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A New Sesquiterpene and Known Alkaloids from *Toddalia asiatica* and Their Inhibitions Against Phosphodiesterase-4

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Abstract: A new sesquiterpene (**1**) and nine known alkaloids (**2**–**10**) were isolated from the roots of *Toddalia asiatica*. The structure of compound **1** were resolved by NMR and HRESI data, as well as ECD calculation for determining the absolute configuration. The known compounds were identified to be 8-acetonyldihydronitidine (**2**), 8-acetonyldihydroavicine (**3**), dihydronitidine (**4**), oxynitidine (**5**), decarine (**6**), skimmianine (**7**), γ-fagarine (**8**), N-methylflindersine (**9**), and 4-methoxy-N-methyl-2-quinolone (**10**) by comparing the NMR data with those in the literature. Compound **1** is the first eremophilane-type sesquiterpenoid isolated from this species. The known compounds **2**, **3**, and **6** were discovered for the first time from the genus *Toddalia*. All the isolated compounds were evaluated for their inhibitory effects against phosphodiesterase-4, as result, compound **2** showed strong inhibitory effect against phosphodiesterase-4 with an IC_{50} value of 5.14 μM.

1. Plant Source

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

2. Previous Studies

Toddalia is a monotypic genus of the Rutaceae family containing the single species *Toddalia asiatica* (L.) Lam. (Synonym: *Toddalia aculeata*), which is a woody climber widely distributed in south China [1]. It has been extensively used in Traditional Chinese Medicine (TCM) for the treatment of

pyogenic infections, dyspepsodynia, rheumatic arthritis, traumatic injury, and hemoptysis. Previous chemical study of this plant led to the identification of coumarins [2-10], a rare dihydrochelerythrine-cadinane derivative [9], benzophenanthridine alkaloids [6, 12, 13], amides [5, 14], and essential oil (sesquiterpenoids and monoterpenoids) [15], some compounds exhibited cytotoxic, antimicrobial, antifungal, antiviral, anti-inflammatory, and insect-resistant effects. In our efforts to discover bioactive molecules from natural resources, a series of prenylated coumarins possessing inhibitions against phosphodiesterase-4 were obtained from the roots of *T. asiatica* [7], further isolation resulted in a new eremophilane-type sesquiterpenoid (**1**), five known benzophenanthridine alkaloids (**2–6**), and four quinoline alkaloids (**7–10**) (Figure 1). All ten compounds were evaluated for their inhibitory effects toward phosphodiesterase-4 (PDE4), compound **2** exhibited strong inhibitory effect with an IC_{50} value of 5.14 μM. Herein, the isolation, structural elucidation, and the inhibitory activities of compounds **1−10** against PED4 are described.

