SHORT REPORT

A C G publications

Rec. Nat. Prod. 18:2 (2024) 296-301

records of natural products

A New Prenylated Coumarin from the Roots of *Toddalia asiatica*

Aifen Zeng [1], Zhengkuan Zhang [1], Guobo Xu [1, 2], Shanggao Liao [1, 2], Qinfeng Zhu [*1, 2] and Xun He [*1, 2]

[1] *School of Pharmacy, Guizhou Medical University, Guian New District, Guizhou 561113, P. R. China*
[2] *University Engineering Research Center for the Prevention and Treatment of Chronic Diseases by Authentic Medicinal Materials in Guizhou Province, Guizhou Medical University, Guian New District, Guizhou 561113, P. R. China*

(Received January 06, 2024; Revised February 28, 2024; Accepted March 01, 2024)

Abstract: A new prenylated coumarin, 5, 7-dimethoxy-6-[(*Z*)-3'-methylbutan-1',3'-d-ienyl] coumarin (**1**), along with ten known compounds (**2**-**11**), was obtained from the roots of *Toddalia asiatica* (Linn.) Lam. Their structures were determined based on 1 D and 2 D NMR spectroscopy as well as high-resolution mass spectrometry. Notably, the structure of (3*S*, 4*R*)-3,4-epoxypimpinellin was revised as its *cis*-epimer, compounds (**2**-**6**) were isolated from this genus for the first time. The cytotoxic activities of the isolated compounds **1**-**11** were evaluated against SW480, A549, MDA-MB-231, HepG 2, HEp-2, SGC7901 cancer cell lines. Compound **10** exhibited weak cytotoxicity against HEP 2 with IC_{50} value of 75.19 ± 1.17 μM.

Keywords: *Toddalia asiatica*; coumarins; chemical constituents; cytotoxic activity.

1. Plant Source

The roots of *T. asiatica* were collected from Guiyang, Guizhou Province, China, in July 2021 and authenticated by Prof. Qingde Long (Herbarium of Guizhou Medical University). A voucher specimen (No.20210718) has been placed in the school of pharmacy, Guizhou Medical University.

2. Previous Studies

Toddalia asiatica (L.) Lam., family Rutaceae, is the sole species from the genus *Toddalia*. It is a woody climber widely distributed in the south of Qinling Mountains in China, most frequently on secondary forests and thickets [1]. This plant is pharmacologically meritorious as herbal medicines in many regions. Its roots and barks have been used as a traditional Chinese medicine for the treatment of bruises sprains, rheumatoid arthritis and intercostal neuralgia [2, 3]. Previous phytochemical studies of *T. asiatica* showed the isolation of alkaloids, coumarins, triterpenes and flavonoids, some of which exhibited anti-inflammatory, antitumor and antimicrobial activities [4-8].

[*] Corresponding authors: E-Mail: zhuqinfeng@gmc.edu.cn (Qingfeng Zhu); hexun224@gmc.edu.cn (Xun He).

The article was published by ACG Publications
http://www.acgpubs.org/journal/records-of-natural-products March-April 2024 EISSN:1307-6167
DOI: http://doi.org/10.25135/rnp.449.2401.3015
Available online: March 14, 2024

3. Present Study

In our continuing exploration for bioactive compounds from this genus, the chemical constituents of the roots of *T. asiatica* were studied. The investigation led to the isolation of a previously undescribed prenylated coumarin, together with ten known compounds. Herein, we report the isolation, structure elucidation as well as cytotoxic activities of these compound.

