

Article

A New *O*-Terpenoidal Coumarin from *Clausena anisum-olens* Merr.

Yun-Song Wang ¹, Hong-Yi Xu ², Da-Xiang Wang ¹ and Jing-Hua Yang ^{1,*}

¹ Key Laboratory of Medicinal Chemistry for Natural Resource, Ministry of Education, School of Chemical Science and Technology, Yunnan University, Kunming 650091, P.R. China; E-mails: wangys@ynu.edu.cn (Y.-S.W.); yangjh@ynu.edu.cn (J.-H.Y.)

² Department of Public Security of Yunnan Province, Kunming 650031, P. R. China; E-mails: hongyix@sina.com (H.-Y. X.)

* Author to whom correspondence may be addressed; E-mail: yangjh@ynu.edu.cn; Tel: +86-871-6598387; Fax: +86-871-5035538.

Received: 5 December 2008; in revised form: 6 February 2009 / Accepted: 9 February 2009 / Published: 13 February 2009

Abstract: A new *O*-terpenoidal coumarin **1**, named hekumarone, was isolated from the leaves and twigs of *Clausena anisum-olens* Merr. (Rutaceae) collected in Hekou County in Yunnan Province, P. R. China. Structure elucidation and unambiguous NMR assignments for the title compound was carried out on the basis of 1D and 2D NMR experiments.

Keywords: Rutaceae; *Clausena anisum-olens* Merr.; Hekumarone; Monoterpenoid coumarins.

Introduction

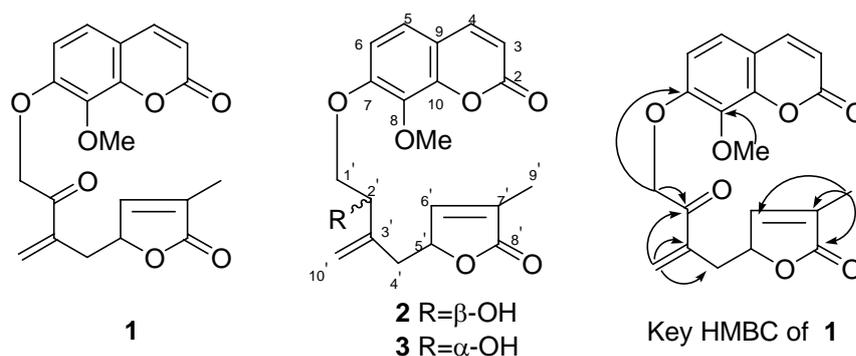
The plants of the Rutaceae family are well-known to contain structurally diverse and biologically active coumarins [1-6]. Within this family, the plants of the genus *Clausena* are shrubs, widely distributed in South and Southeast Asia and many species are used in Chinese folk medicine for various indications [7]. Previous phytochemical studies have indicated that *Clausena* species are the

rich sources of carbazole alkaloids and coumarins [4-6, 8-10]. *Clausena anisum-olens* Merr. is a shrub that both grows wild and is cultivated from Philippines and South China through Southeast Asia. The aerial parts of this plant have been used for the treatment of dysentery and arthritis [7]. Our previous phytochemical studies on *C. anisum-olens* Merr., collected in Hekou County in Yunnan Province, P. R. China, resulted in the isolation of new cyclic peptides and monoterpene coumarins [11-13]. In our ongoing search for bioactive compounds from this medicinally important genus, a new *O*-terpenoidal coumarin **1**, which we have named hekumarone, was isolated from *C. anisum-olens* Merr. In this report, we describe the isolation and structure elucidation of **1** using spectroscopic data analysis, including 1D and 2D NMR techniques (COSY, HSQC and HMBC).

