936 $[M]^+$, $\Omega = 11$. The ^{1}H and ^{13}C NMR data were quite similar to those of **1** (Table 1), suggesting that **2** is a coumarin too. The difference in **2** was worthy noted by the presence of characteristic signals of a 2-methylbut-3-en-2-yl side chain [δ_H 6.18 (1H, dd, 17.5, 10.6), 5.08 (1H, d, 17.5), 5.05 (1H, d,

Table 1. ^{1}H and ^{13}C NMR spectral data of compounds **1** and **2**.

	1[a]		**2**[b]	
No.	δ_H	δ_C	δ_H	δ_C
2		160.2 (s)		159.7 (s)
3	6.05 (1H, d, 9.7)	110.7 (d)		129.0 (s)
4	7.96 (1H, d, 9.7)	139.7 (d)	7.78 (1H, s)	133.0 (d)
4a		104.2 (s)		104.0 (s)
5		151.1 (s)		150.0 (s)
6		102.5 (s)		101.8 (s)
7		158.7 (s)		156.4 (s)
8		114.8 (s)		112.8 (s)
8a		151.9 (s)		150.1 (s)
2′		78.8 (s)		77.8 (s)
3′	5.69 (1H, d, 9.9)	129.0 (d)	5.55 (1H, d, 9.9)	127.8 (d)
4′	6.45 (1H, d, 9.9)	116.3 (d)	6.47 (1H, d, 9.9)	115.9 (d)
5′	1.46 (3H, s)	28.0 (q)	1.47 (3H, s)	27.9 (q)
6′	1.46 (3H, s)	28.0 (q)	1.47 (3H, s)	28.0 (q)
2″	4.49 (1H, t, 5.8)	95.5 (d)	4.47 (1H, dd, 8.2, 3.4)	94.4 (d)
3″		44.1 (s)		43.3 (s)
4″	3.91 (2H, d, 5.8)	61.3 (t)	3.92 (1H, dd, 12.0, 8.2) 3.87 (1H, dd, 12.0, 3.4)	61.7 (t)
5″	1.57 (3H, s)	27.1 (q)	1.57 (3H, s)	26.9 (q)
6″	1.36 (3H, s)	21.1 (q)	1.25 (3H, s)	20.9 (q)
1‴				40.3 (s)
2‴			6.18 (1H, dd, 17.5, 10.6)	145.7 (d)
3‴			5.08 (1H, d, 17.5) 5.05 (1H, d, 10.6)	111.8 (t)
4‴			1.47 (3H, s)	26.2 (q)
5‴			1.47 (3H, s)	26.2 (q)

[a] In acetone-d_6, δ_H and δ_C were recorded at 400 and 100 MHz, respectively.
[b] In $CDCl_3$, δ_H and δ_C were recorded at 500 and 125 MHz, respectively.

10.6), and 1.47 (6H, s); δ_C 145.7, 111.8, 40.3, and 26.2 × 2]. The existence of this side chain [—$C(CH_3)_2$—CH═CH_2] was confirmed by the correlations of H-3‴/C-1‴ and (H-4‴, H-5‴)/C-2‴ in HMBC spectrum (Figure 2), and the correlation of H-2‴/H-3‴ in $^{1}H-^{1}H$ COSY spectrum. Furthermore, the correlations of H-4/C-1‴ and (H-4‴, H-5‴)/C-3 in HMBC spectrum allowed the location of this additional C_5



1 **2**

Figure 2. HMBC correlations of compounds **1** and **2**.

unit at the position C-3 of the coumarin skeleton. For the optical rotation of **2** was found to be identical to that of **1**, the absolute configuration of **2** at C-2″ was assigned as *S*. Therefore, the structure of **2** was established as shown in Figure 1.

Citrusarin A (**3**), a known coumarin similar to **1** and **2**, has been reported previously [15], but the absolute configuration at C-2″ was not determined. The measurement of its optical rotation ($[\alpha]_D^{22.4}$ − 8.4 (*c* 0.13, $CHCl_3$) showed that it is identical with those of **1** and **2**. So the absolute configuration of **3** at C-2″ was found to be *S*.