The air dried powder of *T. asiatica* (10 kg) was extracted with 95% ethanol (3 × 20 L, 3 × 24 h) to acquire a crude extract (1.07 kg), which was chromatographed over macroporous resin eluting with gradient solvents of EtOH : H_2O (30%→95%) to give 6 fractions (*Fr.A-Fr.F*). Fraction B (101.4 g) was separated on a silica gel column (PE-EtOAc, 10 : 1 → 0 : 1, v/v) to afford 15 fractions (*Fr.B1-Fr.B15*). *Fr.B2* (10 g) was separated *via* silica gel column chromatography (PE-CH_2Cl_2, 100 : 1, v/v) to give ten fractions *Fr.B2.1-Fr.B2.10*. The *Fr.B2.1* fraction (300 mg) was purified by semi-preparative HPLC (PE-CH_2Cl_2, 80 : 20, v/v, 3 mL/min) to yield compound **2** (4 mg, t_R = 15 min) and **3** (2 mg, t_R = 19 min). Compound **4** (4 mg, t_R = 15 min) was obtained from the *Fr.B2.3* (56 mg) fraction by the separation using semi-preparative HPLC with (PE-CH_2Cl_2, 73:27, 3 mL/min) as the mobile phase. *Fr.B2.4* (1.8 g) was fractionated on a silica gel chromatography (PE-EtOAc, 100 : 1 to 10 : 1, v/v) to afford *Fr.B2.4.1-Fr.B2.4.4*. *Fr.B2.4.2* (16 mg) was separated by semi-preparative HPLC eluting with 72% MeOH in water (2.5 mL/min) to give **1** (3.2 mg, t_R = 20 min) and compound **11** (4.7 mg, t_R = 22 min). *Fr.B2.4.3* (20 mg) was separated over *Sephadex LH-20* eluting with $CHCl_3$-MeOH (3 : 2, v/v) to acquire compound **5** (2.4 mg). *Fr.B2.5* (5.6 g) was subjected to silica gel chromatography (PE-Acetone, 10 : 1, v/v) to obtain eight fraction. Subfraction *Fr.B2.5.1* (105 mg) was prepared by semi-preparative HPLC (PE-EtOAc, 70 : 30, 3.0 mL/min) to get compound **10** (6 mg, t_R = 16 min).Compound **6** (4.5 mg, t_R = 27 min) and **7** (4 mg, t_R = 36 min) were obtained from the *Fr.B2.5.2* (120 mg) fraction by purifying on semi-preparative HPLC eluting with CH_2Cl_2: EtOAc (65:35, v/v, 3.0 mL/min). *Fr.B2.5.4* (460 mg) was separated by CC eluting with PE-$CHCl_3$ (3 : 1, v/v) as eluents to yield compound **8** (5 mg). Compound **9** (6 mg, t_R = 17 min) was separated from *Fr.B2.5.5* (460 mg) by semi-preparative HPLC (CH_2Cl_2-EtOAc, 80: 20, 3.0 mL/min).

*Compound (**1**)*: Colorless oil, UV (MeOH) λ_{max} (log ε): 206 (3.07), 273 (2.72), 320 (2.69) nm; IR (KBr, ν_{max},): 3423, 1723, 1700, 1612, 1384, 1139 cm^{-1}; HR-ESI-MS *m/z* 273.1119 (calcd. for $C_{16}H_{17}O_4^+$ $[M+H]^+$, 273.1121); ^1H (600 MHz) and ^{13}C (150 MHz) NMR data see Table 1.

Cytotoxicity assay: Six human cancer lines (SW480, A549, MDA-MB-231, HepG 2, HEp-2, SGC7901) were got from the cell bank of Chinese Academy of Science. Six cancer lines except SW480 were cultivated in DMEM containing 10% FBS, 1% penicillin and streptomycin at an atmosphere with 5% CO_2 at 37°C while SW480 was cultivated in RPMI-1640 (Gibco, Germany). The cytotoxicity for compounds **1-11** was measured using MTT method as our previously described [9].

Compound **1** was isolated as a colorless oil. Its molecular formula $C_{16}H_{16}O_4$ was confirmed by its HR-ESI-MS ion at *m/z* 273.1119 $[M + H]^+$ (calcd. 273.1121), indicating 10 degrees of unsaturation. The IR spectrum revealed the presence of carbonyl (1723 cm^{-1}), and aromatic system (1612, 1563, 1461 cm^{-1}). The ^1H NMR spectrum ($CDCl_3$, 600 MHz, Table 1) showed the presence of two methoxy protons [δ_H 3.81 (3H, s, -OCH_3) and 3.86 (3H, s, -OCH_3)]; one methyl proton [δ_H 1.58 (3H, s, 4'-CH_3)]. The coupling constants of two doublet protons at δ_H 6.17 (1H, d, *J* = 12.2 Hz) and 6.40 (1H, d, *J* =12.2 Hz) indicated a *cis* geometry double bond. The remaining ^1H NMR signals at δ_H 6.24 (1H, d, *J* = 9.6 Hz), 7.93 (1H, d, *J* = 9.6 Hz) and δ_H 4.93 (s, 2H) were readily assigned to olefinic protons and methylene respectively. The ^{13}C NMR spectrum ($CDCl_3$, 150 MHz) revealed the presence of sixteen carbon atoms corresponding to a carbonyl (δ_C 161.34, C-2), two methoxy groups (δ_C 61.79, 58.28), a methyl (δ_C 20.59, C-5), six quaternary carbons (δ_C 155.2, 161.3, 107.3, 155.6, 117.3, 142.6), a methylene (δ_C 118.15, C-1′), and five methines (δ_C 112.6, 118.6, 136.5, 139.2, 95.0) (Table 1).

