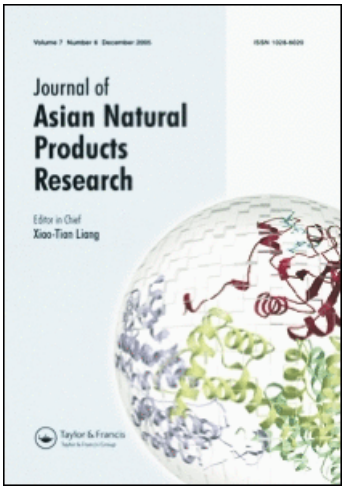

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New geranyloxycoumarins from *Toddalia asiatica*

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A phytochemical study on the twigs of *Toddalia asiatica* led to the isolation of two new geranyloxycoumarins, 7-geranyloxy-5-methoxycoumarin (**1**) and 8-geranyloxy-5,7-dimethoxycoumarin (**2**). Their structures were elucidated on the basis of extensive spectroscopic analysis.

Keywords: *Toddalia asiatica*; Rutaceae; geranyloxycoumarin

1. Introduction

Toddalia asiatica (L.) Lam. (Rutaceae), a woody liana growing in subtropical and tropical areas of China, has been widely used in traditional Chinese herbal medicine to treat cold, stomachache, intention neuralgia, rheumatalgia, and injuries from falls [1]. All parts of the plant are claimed to have medicinal value, but the roots, in particular, are believed to be more potent. Decoctions or infusions of the root bark have good efficacy in the treatment of chronic lumbago and scelalgia [1]. Previously, several research groups have reported some bioactive components from this medicinal plant, including coumarins [2–7], alkaloids [6,8–10], coumarin–quinolone dimer [11], and coumarin–naphthoquinone dimer [12].

As part of our effort to assemble a natural compound library possessing thousands of structures coming from plants and micro-organisms, further chemical investigation on this plant led to the isolation of two new coumarin–monoterpene ethers, 7-geranyloxy-5-methoxycoumarin (**1**) and 8-geranyloxy-5,7-dimethoxycoumarin (**2**), together with four known coumarins, artanin (**3**) [4], norbraylin (**4**) [2], 5,7,8-trimethoxycoumarin (**5**) [2], and toddalosin (**6**) [5] (Figure 1). Herein, details of the isolation and structural elucidation of compounds **1** and **2** are described.

2. Results and discussion

Compound **1** was obtained as an amorphous powder. Its molecular formula was determined to be $C_{20}H_{24}O_4$ on the basis of positive ESI-MS at *m/z* 351 $[M+Na]^+$, in combination with the ^{13}C NMR (DEPT) spectrum. The UV spectrum showed the absorption maxima at 248 (3.90), 257 (3.89), and 329 (4.28) nm, typical of a coumarin chromophore. The ^1H NMR spectrum (Table 1) exhibited characteristic AB system signals of the lactone ring of a coumarin at δ_H 6.15 and 7.96 (each 1H, d, $J = 9.6$ Hz) assigned to H-3 and H-4, respectively. The ^{13}C NMR spectrum (Table 1) exhibited 20 carbon signals,

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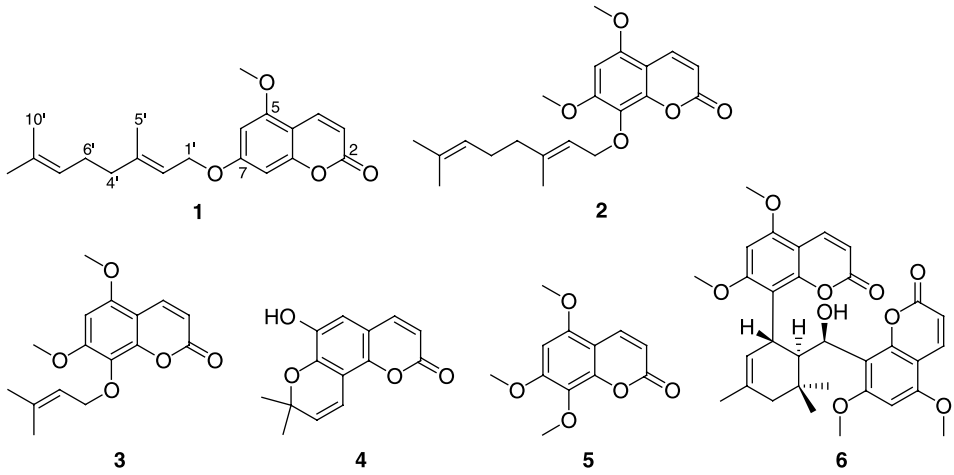

Figure 1. Structures of compounds **1**–**6**.

