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Note

Coumarins from *Clausena anisum-olens* Merr.

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Seven coumarins, including a new *O*-terpenoidal coumarin, named anisumarin (1), were isolated from *Clausena anisum-olens* Merr. The structure of this new coumarin was identified as 7-{(*E*)-4-[(4-acetoxymethyl-1,5-dihydro-5-oxo)-2H-furylium-2-yl]-3-methyl-2-butenoxy}-8-methoxycoumarin on the basis of an extensive spectroscopic analysis.

Key words: coumarins; anisumarin; Rutaceae; *Clausena anisum-olens* Merr.

The plants of the Rutaceae family are well-known to contain structurally diverse and biologically active coumarins.[1–6] *Clausena anisum-olens* Merr. is a shrub growing wild and cultivated from Philippines and South China through Southeast Asia, and the aerial parts of this plant have been used for treating dysentery and arthritis.[7] Our previous phytochemical studies on *C. anisum-olens* Merr., which was collected from Hekou County in Yunnan province, P.R. China, resulted in the isolation of new monoterpenoid coumarins.[8–10] Our ongoing search for bioactive metabolites from the genus *Clausena* isolated seven coumarins, including a new *O*-terpenoidal coumarin (**1**), from *C. anisum-olens* Merr.

Powdered plant material of *C. 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 H_2O and sequentially partitioned with petroleum ether (PE), EtOAc, and *n*-BuOH. The EtOAc extract (110.5 g) was subjected to silica gel column chromatography, eluting with PE-EtOAc (4:1–2:3), EtOAc, EtOAc–MeOH (8:2–1:1) and MeOH, and a total of nine fractions (I-IX) were obtained. Fraction III was subjected to column chromatography over silica gel by gradient elution, using PE-EtOAc (5:1 and 3:1), to yield **2** (10 mg). Fraction IV was resubmitted to column chromatography over silica gel by gradient elution, using $CHCl_3$–EtOAc, and then to Sephadex LH-20 (using MeOH as the eluent) to yield compounds **1** (5 mg) and **5** (25 mg). Fraction V was purified on silica gel, eluting with PE-acetone (3:1), and purified further by CC, developed with $CHCl_3$-acetone (96:4) to yield **3** (12 mg) and **7** (15 mg). Fraction VI was applied to a silica gel column, eluting with $CHCl_3$-acetone (5:1, 3:1), and then to Sephadex LH-20 (MeOH) to yield **6** (11 mg) and **4** (8 mg).

Compound **1**, $[\alpha]_D^{20.7}$ -16.7° (*c* 0.12, CH_3OH), was isolated as a colorless oil. The molecular formula of $C_{22}H_{22}O_8$ was deduced from the HR-ESI-MS data, exhibiting the molecular ion at m/z 415.1386 $[M]^+$ (calcd. for 415.1392), which was also confirmed by NMR spectra. Its UV spectrum with λ_{max} 256, 319 nm is characteristic of a coumarin derivative. The IR spectra showed an additional C=O stretching band at 1,763 cm^{-1} together with the coumarin carbonyl band in the vicinity of 1,728 cm^{-1}, indicating the presence of a γ-lactone ring. The ^{1}H-NMR spectrum of **1** displayed two doublets at δ 6.25 and 7.86 ($J = 9.6$ Hz) characteristic of H-3 and H-4 of coumarins. The presence of a further two proton doublets at δ_H 7.31 and 7.03 (each d, $J = 8.7$ Hz) in the ^{1}H-NMR spectrum, and the resonance of carbons bearing two oxygen moieties at δ_C 156.3 s and 137.4 s indicated a 7,8-disubstituted coumarin.[11]

An analysis of the ^{1}H- and ^{13}C-NMR spectra, including COSY and HMQC, suggested the presence of a C_{10} terpenoid side chain, similar to the previously isolated coumarins from *C. anisum-olens* Merr. The location of the side chain at C-7 was based on the observation of long-range C–H correlation between the methylene protons at δ_H 4.73 (H-1′) and the oxygenated carbon (δ_C 156.3, C-7). The presence of only one methoxyl signal at δ_H 3.92 and significant HMBC correlation with a quaternary carbon signal at δ_C 137.4 s revealed the methoxyl group to be unequivocally located on C-8. The E configuration of the C-2′/C-3′ double bond was suggested by the ^{13}C-NMR chemical shift value of C-10′ (δ_C 17.5) and the HMBC analysis (Fig. 1). The NOE correlations between H-1′ and H-10′ and between H-2′ and H-4′ further supported this.

