

Todasinoid A, a New Eremophilane-type Sesquiterpene from the Plant *Toddalia asiatica*

Lijing Cai , Mengying Zhang , Jie He  and Tingting Lin *

Jiangxi Provincial People's Hospital, The First Affiliated Hospital of Nanchang Medical College,
Nanchang 330006, People's Republic of China

(Received June 09, 2022; Revised August 15, 2022; Accepted August 23, 2022)

Abstract: A new sesquiterpene, named todasinoid A (**1**), together with eight known compounds (**2–9**) were isolated from the roots of *Toddalia asiatica*. The gross structure of todasinoid A were established by analyses of the NMR and HRESIMS data. A comparison of the experimental ECD spectrum of **1** with the calculated ECD spectra for a model compound (**1a**) resolved the absolute configuration of **1**. The known compounds were identified to be bullatantriol (**2**), aculeatin (**3**), 6-(3-ethoxy-2-hydroxy-3-methylbutyl)-5,7-dimethoxy-2H-1-benzopyran-2-one (**4**), *trans*-N-p-coumaroyltyramine (**5**), feruloyltyramine (**6**), β -sitosterol (**7**), sitosterol *D*-glucoside(**8**), 7α -hydroxysitosterol (**9**) by comparing the NMR data and specific rotations with reported data in literature. Compound **1** is an eremophilane-type sesquiterpenoid containing a mercaptolactate side-chain that is rarely found in nature. Compounds **2**, **5**, and **9** were isolated from this plant for the first time. Bioassay study revealed that compound **5** exhibited inhibitory effects against α -glucosidase with an IC_{50} of 320 μ M, being more active than the positive control acarbose.

Keywords: *Toddalia asiatica*; Todasinoid A; sesquiterpenoid; ECD calculation. © 2022 ACG Publications. All rights reserved.

1. Plant Source

The roots of *Toddalia asiatica* were collected in October 2012 in Yunnan Province, P. R. China. The sample of roots was identified by Prof. You-Kai Xu of Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences. A voucher specimen (accession number: FLZX201210) was deposited at the School of Pharmaceutical Sciences, Sun Yat-sen University.

2. Previous Studies

The plant *T. asiatica* (L.) Lam. (Rutaceae) has a wide distribution in south China. In Traditional Chinese Medicine (TCM), the roots of *T. asiatica* were used to treat pyogenic infections,

*Corresponding author: E-Mail: lintingting_1988@163.com

A new eremophilane-type sesquiterpene

dyspepsodynia, and rheumatic arthritis. A recent review revealed that the chemical constituents of the plant were coumarins, alkaloids, terpenoids, some of which exhibited remarkable pharmacological effects, such as anti-inflammatory analgesic, bacteriostatic, and cardiovascular protective effects. [1]. In our previous study of *T. asiatica*, a series of prenylated coumarins and benzophenanthridine alkaloids possessed potent inhibitory effects against phosphodiesterase-4 were reported [2, 3]. In continuing study of the plant, two sesquiterpenes (**1** and **2**), two isocoumarins (**3** and **4**), two amides (**5** and **6**) and three steroids (**7–9**) were obtained (Figure 1), the α -glucosidase inhibitory effects were tested. Herein, the isolation, structural elucidation, and the α -glucosidase inhibitory effects of compounds **1–9** are described.