including nine carbon signals due to the coumarin skeleton at δ_C 162.9 (s), 161.6 (s), 156.9 (s), 156.7 (s), 138.8 (d), 110.8 (d), 103.9 (s), 95.3 (d), and 93.5 (d), 10 typical geranyloxy carbon resonances at δ_C 142.5 (s), 132.0 (s), 123.6 (d), 118.2 (d), 65.4 (t), 39.5 (t), 26.2 (t), 25.6 (q), 17.7 (q), and 16.7 (q), and one methoxy carbon at δ_C 55.9 (q), indicative of a 5,7-dioxygenated coumarin derivative. The following important HMBC correlations (Table 1) were observed: from the protons at δ_H 7.96 (1H, d, $J = 9.6$ Hz, H-4) and 3.87 (3H, s, OMe) to the carbon at δ_C 156.9 (s, C-5), and from the protons at δ_H 4.57 (2H, d, $J = 6.6$ Hz, H-1′) to the carbon at δ_C 162.9 (s, C-7), which confirmed that the methoxy and geranyloxy groups were unambiguously attached at C-5 and C-7, respectively. Therefore, the structure of **1** was elucidated as 7-geranyloxy-5-methoxycoumarin.

Compound **2**, an amorphous powder, possessed a molecular formula of $C_{21}H_{26}O_5$ based on the positive ESI-MS at *m/z* 381 $[M+Na]^+$ and supported by the ^{13}C NMR (DEPT) spectrum. The UV spectrum also showed the absorption maxima of a coumarin chromophore at 264 (4.00) and 322 (4.07) nm. The NMR spectra (Table 1) were very similar to those of **1**, and there were only two obvious differences as follows: (1) an additional methoxy signal was observed and (2) one aromatic proton within the coumarin skeleton was absent, suggesting that this compound was a trioxygenated coumarin substituted by one geranyloxy and two methoxy groups. The following significant HMBC correlations (Table 1): from the protons at δ_H 7.97 (1H, d, $J = 9.5$ Hz, H-4) and 3.91 (3H, s, 5-OMe) to the carbon at δ_C 152.2 (s, C-5), from the protons at δ_H 3.91 (3H, s, 5-OMe) and 3.95 (3H, s, 7-OMe) to the carbon at δ_C 91.2 (d, C-6), and from the protons at δ_H 4.60 (2H, d, $J = 7.0$ Hz, H-1′) to the carbon at δ_C 128.7 (s, C-8) were detected, which indicated that two methoxy groups and one geranyloxy must be linked at C-5, C-7, and C-8, respectively. Accordingly, the structure of **2** was characterized as 8-geranyloxy-5,7-dimethoxycoumarin.

3. Experimental

3.1 General experimental procedures

UV spectra were obtained on a Shimadzu UV-2401PC spectrophotometer. IR spectra were performed on a Bruker Tensor 27 FT-IR spectrometer with KBr pellets. 1D and 2D NMR spectra were respectively recorded on Bruker AV-400 and DRX-500

Table 1. ^{1}H and ^{13}C NMR spectroscopic data for compounds **1** and **2** in $CDCl_3$.

	1			**2**		
No.	δ_H	δ_C	HMBC	δ_H	δ_C	HMBC
2	–	161.6 (s)		–	160.9 (s)	
3	6.15 (1H, d, 9.6)	110.8 (d)	2, 4a	6.15 (1H, d, 9.5)	111.2 (d)	2, 4a
4	7.96 (1H, d, 9.6)	138.8 (d)	5, 8[a], 8a	7.97 (1H, d, 9.5)	138.7 (d)	5, 8[a], 8a
4a	–	103.9 (s)		–	103.9 (s)	
5	–	156.9 (s)		–	152.2 (s)	
6	6.30 (1H, d, 1.3)	95.3 (d)	4a, 8	6.32 (1H, s)	91.2 (d)	4a, 8
7	–	162.9 (s)		–	156.5 (s)	
8	6.41 (1H, d, 1.3)	93.5 (d)	4a, 6	–	128.7 (s)	
8a	–	156.7 (s)		–	149.1 (s)	
1′	4.57 (2H, d, 6.6)	65.4 (t)	7, 3′	4.60 (2H, d, 7.0)	69.9 (t)	8, 3′
2′	5.46 (1H, br t, 6.6)	118.2 (d)	4′, 5′	5.58 (1H, br t, 7.0)	119.7 (d)	4′, 5′
3′	–	142.5 (s)		–	142.5 (s)	
4′	2.09 (2H, m)	39.5 (t)	2′, 5′, 7′	2.02 (2H, m)	39.6 (t)	2′, 5′, 7′
5′	1.76 (3H, s)	16.7 (q)	2′, 4′	1.67 (3H, s)	16.4 (q)	2′, 4′
6′	2.13 (2H, m)	26.2 (t)	3′, 8′	2.05 (2H, m)	26.4 (t)	3′, 8′
7′	5.08 (1H, br t, 5.9)	123.6 (d)	4′, 9′, 10′	5.06 (1H, br t, 6.0)	123.9 (d)	
8′	–	132.0 (s)		–	131.6 (s)	
9′	1.67 (3H, br s)	25.6 (q)	7′, 10′	1.66 (3H, br s)	25.7 (q)	7′, 10′
10′	1.60 (3H, br s)	17.7 (q)	7′, 9′	1.58 (3H, br s)	17.7 (q)	7′, 9′
OCH_3	3.87 (3H, s, 5-OMe)	55.9 (q)	5, 6[a]	3.91 (3H, s, 5-OMe)	56.0 (q)	5, 6[a]
				3.95 (3H, s, 7-OMe)	56.4 (q)	6[a], 7