In addition, the presence of a lone 2H-broad singlet at δ 4.77 in the ^{1}H spectrum, and the long-distance correlations between a 2H-broad singlet at δ 4.77 and δ 130.5 (s, C-7′), 154.0 (d, C-6′), 173.6 (s, C-8′) indicated the presence of a 3-hydroxymethyl-3,4-unsaturated-γ-lactone moiety in the molecule. In the ^{1}H spectrum, except for the signals of *O*-terpenoidal coumarin, there was an additional signal (δ 2.06, 3H) for an acetoxyl group. The clear long-range C–H correlation between the acetoxyl signals and oxymethylene at δ 58.4 suggested the position of the acetoxyl group at C-9′. The foregoing evidence enabled compound **1** to be identified as 7-{(*E*)-4-[(4-acetoxymethyl-

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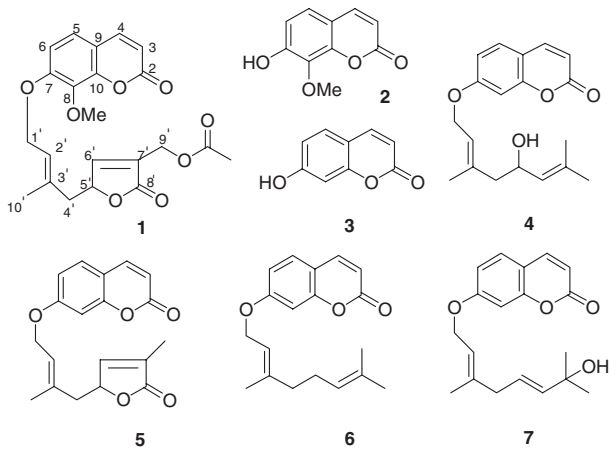

Fig. 1. Structures of Coumarins **1**–**7**.

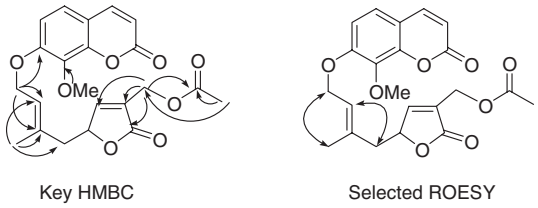

Fig. 2. Key HMBC Correlations, and Selected ROESY Correlations for **1**.

1,5-dihydro-5-oxo)-2H-furylium-2-yl]-3-methyl-2-butenoxy}-8-methoxycoumarin, named anisumarin (Fig. 1).

The configuration pertaining to the C-5′ position of *O*-terpenoidal coumarin **1** remains to be determined. The stereochemistry of this type of *O*-terpenoidal coumarin reported previously has not so far been resolved.[2,4,9] Compound **1** was assayed for its cytotoxicity against human cervical cancer (Hela) cell lines, and was found to be inactive. A further bioassay is being continued.

The structures of known compounds **2**–**7** have been identified as isoscopoletin (**2**),[12] umbelliferone (**3**),[13] anisocoumarin H (**4**),[14] capnolactone (**5**),[15] aurapten (**6**)[16] and 7-[(E)-7′-hydroxy-3′,7′-dimethylocta-2′,5′-dienyloxy]-coumarin (**7**) (see Fig. 1).[17] All of these known compounds were identified by comparing their IR, UV, ^{1}H- and ^{13}C-NMR, TLC, and/or melting point (mp) data with corresponding authentic samples or literature data.