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 (I−IV). Fraction IV was subjected to silica gel CC (PE/EtOAc, 3:1 → 0:1) to give four fractions (IVa−IVd). IVa was purified by silica gel CC (PE/Acetone, 2:1 →1:1) to give **1**, **2**, and **6**. IVb was subjected to Sephadex LH-20 (ethanol) to obtain two fractions (IVb1−IVb2), further purification of IVb1 by Rp-C18 silica gel CC (MeOH/H_2O, 6:4 → 10:0) yielded **5**, **7**, **8**, **9**, and **10**. IVc was applied to sephadex LH-20 to give two fractions (IVc1−IVc2), fraction IVc2 was subjected successively to HPLC using CH$_3$CN/H_2O (80:20) as eluent to obtain **3** and **4**.
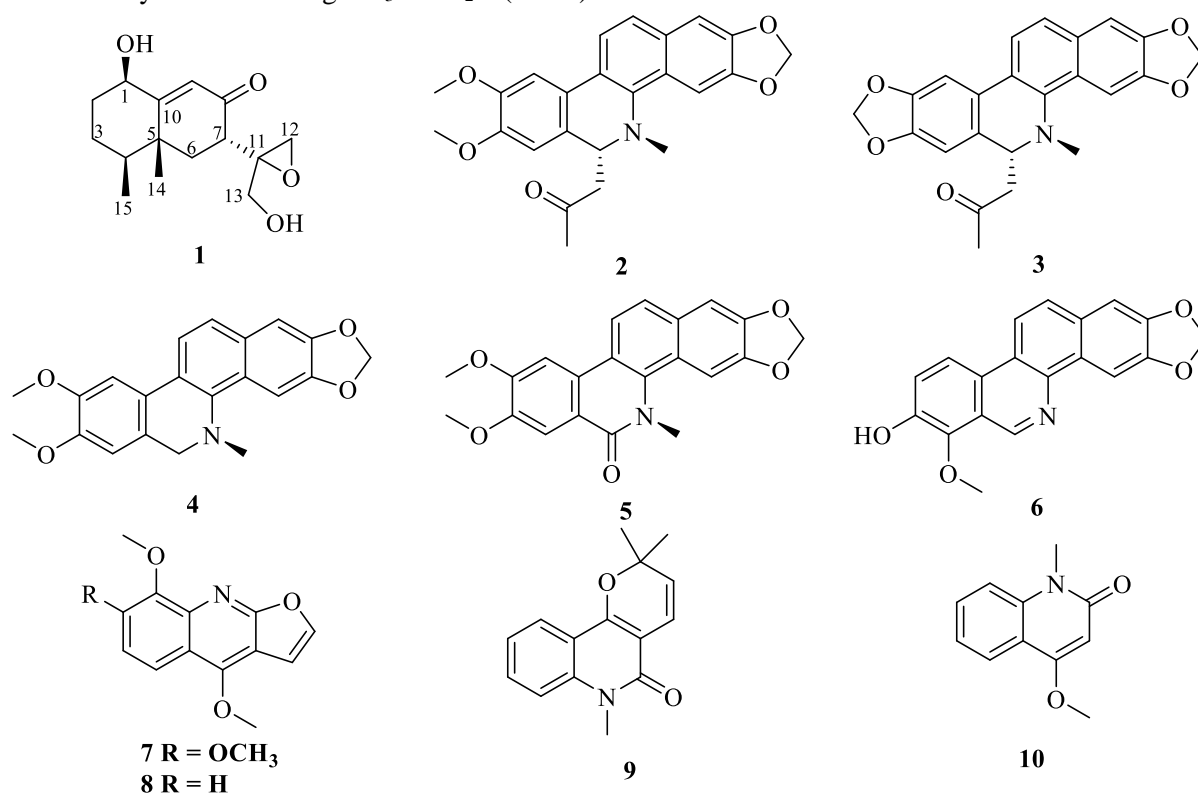

Figure 1. Structures of compounds **1−10** isolated from *T. asiatica*

Compound 1: colorless oil; $[\alpha]^{25}_D$ +154 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 232 (3.91); ECD (*c* 4.5 × 10^{-4} M, MeOH) λ_{max} ($\Delta\varepsilon$) 224 (+8.62), 328 (−0.88), 278 (+0.19) nm; ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ_H 4.15 (1H, br s, H-1), 1.34 (1H, m, H-2β), 1.82 (1H, m, H-2α), 1.27 (1H, m, H-3α), 1.76 (1H, m, H-3β), 1.35 (1H, m, H-4), 1.52 (1H, m, H-6α), 1.80 (1H, m, H-6β), 2.90 (1H, dd, *J* = 14.6, 3.8 Hz, H-7), 5.67 (1H, s, H-9), 2.49 (1H, d, *J* = 4.4 Hz, H-12a), 2.67 (1H, d, *J* = 4.4 Hz, H-12b), 3.41 (1H, dd, J = 12.3, 5.7 Hz, H-13a), 3.76 (1H, dd, *J* = 12.3, 5.9 Hz, H-13b), 1.22 (3H, s, H$_3$-14), 0.86 (3H, d, *J* = 6.7 Hz, H$_3$-15), 5.05 (1H, br s, 1-OH), 4.80 (1H, dd, *J* = 5.9, 5.7 Hz, 13-OH). ^{13}C NMR (100 MHz, DMSO-*d*$_6$) δ_C 70.7 (C-1), 37.8 (C-2), 24.7 (C-3), 42.8 (C-4), 38.3 (C-5), 32.9 (C-6), 42.4 (C-7), 199.2 (C-8), 125.2 (C-9), 168.5 (C-10), 59.3 (C-11), 46.1 (C-12), 63.9 (C-13), 17.5 (C-14), 15.1 (C-15). HRESIMS *m/z* 289.1418 [M + Na]$^+$ (calcd for $C_{15}H_{22}O_4Na^+$, 289.1410), 555.2944 [2M + Na]$^+$ (calcd for $C_{30}H_{44}O_8Na^+$, 555.2928).