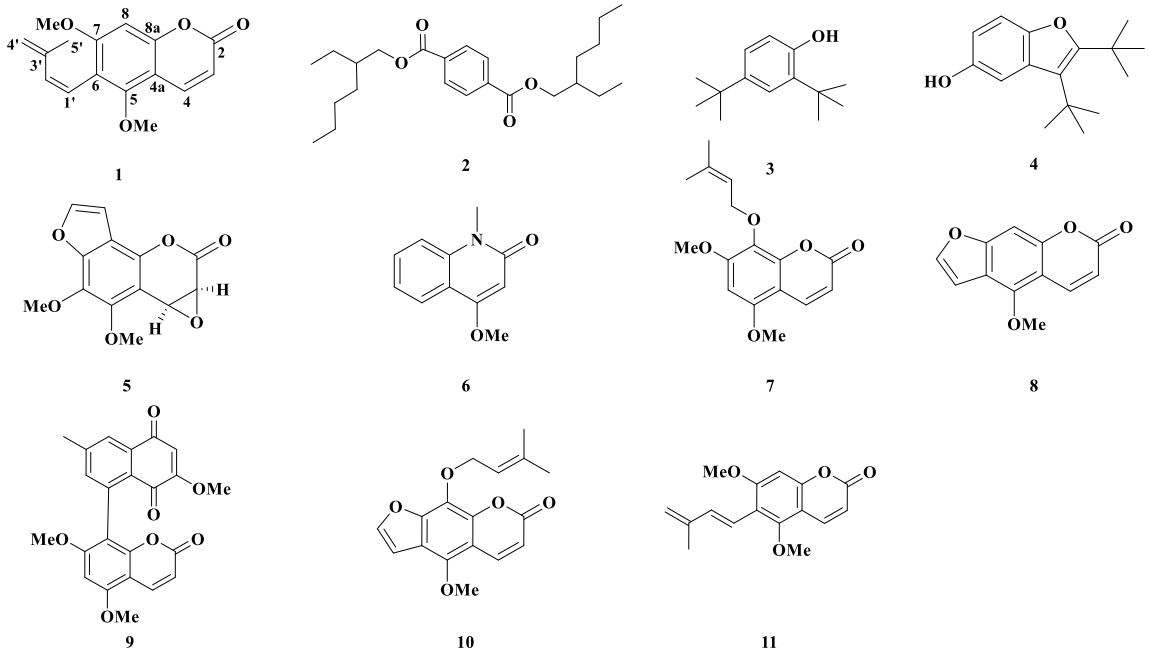

Figure 1. Chemical structures of compounds **1-11**

The aforementioned spectroscopic data and the HMBC correlations of H-3/C-4a, H-4/C-2, H-4/C-5, H-8/C-4a and H-8/C-6 together with the apparent ^{1}H-^{1}H COSY correlations of H-3 with H-4 declared that compound **1** was a triple substituent coumarin derivative (Figure 2). The additional HMBC signals from H-1' to C-5, C-7, C-3', from H-2' to C-6, C-5' and from CH_2-4' to C-2', C-5' were observed, too. All the data of compound **1** were very similar to those of 6-(3'-methyl-1', 3'-butadienyl)-5,7-dimethoxycoumarin (**11**) [10], which was isolated at the same time; however, the coupling constants of H-1'/H-2', and ^{1}H-NMR signals for the side chain protons have some differences for these two compounds. The coupling constants in 6-(3'-methyl-1', 3'-butadienyl)-5,7-dimethoxycoumarin is 16.8 Hz, which is much larger than that of compound **1**. The NOESY spectroscopy correlation from H-2' to H-4' confirmed that these conjugated double bonds were a transoid conformation. Thus, compound **1** was determined.

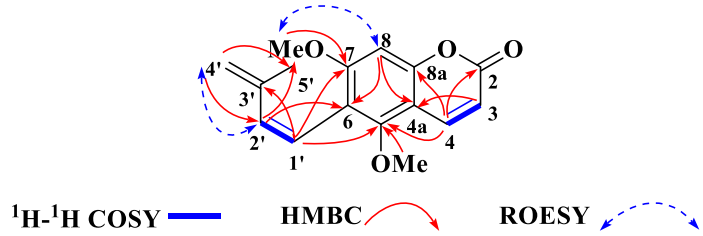

Figure 2. ^{1}H-^{1}H COSY correlations and the selected HMBC correlations of compound **1**

The NMR spectroscopic data of compound **5** are same with those of (3*S*, 4*R*)-3,4-epoxypimpinellin, a previously reported coumarin [11]. Both hydrogens attached to the epoxide ring were assigned as *trans*-configuration in the literature. However, the *trans*-configurations of H-3 and H-4 on the epoxide ring seem to be not stabilized in cyclic molecules due to excessive molecular strain. ^{13}C NMR calculation was applied to further confirm the structure of compound **5** at the B3LYP/6-311+G (d, p) level in PCM model. The results indicated that the predicted chemical shifts of *cis*-**5** are approximately identical to the test data with a correlation coefficient (R^2) of 0.989, which validated the *cis*-configuration of compound **5**. Therefore, the structure of (3*S*, 4*R*)-3,4-epoxypimpinellin was revised as *cis*-3,4-epoxypimpinellin.