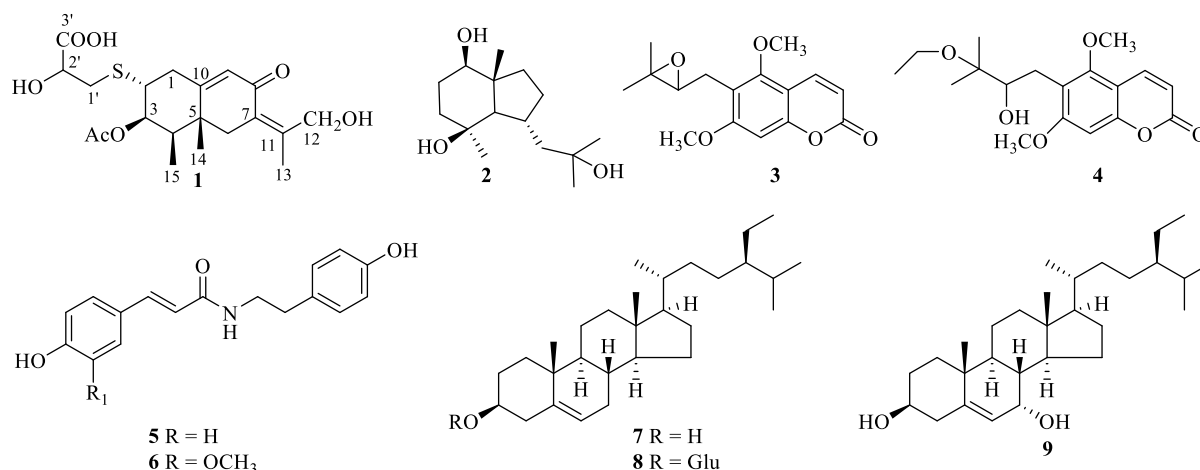


Figure 1. Structures of compounds **1–9** from *Toddalia asiatica*

3. Present Study

The air-dried powder of the roots of *T. asiatica* (1 kg) was extracted with 95% EtOH (3 × 10 L) at room temperature (rt) to give 85 g of crude extract. The extract was suspended in H₂O (1 L) and successively partitioned with petroleum ether (PE, 3 × 1 L) and EtOAc (3 × 1 L), respectively. The EtOAc extract (63 g) was subjected to MCI gel CC eluted with a MeOH/H₂O gradient (3:7 → 10:0) to afford four fractions (Fr. I–Fr. IV). Fr. III was subjected to silica gel CC (PE/EtOAc, 3:1 → 0:1) to give four fractions (Fr. IIIa–Fr. IIId). Fr. IIIa was applied to silica gel CC (PE/EtOAc, 10:1 → 2:1) to afford **3**, **4**, and **7**. Fr. IIIc was subjected to silica gel CC (PE/Acetone, 5:1 → 1:1) to yield **5**, **6**, and **9**. Fr. IIIId was purified by silica gel CC (CH₂Cl/MeOH, 20:1 → 5:1) to give **1**, **2**, and **8**.

Todasinoid A (**1**): Colorless oil; $[\alpha]_D^{25} +59$ (*c* 0.15, MeOH); UV (MeOH) λ_{\max} (log ϵ) 251 (4.16), 282 (3.94); ECD (*c* 2.7 × 10⁻⁴ M, MeOH) λ_{\max} ($\Delta\epsilon$) 240 (+7.10), 284 (−5.41); ¹H and ¹³C NMR data, see Table 1; HRESIMS *m/z* 435.1445 [M + Na]⁺ (calcd for C₂₀H₂₈O₇Na⁺, 435.1448); HRESIMS *m/z* 413.1635 [M + H]⁺ (calcd. for C₂₀H₂₉O₇S⁺, 413.1629).

Compound **1** had the molecular formula C₂₀H₂₈O₇S as determined by the HRESIMS data, requiring 7 degrees of unsaturation. The ¹H NMR spectrum showed four methyl groups including an acetoxy group (δ_H 2.08) and an olefinic methyl group (δ_H 1.99), five protons attached to carbons bearing heteroatoms (δ_H 4.89, 4.10, 4.08, 3.99, 3.31), an olefinic proton (δ_H 5.72), and seven alkyl protons. The ¹³C NMR and HSQC spectra revealed the presences of an α,β -unsaturated ketone group (δ_C 190.5, 127.6, 164.4), four methyl carbons (δ_C 11.1, 17.4, 17.9, 20.8), a tetrasubstituted double bond (δ_C 164.4, 145.4), four sp³ methylenes including an hydroxymethyl group (δ_C 61.5), four methines (δ_C 37.9, 44.5, 70.2, 74.8), one sp³ quaternary carbon (δ_C 40.4), and a carboxylic acid group (δ_C 173.9), and an ester carbon (δ_C 169.9). The functional groups above accounted for 5 of the 7

degrees of unsaturation, indicating a bicyclic nucleus. The above-mentioned information was similar to that of a reported analogue (3*S*)-3-acetoxyeremophil-7(11),9(10)-dien-8-one [4], suggestive of an eremophilane-type sesquiterpenoid. The obvious differences were owing to the presence of a hydroxymethyl and three additional carbons in **1** instead of one olefinic methyl in (3*S*)-3-acetoxyeremophil-7(11),9(10)-dien-8-one.