Seven known coumarins were identified by comparing their spectroscopic data (UV, IR, NMR, and mass spectrometry) with the data in the literatures: citrusarin A (**3**) [15], clausenidin (**4**) [16], clausenidin methyl ether (**5**) [17], dentatin (**6**) [18], nordentatin (**7**) [19], clausarin (**8**) [20], and xanthyletin (**9**) [19].

3. Experimental

3.1 General experimental procedures

Optical rotations were measured with a Horiba SEPA-300 polarimeter (Horiba, Tokyo, Japan). UV spectra were obtained using a shimadzu UV-2401 A spectrophotometer (Shimadzu, Tokyo, Japan). IR spectra were recorded with a Tensor 27 FT-IR spectrometer with KBr pellets (Bio-Rad, Hercules, CA, USA). The ^1H and ^{13}C NMR spectra were acquired with a Bruker AV-400 (^1H: 400 MHz, ^{13}C: 100 MHz) or DRX-500 (^1H: 500 MHz, ^{13}C: 125 MHz) spectrometer in acetone-d_6 or $CDCl_3$ with tetramethylsilane as the internal standard at room temperature (Bruker, Bremerhaven, Germany). MS were recorded on an API QSTAR Pular-1 mass spectrometer (VG, Manchester, UK). Semipreparative reversed-phase HPLC was carried out on an Agilent 1100 apparatus equipped with a UV detector and a YMC-Pack ODS-A (YMC, 1 × 15 cm) column at a flow rate of 2 ml/min (Agilent, Paloalto, CA, USA). Column chromatographies were carried out on silica gel (100–200 mesh, 200–300 mesh, and 10–40 μm, Qingdao Marine Chemical, Inc., Qingdao, China) and Lichroprep RP-18 gel (40–63 μm, Merck, Darmstadt, Germany). Thin layer chromatography was carried out on precoated silica gel GF_{254} glass plates (Qingdao Marine Chemical, Inc., Qingdao, China).

3.2 Plant material

The roots of *C. excavata* were collected from Xishuangbanna, Yunnan province, China, in August 2010. They were identified by Prof. Yu-Min Sui of Kunming Institute of Botany. A voucher specimen (no. 2010813) has been deposited in the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences.

3.3 Extraction and isolation

The air-dried and powdered roots of *C. excavate* (13 kg) were extracted with 95% EtOH under reflux for three times. The filtrates were combined and evaporated to a small volume, followed by successive partition with petroleum ether (PE), EtOAc, and BuOH. The EtOAc soluble fraction (600 g) was applied to silica gel (200–300 mesh) column eluting gradiently with $CHCl_3$–MeOH (10:0, 9:1, 8:2, 7:3, 1:1, 0:1), to give six fractions, A–F. The separation of fraction B (38 g) over silica gel column was eluted with PE–acetone (10:1–1:2) to yield fractions B1–B6. Fraction B2 (1.2 g) was subjected to a silica gel column eluted with PE–acetone (5:1–1:1) to give four subfractions (B2-1–B2-4). B2-1 (0.3 g) was subjected to a reversed-phase column (RP-18) eluting with MeOH–water (20–90%) to afford three subfractions (B2-1-1–B2-1-3). B2-1-2 (0.1 g) was subjected to semipreparative reversed-phase HPLC (80% MeOH–H_2O for 17