The other nine compounds were identified as *bis*(2-ethylhexyl) terephthalate (**2**) [12], 2,4-di-tert-butylphenol (**3**)[13] , 2,3-di-tert-butylbenzofuran-5-ol (**4**) [14], 4-methoxy-*N*-methyl-2-quinolone (**6)** [15], artanin (**7**) [16], bergapten (**8**) [17], toddacoumaquinone (**9**) [18], phellopterin (**10**) [19], 6-(3'-methyl-1', 3'-butadienyl)-5,7-dimethoxycoumarin (**11**) [10], by comparing their NMR data with published data. Compounds **2**-**6** were isolated from *T. asiatica* for the first time.

Table 1. The NMR data of compound **1** in $CDCl_3$ (δ in ppm, J in Hz).

Position	δ_H	δ_C
2		161.3
3	6.24 (d, J = 9.6 Hz, 1H)	112.6
4	7.93 (d, J = 9.6 Hz, 1H)	139.2
4a		107.3
5		155.2
6		117.3
7		161.1
8	6.60 (s, 1H)	95.0
8a		155.6
1′	6.17 (d, J = 12.2 Hz, 1H)	118.6
2′	6.40 (d, J = 12.2 Hz, 1H)	136.5
3′		142.6
4′	4.93 (s, 2H)	118.2
5′	1.58 (s, 3H)	20.6
5-OCH_3	3.81 (s, 3H)	61.8
7-OCH_3	3.86 (s, 3H)	56.3

The effects of compounds **1**-**11** against SW480, A549, MDA-MB-231, HepG 2, HEP 2 and SGC7901 cell lines were evaluated by MTT assay and 5-fluorouracil was used as the positive control. As a results, all the isolated compounds showed no cytotoxicity against A549 and HepG 2 at the concentration of 100 μM. Compound **10** exhibited weak cytotoxic activity against HEp-2 with IC_{50} values of 75.19 ± 1.17 μM (5-fluorouracil, IC_{50} = 3.61 ± 0.84 mM), while other compounds were inactive (IC_{50} > 100 μM).

In conclusion, our investigation towards the roots of *T. asiatica* lead to the isolation of a new prenylated coumarin, 5, 7-dimethoxy-6-[(*Z*)-3'-methylbutan-1',3'-d-ienyl] coumarin (**1**) and ten known constituents. Compound **1** represents a rare example of naturally occurring coumarin with a (*Z*)-pentene derivative moiety while compounds (**2**-**6**) were isolated from this genus for the first time. The cytotoxicity of all the isolates against six human tumor cell lines were evaluated. This study enriched the chemistry and structure diversity of natural products derived from *T. asiatica*.

Acknowledgments

This work was supported by the Science and Technology Department of Guizhou Province (ZK[2021]554), the University Engineering Research Center for the Prevention and Treatment of Chronic Diseases by Authentic Medicinal Materials in Guizhou Province ([2023]035), the National Natural Science Foundation of China (U1812403-5-1) and the Scientific Research Foundation for Innovative Talent of Guizhou Province (2020)6011-2.

Supporting Information

Supporting Information accompanies this paper on http://www.acgpubs.org/journal/records-of-natural-products

ORCID
Aifen Zeng: 0009-0002-0802-8238
Zhengkuan Zhang: 0009-0008-7311-6799
Guobo Xu: 0000-0001-9121-4618
Shanggao Liao: 0000-0001-9008-0371
Qinfeng Zhu: 0000-0001-6877-1838
Xun He: 0000-0001-5056-3440