Table 1. ¹H (400 Hz) and ¹³C NMR (100 Hz) data of compound **1** in DMSO-*d*₆ (δ in ppm)

No.	δ _H , mult. (J in Hz)	δ _C , type	No.	δ _H , mult. (J in Hz)	δ _C , type
1	β 2.96, d (14.7) α 2.33, d (14.7)	32.3, CH ₂	11		145.4, C
2	3.31, br s	44.4, CH	12	4.10 d (13.1) 3.99 d (13.1)	61.5, CH ₂
3	4.89, br s	74.8, CH	13	1.99, s	17.4, CH ₃
4	2.15, m	37.9, CH	14	1.05, s	17.9, CH ₃
5		40.4, C	15	0.94, d (6.9)	11.1, CH ₃
6	β 2.97, o α 1.98, o	35.7, CH ₂	1'	2.92, m 2.78, dd (13.2, 6.7)	35.7, CH ₂
7		127.6, C	2'	4.08, m	70.2, CH
8		190.5, C	3'		173.9, C
9	5.72, s	127.4, CH	1''	2.08, s	20.8, CH ₃
10		164.4, C	2''		169.9, C

The structure of **1** was established by detailed analyses of the 2DNMR data (Figure 2). The COSY relationship established a spin system containing CH₂-1, CH-2, CH-3, CH-4, and CH₃-15. The HMBCs from the olefinic methyl H₃-13 (δ_H 1.99) to C-7 (δ_C 127.6), C-11 (δ_C 145.4), and C-12 (δ_C 61.5) assigned the tetrasubstituted double bond resided at C-7 and C-11 and a hydromethyl group at C-12. Additional HMBCs from H₂-6 (δ_H 2.96, 1.98) to C-7, C-8 (δ_C 190.5), C-10 (δ_C 164.4), and C-11 and from H-9 (δ_H 5.72) to C-8 located the α,β-unsaturated ketone group at C-8, C-9 (δ_C 127.4), C-10. The acetoxy group (δ_H 2.08; δ_C 20.8, 169.9) was attached to C-3 (δ_C 74.8) by HMBC correlations from H-3 (δ_H 4.89) to the carbonyl carbon of the acetyl group (δ_C 169.9). Thus, the gross structure of **1** was established except for the substituent group bonded to C-2.

The remaining groups including an oxymethine (δ_H 4.08, δ_C 70.2), a methylene (δ_H 2.92, 2.78; δ_C 35.7), and a carboxylic acid group (δ_C 173.9) were assembled to a 2-hydroxypropanoic acid unit by the ¹H-¹H COSY correlations between H₂-1' (δ_H 2.92, 2.78) with H-2' (δ_H 4.08) and HMBCs from H₂-1' and H-2' to the carboxylic acid carbon C-3' (δ_C 173.9). Based on the molecular formula of **1**, the substituent group at C-2 has a molecular formula of C₃H₅O₃S, bearing a sulfur atom. Thus, the methine C-2 (δ_C 44.4) of the 2-hydroxypropanoic acid unit should be connected to a sulfur atom to form a mercaptolactate side-chain, which was attached to C-2 of the eremophilane nucleus by HMBC from H-1' to C-2. Besides, the mercaptolactate side-chain was confirmed by comparing its chemical shifts with those of the mercaptolactate side-chain in sumalarin A and B [5], whose structures were confirmed by X-ray analysis.