Compound **1** was obtained as a colorless oil, its molecular formula was determined as $C_{15}H_{22}O_4$ according to its HR-ESI-MS peak at m/z [M + Na]+, suggesting five degrees of unsaturation. The ^{1}H NMR spectrum displayed signals for an olefinic proton [δ_H 5.67 (s, 3H)], a methyl doublet [δ_H 0.86 (d, *J* = 6.7 Hz, 3H)], a methyl singlet [δ_H 1.22 (s, 3H)], five oxygenated protons (δ_H 2.49, 2.67, 3.41, 3.76, 4.15), and several alkyl protons. The ^{13}C NMR spectrum resolved 15 carbon signals attributable to one carbonyl (δ_C 199.2), two olefinic carbon (δ_C 125.2, 168.5), two methyls (δ_C 15.1, 17.5), five methylenes (δ_C 24.7, 32.9, 37.8, 46.1, 63.9), three sp^3 methines (δ_C 42.4, 42.8, 70.7), and two sp^3 quaternary carbons (δ_C 38.3, 59.3). The carbonyl group and the double bond covered two degrees of unsaturation, the remaining three degrees of unsaturation required that **1** was tricyclic. The aforementioned structural features were very similar to those of 7β-H-9(10)-ene-11,12-epoxy-8-oxoeremophilane (an eremophilane sesquiterpene isolated from *Aquilaria sinensis*), the obvious distinctions were due to the presences of an oxygenated methine and a hydroxymethyl group [16]. The oxygenated methine group was located at C-1 by the ^{1}H-^{1}H COSY correlation from 1-HO (δ_H 5.05) to H-1 (δ_H 4.15) in addition to the HMBC correlation from H-9 (δ_H 5.67) to C-1 (δ_H 70.7), while the hydroxymethyl group was positioned at C-11 by the HMBC correlations from H-7 (δ_H 2.90) to C-13 (δ_C 63.9) and from H$_2$-13 (δ_H 3.41, 3.76) to C-11 (δ_C 59.3) and C-12 (δ_C 46.1). Detailed interpretation of the 2D NMR spectra confirmed the gross structure of **1** (Figure 2).
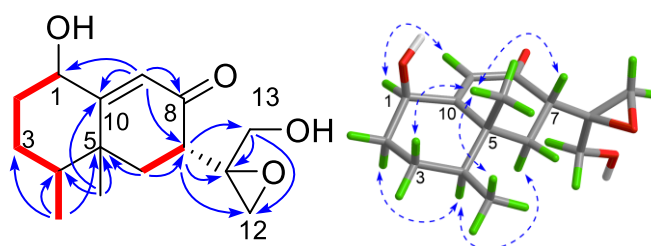

Figure 2. ^{1}H-^{1}H COSY (——), HMBC (→), and NOESY (←→) correlations of **1**

The relative configuration of **1** was further determined by NOESY correlations and *J* values. The β-axial orientation of the 1-OH was suggested by the small α-equatorial coupling constant of H-1 (δ_H 4.15, br s). The coupling constant between H-7 and H-6a ($J_{H-7/H-6a}$ = 14.6 Hz) was indicative of the *trans*-relationship of H-7 and H-6a in an axial orientation. The NOE correlations from H$_3$-14 (δ_H 1.22, s) to H$_3$-15 (δ_H 0.86, d) and H-7 (δ_H 2.90, dd, *J* = 3.8, 14.6 Hz) and between H-6a (δ_H 1.82)/H-4, H$_3$-15/H-6b (δ_H 1.90) (Figure 2) determined the same orientation of H$_3$-14, H$_3$-15, and H-7, while H-4 was in an the other orientation (axial orientation). The $J_{H-3/H-4}$ value (4.8 Hz) suggested an equatorial−axial relationship between H-3 and H-4. Unfortunately, the relative configuration of C-11 could not be resolved by the NOESY correlations, as the chiral center at C-11 located on a freely rotating side chain.

The absolute configurations of C-1, C-4, C-5, and C-7 were determined to be *R*, *S*, *R*, and *S* by comparing its ECD spectrum with those of the calculated model molecules **1a** (1*R*, 4*S*, 5*R*, 7*S*-**1**) and **1b** (1*S*, 4*R*, 5*S*, 7*R*-**1**). The experimental ECD spectrum of **1** showed a curve with Cotton effects around