It is well-known that coumarins are an important and well-recognized group of compounds in the genus *Clausena*, and significant diversification in the coumarin composition among species has been observed.[1–6] Of major coumarin classes, mono- and di-oxygenated coumarins are often widely distributed. All the isolated coumarins in this study were oxygenated at C-7, most with the normal geranyl or prenyl side-chain. Hence, the results of the present study support the notion that coumarins are a distinguishable chemical marker for the genus *Clausena*. Moreover, it seems that the ability to oxidize the coumarin nucleus at C-7 is a common factor for *C. anisum-olens* Merr.

Table 1. ^{13}C- (125 MHz) and ^{1}H- (500 MHz) NMR Data for **1** (in CD_3OD)

Position	δ_C	δ_H (integral, mult, *J* in Hz)	Position	δ_C	δ_H (integral, mult, *J* in Hz)
2	162.9	/	3′	137.7	/
3	113.8	6.25 (1H, d, 9.6)	4′a	43.3	2.59 (1H, dd, 14.8, 5.4)
4	146.0	7.86 (1H, d, 9.6)	4′b	43.3	2.54 (1H, dd, 14.8, 7.3)
5	124.6	7.31 (1H, d, 8.7)	5′	82.2	5.24 (1H, m)
6	111.8	7.03 (1H, d, 8.7)	6′	154.0	7.53 (1H, d, 1.5)
7	156.3	/	7′	130.5	/
8	137.4	/	8′	173.6	/
9	115.3	/	9′	58.4	4.77 (2H, s)
10	149.2	/	10′	17.5	1.84 (3H, s)
1′	66.9	4.73 (2H, dd, 6.4, 2.3)	OMe	61.7	3.93 (3H, s)
2′	124.9	5.63 (1H, t, 6.4)	OAc	20.5 172.1	2.06 (3H, s)

Acknowledgments

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References

1) Chlouchi A, Muyard F, Girard C, Waterman PG, and Bévalot F, *Biochem. Syst. Ecol.*, **33**, 967–969 (2005).
2) He HP, Shen YM, He YN, Yang XS, Zhu WM, and Hao XJ, *Heterocycles*, **53**, 2067–2070 (2000).
3) Huang SC, Wu PL, and Wu TS, *Phytochemistry*, **44**, 179–181 (1997).
4) Ito C, Itoigawa M, Katsuno S, Omura M, Tokuda H, Nishino H, and Furukawa H, *J. Nat. Prod.*, **63**, 1218–1224 (2000).
5) Takemura Y, Nakamura K, Hirusawa T, Ju-ichi M, Ito C, and Furukawa H, *Chem. Pharm. Bull.*, **48**, 582–584 (2000).
6) Phuwapraisirisan P, Surapinit S, Sombund S, Siripong P, and Tip-pyang S, *Tedrahedron Lett.*, **47**, 3685–3688 (2006).
7) Wu ZY, "Flora Yunnanica" (in Chinese) Vol. 6, Science Press, Beijing, p. 767 (1995).
8) Wang YS, Huang R, Li L, Zhang HB, and Yang JH, *Biochem. Syst. Ecol.*, **36**, 801–803 (2008).
9) Wang YS, He HP, Yang JH, Di YT, and Hao XJ, *Molecules*, **13**, 931–937 (2008).
10) Wang YS, Xu HY, Wang DX, and Yang JH, *Molecules*, **14**, 771–776 (2009).
11) Rosa SD, Mitova M, Handjieva N, and Calis I, *Phytochemistry*, **59**, 447–450 (2002).
12) Kong YC, Lau CP, Wat KH, Ng KH, But PPH, Cheng KF, and Waterman PG, *Planta Med.*, **55**, 176–178 (1989).
13) Ito C and Furukawa H, *Chem. Pharm. Bull.*, **35**, 4277–4285 (1987).
14) Ngadjui BT, Ayafor JF, Sondengam BL, and Connolly JD, *J. Nat. Prod.*, **52**, 243–247 (1989).
15) Bohlmann F and Clausen E, *Chem. Ber.*, **103**, 3619–3622 (1970).
16) Ishii H, Ishikawa T, Mihara M, and Akaike M, *Yakugaku Zasshi*, **103**, 279–292 (1983).
17) Quader MA, El-Turbi JA, Armstrong JA, Gray AI, and Waterman PG, *Phytochemistry*, **31**, 3083–3089 (1992).