*SHORT REPORT*

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*Rec. Nat. Prod.* 17:2 (2023) 382-387

records of natural products

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

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*(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**), 7*α*-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 μM, being more active than the positive control acarbose.

**Keywords:** *Toddalia asiatica*; Todasinoid A; sesquiterpenoid; ECD calculation. 

## 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,

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The article was published by ACG Publications
http://www.acgpubs.org/journal/records-of-natural-products March-April 2023 EISSN:1307-6167
DOI: http://doi.org/10.25135/rnp.356.2206.2486
Available online:August 30, 2022

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.

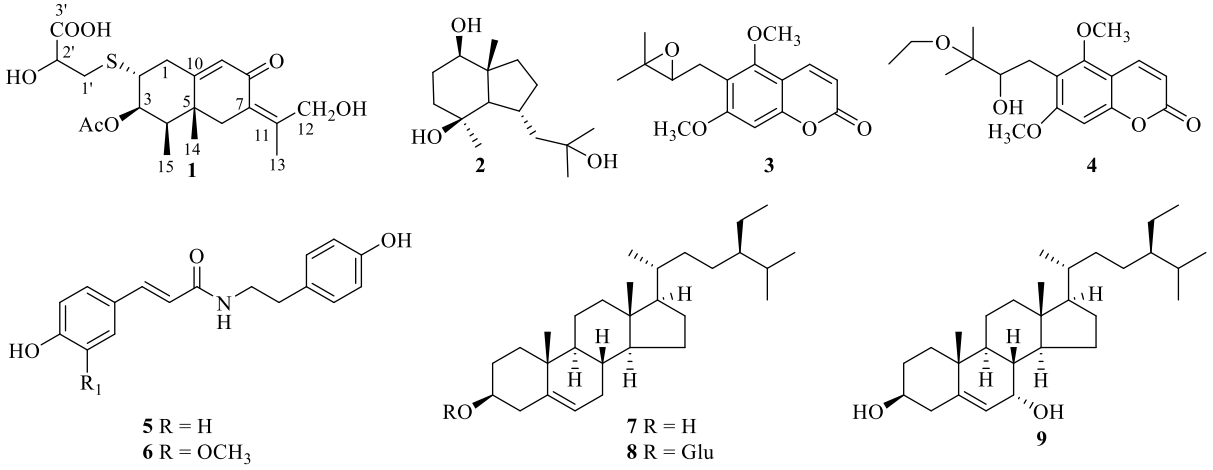


**Figure 1.** Structures of compounds **1**–**9** from *Toddalia asiatica*

## 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 $H_2O$ (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/$H_2O$ 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. IIId was purified by silica gel CC ($CH_2Cl$/MeOH, 20:1 →5:1) to give **1**, **2**, and **8**.

*Todasinoid A* (**1**)*:* Colorless oil; $[\alpha]^{25}_D$ +59 (*c* 0.15, MeOH); UV (MeOH) $\lambda_{max}$ (log $\varepsilon$) 251 (4.16), 282 (3.94); ECD (*c* 2.7 × $10^{-4}$ M, MeOH) $\lambda_{max}$ ($\Delta\varepsilon$) 240 (+7.10), 284 (–5.41); $^1$H and $^{13}$C NMR data, see Table 1; HRESIMS *m/z* 435.1445 $[M + Na]^+$ (calcd for $C_{20}H_{28}O_7SNa^+$, 435.1448); HRESIMS *m/z* 413.1635 $[M + H]^+$ (calcd. for $C_{20}H_{29}O_7S^+$, 413.1629).

Compound **1** had the molecular formula $C_{20}H_{28}O_7S$ as determined by the HRESIMS data, requiring 7 degrees of unsaturation. The $^1$H NMR spectrum showed four methyl groups including an acetoxy group (δ 2.08) and an olefinic methyl group ($\delta_H$ 1.99), five protons attached to carbons bearing heteroatoms ($\delta_H$ 4.89, 4.10, 4.08, 3.99, 3.31), an olefinic proton ($\delta_H$ 5.72), and seven alkyl protons. The $^{13}$C NMR and HSQC spectra revealed the presences of an *α,β*-unsaturated ketone group ($\delta_C$ 190.5, 127.6, 164.4), four methyl carbons ($\delta_C$ 11.1, 17.4, 17.9, 20.8), a tetrasubstituted double bond ($\delta_C$ 164.4, 145.4), four $sp^3$ methylenes including an hydroxymethyl group ($\delta_C$ 61.5), four methines ($\delta_C$ 37.9, 44.5, 70.2, 74.8), one $sp^3$ quaternary carbon ($\delta_C$ 40.4), and a carboxylic acid group ($\delta_C$ 173.9), and an ester carbon ($\delta_C$ 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.** $^{1}$H (400 Hz) and $^{13}$C NMR (100 Hz) data of compound **1** in DMSO-$d_6$ ($\delta$ in ppm)