A new eremophilane-type sesquiterpene

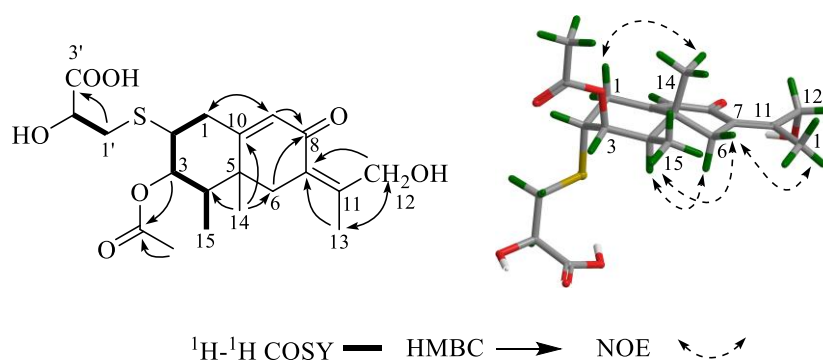


Figure 2. Key ^1H - ^1H COSY, HMBC, and NOE correlations of **1**

The relative configuration of **1** was assigned by NOESY experiment in association with J values. In the NOESY spectrum (Figure 2), the strong correlations between H_3 -14 (δ_{H} 1.05) and H -1 β (δ_{H} 2.96), H_3 -1'' (δ_{H} 2.08), H -6 β and from H -4 (δ_{H} 2.15) to H -6 α (δ_{H} 1.98) and the small coupling constants of $J_{2,3}$ (δ_{H} 3.31, br s, H -2) and $J_{3,4}$ (δ_{H} 4.89, br s, H -3) clarified that the bicyclic nucleus adopted a half-chair-chair conformation, and H_3 -14, H_3 -15 and the acetoxy group were β -orientated, while the mercaptolactate side-chain was α -orientated. The strong NOE correlations between H_3 -13 (δ_{H} 1.99) and H -6 β (δ_{H} 2.97) indicated that the double bond Δ^{13} had a Z configuration. As for the absolute configuration of **1**, the ECD calculation of the model compounds **2R**, **3R**, **4R**, **5R-1a** and **2S**, **3S**, **4S**, **5S-1a** were carried out using b3lyp/6-31+g(d,p) optimized geometries at the b3lyp/6-31+g(d,p) level in methanol. The experimental ECD spectrum of **1** showed an ECD curve similar to that of **1a** (Figure 3), suggesting that **1** had a **2R**, **3R**, **4R**, **5R**-configuration. Thus, the structure of **1** was determined as depicted and was named todasinoid A. The mercaptolactate side-chain was rarely found in natural product. Biogenetically, the mercaptolactate unit may derive from cysteine by oxidation [6].

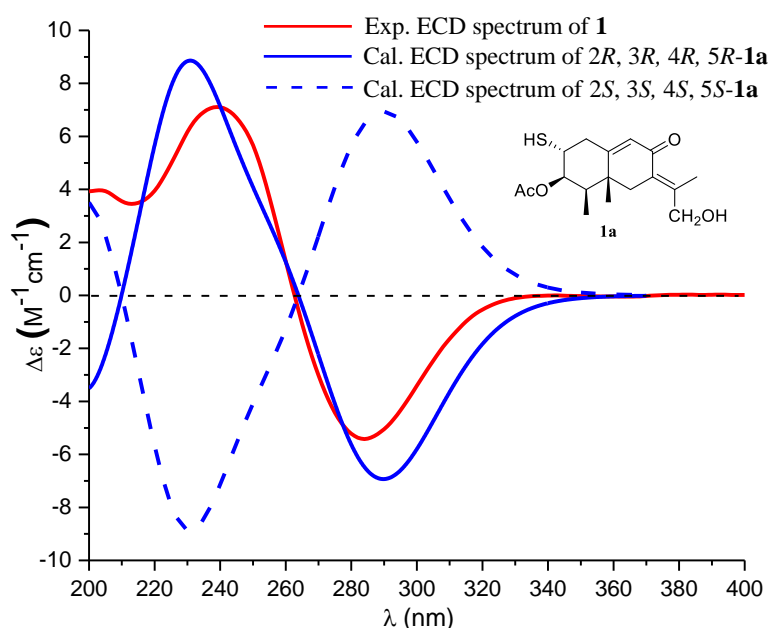


Figure 3. Experimental spectrum of **1** and calculated ECD spectra of **1a** in MeOH

Additionally, the known compounds **2–9** were identified to be bullatantriol (**2**) [7], aculeatin (**3**) [8], 6-(3-ethoxy-2-hydroxy-3-methylbutyl)-5,7-dimethoxy-2H-1-benzopyran-2-one (**4**) [8], *trans*-N-p-coumaroyltyramine (**5**) [9], feruloyltyramine (**6**) [10], β -sitosterol (**7**) [11], sitosterol *D*-glucoside (**8**) [12], 7 α -hydroxysitosterol (**9**) [13] by comparing their NMR data with those in the literature.

The isolated compounds were evaluated for their inhibitory effect toward the α -glucosidase activity assay [14] as results, compound **5** exhibited inhibitory effects against α -glucosidase with an IC₅₀ value of 320 μ M, being more active than the positive control acarbose (670 μ M).