Results and Discussion

The powdered plant material of *Clausena anisum-olens* Merr. (22.5 kg) was repeatedly extracted with EtOH at room temperature. The extract was then concentrated under reduced pressure to give a brown syrup, which was suspended in water and successively partitioned with petroleum ether, ethyl acetate (EtOAc) and *n*-butanol (*n*-BuOH). The EtOAc fraction was subjected to silica gel column chromatography, Pharmadex LH-20 and RP C-18 to yield compound **1**, which was isolated as a light yellow oil, $[\alpha]_D^{18.8} - 6.67$ (*c* 0.75, CH₃OH). The molecular formula C₂₀H₁₈O₇ deduced from its ¹³C-NMR DEPT spectrum and HR-ESI-MS, which exhibited a molecular ion at *m/z* 369.1000 [M-1]⁺, indicated twelve degrees of unsaturation. Strong UV bands at λ_{max} 256 and 319 and an IR band at 1,730 cm⁻¹ indicated the presence of a 7,8-dioxygenated coumarin [5].

Figure 1. Structures of compounds **1-3** and key HMBC correlations of **1**.



The ¹H-NMR spectrum (Table 1) showed resonances for two pairs of typical AB system characteristic doublets at δ_H 6.32 and 7.64 (each *J* = 9.5 Hz) and δ_H 6.98 and 7.20 (each *J* = 8.6 Hz), corresponding to H-3, H-4, H-5 and H-6 in the coumarin nucleus. Additional signals in the spectrum were consistent with three olefinic protons δ 6.37, 6.09 (each 1H, s) and 7.01 (1H, d, *J* = 1.6 Hz), one oxymethylene group δ 5.57 (2H, s), two methylene protons δ 2.80, 2.62 and one olefinic methyl δ 1.77 could be seen.

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

No.	1		2		3	
	δ_{H}	δ_{C}	δ_{H}	δ_{C}	δ_{H}	δ_{C}
2	/	159.2 (s)	/	160.4 (s)	/	160.4 (s)
3	6.32 (d,9.5)	112.7 (d)	6.33 (d, 9.8)	113.6 (d)	6.33 (d, 9.8)	113.6 (d)
4	7.64 (d,9.5)	142.9 (d)	7.71 (d, 9.8)	143.6 (d)	7.71 (d, 9.8)	143.6 (d)
5	7.20 (d,8.6)	122.3 (d)	7.36(d, 8.6)	123.0 (d)	7.36 (d, 8.6)	123.0 (d)
6	6.98 (d,8.6)	109.3 (d)	6.97 (d, 8.6)	110.4 (d)	6.97 (d, 8.6)	110.4 (d)
7	/	153.5 (s)	/	154.3 (s)	/	154.3 (s)
8	/	135.6 (s)	/	136.3 (s)	/	136.3 (s)
9	/	113.4 (s)	/	114.1 (s)	/	114.1(s)
10	/	147.7 (s)	/	147.9 (s)	/	147.9 (s)
1'a			4.23 (m)	72.8 (t)	4.24 (m)	72.8 (t)
1'b	5.57 (s)	69.6 (t)	4.14 (m)	72.8 (t)	4.17 (m)	72.8 (t)
2'	/	193.8 (s)	4.66 (m)	72.7 (d)	4.68 (m)	72.6 (d)
3'	/	139.6 (s)	/	142.0 (s)	/	142.3 (s)
4'a	2.80 (dd,14.1, 4.8)		2.63 (dd, 14.6, 7.3)		2.69 (dd, 14.6, 5.1)	
4'b	2.62 (dd, 14.1, 9.4)	33.5 (t)	2.54 (dd, 14.6, 6.4)	36.4 (t)	2.45 (dd, 14.6, 8.1)	36.4 (t)
5'	5.11 (m)	78.2 (d)	5.25 (m)	79.8 (d)	5.25 (m)	80.0 (d)
6'	7.01(d,1.6)	147.6 (d)	7.22 (d, 1.7)	148.7 (d)	7.22 (d, 1.7)	148.7 (d)
7'	/	128.8 (s)	/	130.0 (s)	/	130.0 (s)
8'	/	172.7 (s)	/	174.0 (s)	/	174.0 (s)
9'	1.77 (s)	9.3 (q)	1.98 (s)	10.5 (q)	1.98 (s)	10.5 (q)
10'a	6.37 (s)		5.43 (d, 6.8)		5.45 (d, 6.8)	
10'b	6.09 (s)	128.3 (t)	5.28 (d, 6.8)	116.2 (t)	5.28 (d, 6.8)	115.9 (t)
OMe	4.05 (s)	60.0 (q)	3.94 (s)	61.5 (q)	3.94 (s)	61.5 (q)