References

[1] Editorial Committee of the Flora of China (1999). Flora of China. Science Press, Beijing.
[2] G. Q. Wang (2014). The Compilation of National Chinese Herbal Medicine. People's Medical Publishing House, Beijing.
[3] Nangjing University of Chinese Medicine (2006). Dictionary of Chinese Medicine. Shanghai Scientific & Technical Publishers, Shanghai.
[4] J. Hu, X. Shi, J. Chen, X. Mao, L. Zhu, L. Yu and J. Shi (2014). Alkaloids from *Toddalia asiatica* and their cytotoxic, antimicrobial and antifungal activities, *Food Chem.* **148**, 437-444.
[5] Z. H. Tang, Y. B. Liu, S. G. Ma, L. Li, Y. Li, J. D. Jiang, J. Qu and S. S. Yu (2016). Antiviral spirotriscoumarins A and B: two pairs of oligomeric coumarin enantiomers with a spirodienone–sesquiterpene skeleton from *Toddalia asiatica*, *Org. Lett.* **18**, 5146-5149.
[6] Z. Zeng, R. Tian, J. Feng, N. A. Yang and L. Yuan (2021). A systematic review on traditional medicine *Toddalia asiatica* (L.) Lam.: Chemistry and medicinal potential, *Saudi Pharm. J* .**29**, 781-798.
[7] W. Li, J. S. Zhang, J. L. Huang, M. H. Jiang, Y. K. Xu, A. Ahmed, S. Yin and G. H. Tang (2017). New prenylated coumarins from the stems of *Toddalia asiatica*, *RSC Adv.* **7**, 31061-31068.
[8] J. K. Reinhardt, A. M. Zimmermann-Klemd, O. Danton, M. Smieško, C. Gründemann and M. Hamburger (2020). Compounds from *Toddalia asiatica*: Immunosuppressant activity and absolute configurations, *J. Nat. Prod.* **83**, 3012-3020.
[9] Q. F. Zhu, Y. Y. Qi, Z. J. Zhang, M. Fan, R. Bi, J. Su, X. D. Wu, L. D. Shao and Q. S. Zhao (2018). Vibsane-type diterpenoids from *Viburnum odoratissimum* and their cytotoxic and HSP90 inhibitory activities, *Chem. Biodivers.* 1**5**, 1800049.
[10] R. Phatchana and C. Yenjai, Cytotoxic coumarins from *Toddalia asiatica* (2014), *Planta Med.* **80**, 719-722.
[11] S.Y. Park, N. Lee, S. Lee, M.J. Kim, W. Chun, H.P. Kim, H.J. Yang, H. S. Lee and Y. Kwon (2017). A new 3, 4-epoxyfurocoumarin from *Heracleum moellendorffii* Roots, *Nat. Prod. Sci.* **23**, 213-216.
[12] A. Kdimy, S. J. Kim, Z. Ali, I. H. M. Khan, S. K. Tripathi, S. El Hajjaji, H. V. Le (2023). Isolation of two plasticizers, bis(2-ethylhexyl) terephthalate and bis(2-ethylhexyl) phthalate, from *Capparis spinosa* L. leaves, *Chem. Biodivers.* **20**, e202300903.
[13] J. Y. Cho, M. S. Kim, Y. G. Lee, H. Y. Jeong, H. J. Lee, K. S. Ham and J. H. Moon (2016). A phenyl lipid alkaloid and flavone C-diglucosides from *Spergularia marina*, *Food Sci. Biotechnol.* **25**, 63-69.
[14] N. Xu, E. Nyamdavaa, J. Yu, H. Xiao, J. Liu, S. Li and L. Xu (2023). A new benzofuran compound from the resinous exudates of *Commiphora myrrha*, *Nat. Prod. Res*. **37**, 1416-1420.
[15] H. Y. Huang, T. Ishikawa, C. F. Peng, S. Chen and I. S. Chen (2011). Secondary metabolites from the root wood of *Zanthoxylum wutaiense* and their antitubercular activity, *Chem. Biodivers.* **8**, 880-886.
[16] D. Maes, M. E. Riveiro, C. Shayo, C. Davio, S. Debenedetti and N. D. Kimpe (2008). Total synthesis of naturally occurring 5,6,7- and 5,7,8-trioxygenated coumarins, *Tetrahedron* **64**, 4438-4443.
[17] S. W. Yoo, J. S. Kim, S. S. Kang, K. H. Son, H. W. Chang, H. P. Kim, K. Bae and C. O. Lee (2002) Constituents of the fruits and leaves of *Euodia daniellii*, *Arch. Pharm. Res*. **25**, 824-830.
[18] H. Ishii, J. I. Kobayashi, H. Seki and T. Ishikawa (1992). Toddacoumaquinone, a unique coumarin-naphthoquinone dimer, from *Toddalia asiatica* (L.) Lam. (*T. aculeata Pers.*), *Chem. Pharm. Bull.* **40**, 1358-1359.

[19] S. Marumoto and M. Miyazawa (2010). β-secretase inhibitory effects of furanocoumarins from the root of *Angelica dahurica*, *Phytother. Res.* **24**, 510-513.