Supporting Information

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

ORCID

Lijing Cai: [0000-0002-6621-7275](https://orcid.org/0000-0002-6621-7275)

Mengying Zhang: [0000-0002-6487-6680](https://orcid.org/0000-0002-6487-6680)

Jie He: [0000-0001-8510-2409](https://orcid.org/0000-0001-8510-2409)

Tingting Lin: [0000-0002-5183-2267](https://orcid.org/0000-0002-5183-2267)

References

- [1] 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.
- [2] T. T. Lin, Y. Y. Huang, G. H. Tang, Z. B. Cheng, X. Liu, H. B. Luo and S. Yin (2014). Prenylated coumarins: natural phosphodiesterase-4 inhibitors from *Toddalia asiatica*, *J. Nat. Prod.* **77**, 955-962.
- [3] T.-T. Lin and G. Chen (2020). A new sesquiterpene and known alkaloids from *Toddalia asiatica* and their inhibitions against phosphodiesterase-4, *Rec. Nat. Prod.* **14**, 207-212.
- [4] D. Sørensen, A. Raditsis, L. A. Trimble, B. A. Blackwell, M. W. Sumarah and J. D. Miller (2007). Isolation and structure elucidation by LC-MS-SPE/NMR: PR toxin- and cuspidatol-related eremophilane sesquiterpenes from *Penicillium roqueforti*, *J. Nat. Prod.* **70**, 121-123.
- [5] L.-H. Meng, X.-M. Li, C.-T. Lv, C.-S. Li, G.-M. Xu, C.-G. Huang and B.-G. Wang (2013). Sulfur-containing cytotoxic curvularin macrolides from *Penicillium sumatrense* MA-92, a fungus obtained from the rhizosphere of the mangrove *Lumnitzera racemosa*, *J. Nat. Prod.* **76**, 2145-2149.
- [6] E. Adelin, M.T. Martin, M.F. Bricot, S. Cortial, P. Retailleau and Ouazzani (2012). Biotransformation of natural compounds: Unexpected thio conjugation of Sch-642305 with 3-mercaptolactate catalyzed by *Aspergillus niger* ATCC 16404 cells, *Phytochemistry* **84**, 135-140.
- [7] Y. F. Wang, X. Y. Wang, G. F. Lai, C. H. Lu and S. D. Luo (2007). Three new sesquiterpenoids from the aerial parts of *Homalomena occulta*, *Chem. Biodivers.* **4**, 925–931.
- [8] M. Peuchmaur, N. Saidani, C. Botte, E. Marechal, H. Vial and Y. S. Wong (2008). Enhanced Antimalarial activity of novel synthetic aculeatin derivatives, *J. Med. Chem.* **51**, 4870-4873.
- [9] L. Zhang, B. Bai, X. Liu, Y. Wang, M. Li and D. Zhao. α -Glucosidase inhibitors from *Chinese Yam* (*Dioscorea opposita* Thunb.), *Food Chem.* 2011, **126**, 203–206.
- [10] Nagasawa, A. Isogai, A. Suzuki and S. Tamura (1979). ¹³C NMR spectra and stereochemistry of isoechinulins A, B and C, *Agric. Biol. Chem.* 1979, **43**, 1759–1763.
- [11] L. L. Ricardo, D. I. Bernardi, G. C. Mantovanelli, B. P. Moreno, M. S. Mito, A. A. Silva, R. Silverio de Oliveira, E. L. Ishii- Iwamoto, M. H. Sarragiotto and D. C. Baldoqui (2018). Phytochemical investigation and phytotoxic activity of aerial parts of oilseed radish (*Raphanus sativus* var. oleifer Stokes), *Biochem. Syst. Ecol.* **78**, 52-58.
- [12] R. A. El-Shiekh, D. A. Al-Mahdy, M. S. Hifnawy, T. Tzanova, E. Evian-Bana, S. Philippot, D. Bagrel and E. A. Abdelsattar (2017). Chemical and biological investigation of *Ochrosia elliptica* in Egypt, *Rec. Nat. Prod.* **11**, 552-557.

A new eremophilane-type sesquiterpene

- [13] V. R. Hegde, S. Borges, M. Patel, P. R. Das, B. Wu, V. P. Gullo and T. M. Chan (2010). New potential antitumor compounds from the plant *Aristolochia manshuriensis* as inhibitors of the CDK2 enzyme. *Bioorg. Med. Chem. Lett.* **20**, 1344–1346.
- [14] M.N. Azmi, N.A. Saad, M.H.Abu Bakar, M.T.C. Omar, A.N. Aziz, H.A. Wahab, S. Siddiq, M.I. Choudhary, M. Litaudon and K. Awang (2021). Cyclic polyketides with alpha-glucosidase inhibitory activity from *Endiandra kingiana* gamble and molecular docking study, *Rec.Nat.Prod.* **15**, 414-419.

A C G
publications

© 2022 ACG Publications