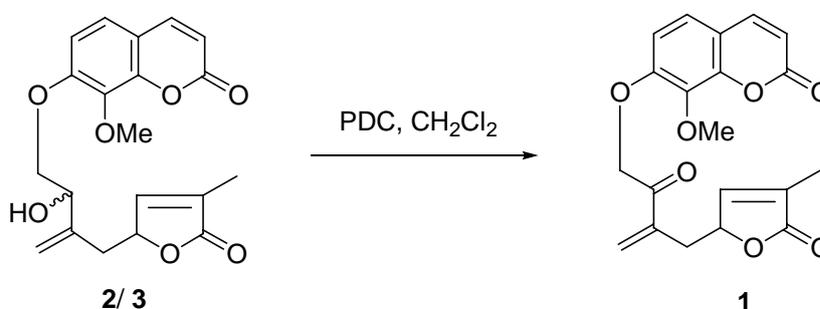
* Compound **1** recorded in pyridine- d_5 , **2/3** in CDCl_3

The ^{13}C -NMR spectrum (Table 1) showed, in addition to the resonances of the carbons belonging to the coumarin nucleus, 10 other signals arising from one methyl, one oxymethylene, one methylene, one oxygen-bearing methine, four olefinic and two carbonyl carbons that could only be located on the side-chain. Joint analysis of HSQC spectra and HMBC correlations (Figure 1) confirmed the presence of a C_{10} terpenoid side-chain attached to the coumarin skeleton. The connection of the side chain to C-7 on the coumarin nucleus was revealed by observation of a three-bond correlation between the oxymethylene proton signals (H-1') and carbon C-7 at δ_{C} 153.5s. The presence of only one methoxyl signal at δ_{H} 4.05 and a significant HMBC correlation with a quaternary carbon signal at δ_{C} 135.6s revealed that the methoxyl group must be unequivocally located on C-8. Meanwhile, the EI-MS spectra also showed characteristic 8-OMe coumarin fragment ion at m/z 193 corresponding to loss of side chain [10].

Carefully analysis of the ^1H and ^{13}C NMR data of **1** (Table 1), showed that the signal patterns were in good agreement with those of previously isolated compounds **2/3** [13], except for some chemical shift differences. IR bands at 1,753, 1,748 and $1,730\text{ cm}^{-1}$ were indicative of the presence of three

carbonyl groups in **1**, suggesting a ketonic group and a lactone in the side chain. The major difference between the ^{13}C -NMR spectra of **1** and **2/3** was the appearance of a characteristic keto quaternary carbon signal at δ_{C} 193.8s (Table 1) in **1** and disappearance of oxygen-bearing carbons at δ_{C} 72.7d/72.6d compared to **2/3**. The proton signal at δ 5.57 (2H, H-1') appeared as a singlet, revealing the ketone (δ_{C} 193.8s) was located at C-2'. In the HMBC spectrum (Figure 1), an informative correlation between the oxymethylene proton signal at δ_{H} 5.57 (2H, H-1') and keto quaternary carbon signal at δ_{C} 193.8s confirmed it. Compared to **2/3**, because the C-2' chiral center disappeared, there were no NMR signals pairs for C-2', C-5' and C-10' in **1**. All these suggested that **1** was the C-2' oxygenation product of **2/3**. To confirm the assigned structure, compound pair **2/3** was oxygenated to afford **1** with $\text{H}_2\text{Cr}_2\text{O}_7$ -2Pyr. (PDC) in CH_2Cl_2 (Figure 2). On the basis of these results, the structure of **1** as shown in Figure 1 is proposed.