[a] Weak but significant four-bond HMBC correlations.

instruments with the deuterated solvent ($CDCl_3$: δ_H 7.26, δ_C 77.0) as an internal standard. EI-MS and ESI-MS (including HR-ESI-MS) were measured on Finnigan-MAT 90 and API QSTAR Pulsar i mass spectrometers, respectively. Silica gel (200–300 mesh; Qingdao Marine Chemical, Inc., Qingdao, China) and Sephadex LH-20 (Amersham Biosciences, Uppsala, Sweden) were used for column chromatography (CC). Fractions were monitored by TLC (Qingdao Marine Chemical, Inc.) in combination with Agilent 1200 HPLC (Eclipse XDB-C18 column, 5 μm, 4.6 × 150 mm, 50–100% MeOH in H_2O over 8 min followed by 100% MeOH to 15 min, 1 ml/min, 25°C).

3.2 Plant material

The twigs of *T. asiatica* were collected from Yunnan Province, China and identified by Prof. Dr Hua Peng. The voucher specimen (No. BBP2009004TA) has been deposited in the Herbarium of Kunming Institute of Botany, Chinese Academy of Sciences.

3.3 Extraction and isolation

The air-dried and powdered twigs (5.0 kg) were extracted with 95% ethanol at room temperature and filtered. The filtrate was concentrated to give a crude extract (~400 g), which was subjected to silica gel CC with a stepwise elution of petroleum ether (PE)–acetone to yield fractions A–I. Fraction B (~5.0 g) eluted by PE–acetone (96:4) was further separated by silica gel CC using PE–acetone (100:1, 50:1, 10:1, 1:1) to yield subfractions A–D, respectively. Subfraction A (~300 mg) was repeatedly isolated and purified by silica gel CC (PE–acetone = 150:1; PE–$CHCl_3$ = 5:1) and Sephadex LH-20 ($CHCl_3$–MeOH = 1:1), and recrystallized to afford compound **1** (91 mg). Similarly, compound **2** (296 mg) was obtained from subfraction B (~750 mg) by silica gel CC (PE–acetone = 50:1; PE–$CHCl_3$ = 3:1) and Sephadex LH-20 ($CHCl_3$–MeOH = 1:1).

3.3.1 7-Geranyloxy-5-methoxycoumarin (*1*)

An amorphous powder; HPLC: t_R = 10.24 min; UV λ_{max} ($CHCl_3$) (log ε): 248 (3.90), 257 (3.89), 329 (4.28) nm; IR (KBr) ν_{max}: 3075, 3016, 1725, 1630, 1612, 1501, 1452, 1364, 1226, 1196, 1164, 1123 cm^{-1}; NMR spectral data: see Table 1; EI-MS *m/z*: 192 $[M-geranyl]^+$ (100), 164 (39), 149 (11), 136 (19); ESI-MS (pos.) *m/z*: 351 $[M+Na]^+$; HR-ESI-MS (pos.) *m/z*: 351.1569 (calcd for $C_{20}H_{24}O_4Na$, 351.1572).

3.3.2 8-Geranyloxy-5,7-dimethoxycoumarin (*2*)

An amorphous powder; HPLC: t_R = 9.25 min; UV λ_{max} ($CHCl_3$) (log ε): 264 (4.00), 322 (4.07) nm; IR (KBr) ν_{max}: 3084, 1730, 1605, 1505, 1438, 1345, 1261, 1221, 1188, 1149, 1120 cm^{-1}; NMR spectral data: see Table 1; EI-MS *m/z*: 222 $[M-geranyl]^+$ (100), 207 (34), 193 (26), 179 (9), 136 (6); ESI-MS (pos.) *m/z*: 381 $[M+Na]^+$; HR-ESI-MS (pos.) *m/z*: 381.1676 (calcd for $C_{21}H_{26}O_5Na$, 381.1677).

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