| No. | $\delta_H$, mult. (*J* in Hz) | $\delta_C$, type | No. | $\delta_H$, mult. (*J* in Hz) | $\delta_C$, type |
|---|---|---|---|---|---|
| 1 | β 2.96, d (14.7)<br>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)<br>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<br>*α* 1.98, o | 35.7, $CH_2$ | 1′ | 2.92, m<br>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 $CH_2$-1, CH-2, CH-3, CH-4, and $CH_3$-15. The HMBCs from the olefinic methyl $H_3$-13 ($\delta_H$ 1.99) to C-7 ($\delta_C$ 127.6), C-11 ($\delta_C$ 145.4), and C-12 ($\delta_C$ 61.5) assigned the tetrasubstituted double bond resided at C-7 and C-11 and a hydromethyl group at C-12. Additional HMBCs from $H_2$-6 ($\delta_H$ 2.96, 1.98) to C-7, C-8 ($\delta_C$ 190.5), C-10 ($\delta_C$ 164.4), and C-11 and from H-9 ($\delta_H$ 5.72) to C-8 located the *α*,*β*-unsaturated ketone group at C-8, C-9 ($\delta_C$ 127.4), C-10. The acetoxy group ($\delta_H$ 2.08; $\delta_C$ 20.8, 169.9) was attached to C-3 ($\delta_C$ 74.8) by HMBC correlations from H-3 ($\delta_H$ 4.89) to the carbonyl carbon of the acetyl group ($\delta_C$ 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 ($\delta_H$ 4.08, $\delta_C$ 70.2), a methylene ($\delta_H$ 2.92, 2.78; $\delta_C$ 35.7), and a carboxylic acid group ($\delta_C$ 173.9) were assembled to a 2-hydroxypropanoic acid unit by the $^{1}$H-$^{1}$H COSY correlations between $H_2$-1′ ($\delta_H$ 2.92, 2.78) with H-2′ ($\delta_H$ 4.08) and HMBCs from $H_2$-1′ and H-2′ to the carboxylic acid carbon C-3′ ($\delta_C$ 173.9). Based on the molecular formula of **1**, the substituent group at C-2 has a molecular formula of $C_3H_5O_3S$, bearing a sulfur atom. Thus, the methine C-2 ($\delta_C$ 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.

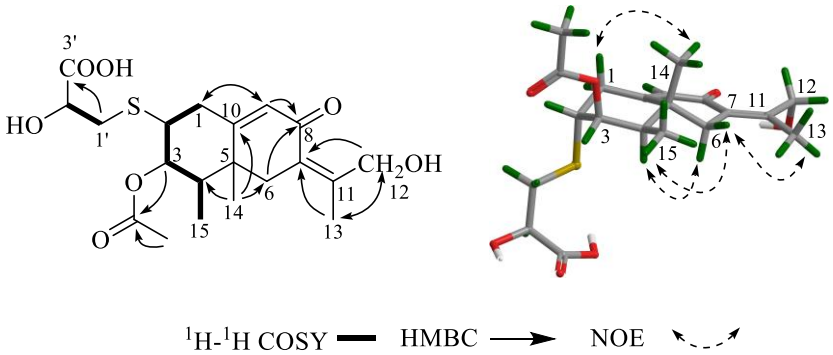


**Figure 2.** Key $^{1}$H-$^{1}$H COSY, HMBC, and NOE correlations of **1**

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 ($\delta_H$ 1.05) and H-1β ($\delta_H$ 2.96), $H_3$-1″ ($\delta_H$ 2.08), H-6β and from H-4 ($\delta_H$ 2.15) to H-6α ($\delta_H$ 1.98) and the small coupling constants of $J_{2,3}$ ($\delta_H$ 3.31, br s, H-2) and $J_{3,4}$ ($\delta_H$ 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 ($\delta_H$ 1.99) and H-6β ($\delta_H$ 2.97) indicated that the double bond $\Delta^{13}$ had a *Z* configuration. As for the absolute configuration of **1**, the ECD calculation of the model compounds 2*R*, 3*R*, 4*R*, 5*R*-**1a** and 2*S*, 3*S*, 4*S*, 5*S*-**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 2*R*, 3*R*, 4*R*, 5*R*-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].

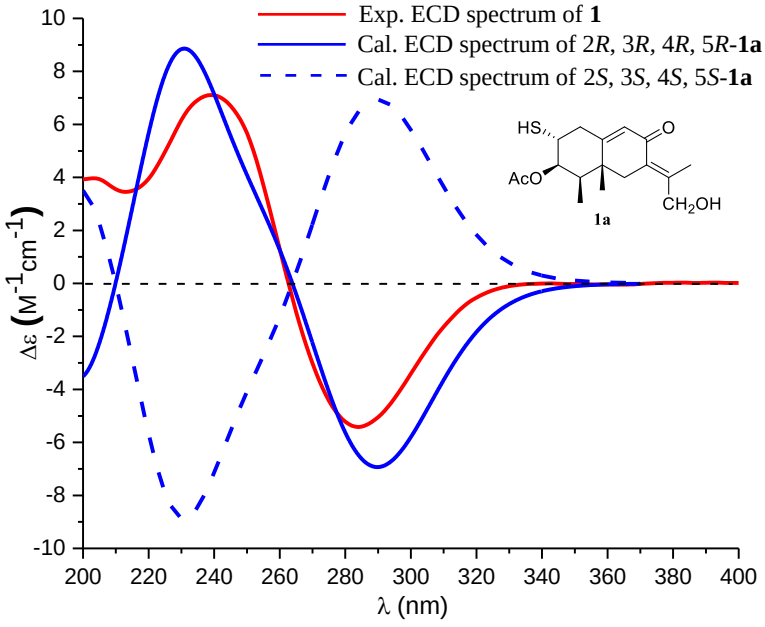


**Figure 3.** Experimental spectrum of **1** and calculated ECD spectra of 1a in MeOH

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], 7α-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 $IC_{50}$ value of 320 μM, being more active than the positive control acarbose (670 μM).

## Supporting Information

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

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