Figure 2. Oxidation of **2/3**.



Conclusions

As a continuation of our phytochemical investigation on *Clausena*, a new *O*-terpenoidal coumarin **1**, named hekumarone, was isolated from the leaves and twigs of *Clausena anisum-olens* Merr., collected in Hekou County in Yunnan Province, P. R. China. It is documented that coumarins are one of the major constituents in plants belonging to the genus *Clausena* [4-6, 9]. The result of the present study supports the conclusion that coumarins are characteristic and distinguishable chemical markers for the Rutaceae family, especially, the genus *Clausena*.

Experimental

General

Commercial silica-gel plates (Qing Dao Marine Chemical Group Co.) were used for TLC analyses. UV/VIS Spectra was measured on a Shimadzu UV-2401PC spectrophotometer; λ_{max} in nm. IR spectra were obtained on a Bio-Rad FTS-135 infrared spectrophotometer, ν_{max} in cm^{-1} . ^1H - and ^{13}C -NMR as well as 2D-NMR spectra were recorded on a Bruker DRX-500 spectrometer with TMS as internal standard, coupling constant J are expressed in Hz. MS spectra were recorded on a VG Autospec-3000 mass spectrometer.

Plant material

The leaves and twigs of *Clausena anisum-olens* Merr. were collected in Hekou County of Yunnan province, P. R. China, in May 2003 and identified by Professor De-Ding Tao of the Kunming Institute of Botany. A voucher specimen (No. 02041705) is deposited in the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences.

Extraction and isolation

The powdered leaves and twigs of *Clausena anisum-olens* Merr. (22.5 kg) was repeatedly extracted with EtOH at room temperature. The extract was then concentrated under reduced pressure to give brown syrup, which was partitioned in H₂O and extracted with solvents into petroleum ether-fraction, EtOAc-fraction and n-BuOH-fraction fractions. The EtOAc extracts (110.5g) were subjected to silica gel column chromatography eluting with PE-EtOAc (4:1, 2:1, 1:1, 2:3), EtOAc, EtOAc–MeOH (8:2, 7:3, 6:4, 1:1), MeOH, by which nine fractions (I–IX) were obtained. Fraction II was resubmitted to silica gel column chromatography, Pharmadex LH-20 (MeOH) and RP C-18 to yield compound **1** (4 mg).

Hekumarone (**1**). Light yellow oil; $[\alpha]_D^{18.8} - 6.67$ (*c* 0.75, CH₃OH); IR (KBr): IR $\nu_{\max}^{\text{KBr}} \text{ cm}^{-1}$: 3,429, 2,923, 2,852, 1,753, 1,730, 1,606; UV (MeOH) λ_{\max} nm: 319, 256, 208; ¹H-NMR (δ ppm, pyridine-d₅) and ¹³C-NMR data are shown in Table 1; EI-MS *m/z*: 370 ([M]⁺, 85), 273 (7), 204 (35), 193 (100), 161 (45); HRESIMS *m/z* 369.1000 [M-1]⁺ (calcd. for C₂₀H₁₈O₇ 369.0974).

Oxidation of **2/3**

PDC (60 mg) was resolved in dry CH₂Cl₂ (2 mL) under N₂. The mixture was stirred 5 min at room temperature. Then a soln. of **2/3** (6 mg) in CH₂Cl₂ (1 mL) was added. The reaction mixture was stirred overnight. The mixture was filtered and H₂O (2 ml) was added, and then extracted with CH₂Cl₂. The org. phase dried (MgSO₄) and evaporated to afford **1** (3 mg) as a light yellow oil.