and 19 min; detection at 254 and 365 nm; flow rate 2 ml/min) to give clauexcavatin A (**1**) (19 mg) and clausenidin (**4**) (61 mg). B2-1-3 (0.12 g) was subjected to semipreparative reversed-phase HPLC (85% MeOH–H_2O for 16 and 18 min; detection at 254 and 365 nm; flow rate 2 ml/min) to give dentatin (**6**) (20 mg) and clauexcavatin B (**2**) (12 mg). Fraction B3 (0.9 g) was subjected to a silica gel column chromatography using PE–acetone (5:1–1:1) to give three subfractions (B3-1–B3-3). B3-1 (0.13 g) was subjected to a reversed-phase column (RP-18) eluting with MeOH–water (30–100%) to afford two subfractions (B3-1-1–B3-1-2). B3-1-1 (0.06 g) was subjected to semipreparative reversed-phase HPLC (80% MeOH–H_2O for 20 min; detection at 254 and 365 nm; flow rate 2 ml/min) to give clausarin (**8**) (32 mg). B3-1-2 (0.07 g) was subjected to semipreparative reversed-phase HPLC (75% MeOH–H_2O for 24 and 27 min; detection at 254 and 365 nm; flow rate 2 ml/min) to afford xanthyletin (**9**) (23 mg) and clausenidin methyl ether (**5**) (15 mg). Fraction B4 (0.6 g) was subjected to a silica gel column chromatography using PE–acetone (4:1–1:1) to give five subfractions (B4-1–B4-5). B4-2 (0.2 g) was subjected to a reversed-phase column (RP-18) eluting with MeOH–water (30–100%) to afford three subfractions (B4-2-1–B4-2-3). B4-2-1 (0.1 g) was subjected to semipreparative reversed-phase HPLC (78% MeOH–H_2O for 26 and 31 min; detection at 254 and 365 nm; flow rate 2 ml/min) to give nordentatin (**7**) (25 mg) and citrusarin A (**3**) (18 mg).

3.3.1 *Clauexcavatin A (**1**)*

Pale yellow gum; $[\alpha]_D^{22.8}$ − 8.9 (*c* 0.38, $CHCl_3$); UV (MeOH) λ_{max} (log ε): 202 (4.45), 225 (4.42), 283 (4.41), 338 (4.25), nm; IR (KBr) ν_{max} cm^{-1}: 3437, 2971, 2929, 1734, 1624, 1603, 1436, 1137, 1122, 1010, 822, 749; ^1H and ^{13}C NMR spectral data, see Table 1; positive ESI-MS: *m/z* 329 $[M + H]^+$; positive HR-ESI-MS: *m/z* 329.1382 $[M + H]^+$ (cacld for $C_{19}H_{21}O_5$, 329.1388).

3.3.2 *Clauexcavatin B (**2**)*

Pale yellow gum; $[\alpha]_D^{22.7}$ − 8.8 (*c* 0.20, $CHCl_3$); UV (MeOH) λ_{max} (logε): 196 (2.91), 208 (2.92), 216 (2.91), 237 (2.96), 286 (3.19), 335 (3.09) nm; IR(KBr) ν_{max} cm^{-1}: 3441, 2967, 2926, 1728, 1643, 1625, 1604, 1463, 1438, 1362, 1305, 1212, 1150, 1121, 1092, 1024, 780, 739, 660; ^1H and ^{13}C NMR spectral data, see Table 1; positive ESI-MS: *m/z* 397 $[M + H]^+$, 419 $[M + Na]^+$; positive HR-EI-MS: *m/z* 396.1936 $[M]^+$ (calcd for $C_{24}H_{28}O_5$, 396.1937).

3.4 *Calculation*

The geometry was optimized using density functional theory at the B3LYP/6-31G* level. The harmonic vibrational frequency was then calculated to confirm its stability at the same level. The optical rotation (589.3 nm) of the optimized geometry was calculated in acetone using the polarizable continuum model (IEF-PCM) at the B3LYP/6-311 + + G(d,p) level. The calculated value is −9.80°. All the calculations were carried out with the Gaussian 03 program [14].

Acknowledgements

This work was financially supported by the National Natural Science Foundation of China (U1032602, 91013002, and 30725048), the National New Drug Innovation Major Project of China (2011ZX09307-002-02), the National Basic Research Program of China (2009CB522300 and 2013CB127505), the Foundation of Chinese Academy of Sciences (Hundred Talents Program), and the Natural Science Foundation of Yunnan Province (2012GA003). The authors are grateful to the members of the analytical group from the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, for measuring the [α], IR, UV, NMR, and mass spectra.

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