Acknowledgements

This work was supported by National Nature Science Foundation of China (No. 20862018), Science Foundation of Yunnan University (Grant No. 2004Q004A and 2005Z001A) and Science Foundation of Yunnan (Grant No. 2006B0003Q and 2007PY01-23).

References

1. Phuwapraisirisan, P.; Surapinit, S.; Sombund, S.; Siripong, P.; Tip-pyang, S. Feroniellins A–C, novel cytotoxic furanocoumarins with highly oxygenated C₁₀ moieties from *Feroniella lucida*. *Tetrahedron Lett.* **2006**, *47*, 3685–3688.

2. Chlouchi, A.; Muyard, F.; Girard, C.; Waterman, P. G.; Bévalot, F. Coumarins from the twigs of *Diplolaena mollis* P. G. Wilson (Rutaceae). *Biochem. Syst. Ecol.* **2005**, *33*, 967-969.
3. Nájera, C.; Yus, M. Natural products with polyene amide structures. *Stud. Nat. Prod. Chem.* **2000**, *21*, 373-455.
4. He, H.; Shen, Y.; He, Y.; Yang, X.; Zhu, W.; Hao, X. Six new *O*-terpenoidal coumarins, excavacoumarins B-G from *Clausena excavata*. *Heterocycles* **2000**, *53*, 2067-2070.
5. Ito, C.; Itoigawa, M.; Katsuno, S.; Omura, M.; Tokuda, H.; Nishino, H.; Furukawa, H. Chemical constituents of *Clausena excavata*: isolation and structure elucidation of novel furanone-coumarins with inhibitory effects for tumor-promotion. *J. Nat. Prod.* **2000**, *63*, 1218-1224.
6. Huang, S.C.; Wu, P.L.; Wu, T.S. Two coumarins from the root bark of *Clausena excavata*. *Phytochemistry* **1997**, *44*, 179-181.
7. Institutum Botanicum Kunmingense Academiae Sinicae. *Flora Yunnanica (Spermatophyta)*; Wu, C.Y., Ed.; Science Press: Beijing, **2001**; Tomus 6, p. 767 (in Chinese).
8. Chakraborty, A.; Chowdhury, B.K.; Bhattacharyya, P. Clausenol and clausenine-two carbazole alkaloids from *Clausena anisata*. *Phytochemistry* **1995**, *40*, 295-298.
9. Wu, T.S.; Huang, S.C.; Wu, P.L. Carbazole-pyrancoumarin dimer and binary carbazole alkaloids from *Clausena excavata*. *Tetrahedron Lett.* **1996**, *37*, 7819-7822.
10. Takemura, Y.; Nakamura, K.; Hirusawa, T.; Ju-ichi, M.; Ito, C.; Furukawa, H. Four new furanone-coumarins from *Clausena excavata*. *Chem. Pharm. Bull.* **2000**, *48*, 582-584.
11. Wang, Y.S.; He, H.P.; Yang, J.H.; Shen, Y.M.; Zhou, J.; HAO, X.J. A new cyclopeptide from *Clausena anisum-olens* Merr. *Helv. Chim. Acta* **2005**, *88*, 2345-2348.
12. Wang, Y.S.; He, H.P.; Yang, J.H.; Di, Y.T.; HAO, X.J. New monoterpenoid coumarins from *Clausena anisum-olens* Merr. *Molecules* **2008**, *13*, 931-937.
13. Wang, Y.S.; Huang, R.; Li, L.; Zhang, H.B.; Yang, J.H. *O*-terpenoidal coumarins from *Clausena anisum-olens* Merr. *Biochem. Syst. Ecol.* **2008**, *36*, 801-803.

Sample Availability: Available from the authors.

© 2009 by the authors; licensee Molecular Diversity Preservation International, Basel, Switzerland. This article is an open-access article distributed under the terms and conditions of the Creative Commons Attribution license (<http://creativecommons.org/licenses/by/3.0/>).