328 (−), 278 (+), 224 (+) nm, respectively (Figure 3). The calculated ECD spectrum for **1a** showed a similar ECD curve with Cotton effects at 334 (−), 268 (+), and 257 (+) nm (Figure 3), indicating that **1** had an 1*R*, 4*S*, 5*R*, 7*S* configuration.
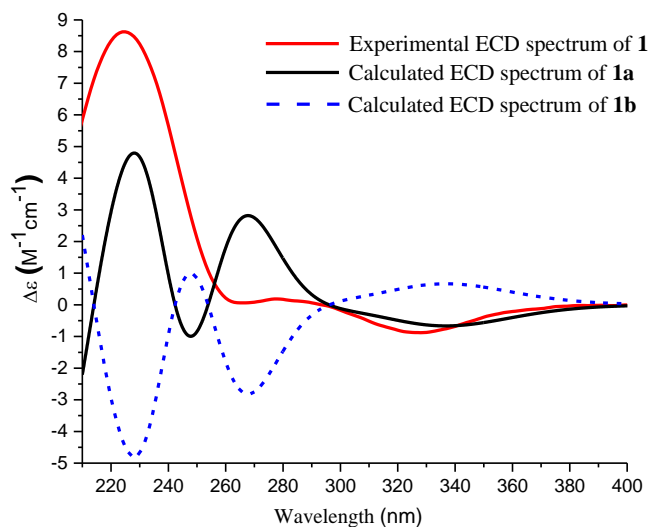

Figure 3. Experimental ECD spectrum of **1** in MeOH and calculated ECD spectra of **1a** and **1b**

The known compounds were identified to be 8-acetonyldihydronitidine (**2**) [17], 8-acetonyldihydroavicine (**3**) [17], dihydronitidine (**4**) [18], oxynitidine (**5**) [18], decarine (**6**) [19], skimmianine (**7**) [20], *γ*-fagarine (**8**) [21], N-methylflindersine (**9**) [22], 4-methoxy-N-methyl-2-quinolone [23] (**10**) by comparison of their NMR data with those in the literature.

All compounds were screened for their inhibitory activity against PED4D2 by using our reported methods [7]. Rolipram, a well-known PDE4 inhibitor, was used as the positive control. The bioassay results showed that compound **2** had strong activity with an IC_{50} value of 5.14 μM toward PDE4D2 (Table 1). A preliminary structure-activity analyses revealed that the coexistence of vicinal methoxyl groups and the acetonyl were essential for the inhibitory activity, as compounds **3–5** showed much weaker activity than that of compound **2**, which contains both vicinal methoxyl and the acetonyl groups. The isolated compounds was also evaluated for their inhibitions against *α*-glucosidase following the procedures in the literature [24, 25], while all of them exhibited inhibitions less than 30% at a concentration of 200 *μ*M.

Table 1. Inhibitory Effects of all compounds against PDE4D2.

NO.	Inhibitory rate (%, 10 *μ*M)	IC_{50} (*μ*M)	Inhibitory rate (%, 10 *μ*M)	IC_{50} (*μ*M)
1	11.21%		**6**	14.53%
2	62.08%	5.14 ± 0.15	**7**	9.74%
3	26.76%		**8**	15.49%
4	21.34%		**9**	13.21%
5	10.47%		**10**	15.09%
rolipram[a]		0.59 ± 0.05		

[a] positive

Acknowledgments

Supporting Information

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

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References

[1]   D. Zhang, G. H. Thomas and J. M. David (2008). Flora of China, Science Press (Beijing) and Missouri Botanical Garden Press (Saint Louis), vol.**11**, pp. 51–97.

[2]   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.

[3]   H. Y. Qiu, X. H. Xiao and G. K. Li (2012). Separation and purification of furanocoumarins from *Toddalia asiatica* (L.) Lam. using microwave-assisted extraction coupled with high-speed counter-current chromatography, *J. Sep. Sci.* **35**, 901-906.

[4]   H. A. Oketch-Rabah, J. W. Mwangi, J. Lisgarten and E. K. Mberu (2000). A new antiplasmodial coumarin from *Toddalia asiatica* roots, *Fitoterapia* **71**, 636-640.

[5]   I. L. Tsai, S. C. Fang, T. Ishikawa, C. T. Chang and I. S. Chen (1997). N-cyclohexyl amides and a dimeric coumarin from formosan *Toddalia asiatica*, *Phytochemistry*, **44**, 1383-1386.

[6]   S. Sukieum, W. Sang-aroon and C. Yenjai (2018). Coumarins and alkaloids from the roots of *Toddalia asiatica*, *Nat. Prod. Res*. **32**, 944-952.

[7]   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 ınhibitors from *Toddalia asiatica*, *J. Nat. Prod.* **77**, 955-962.

[8]   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.

[9]   C. Hirunwong, S. Sukieum, R. Phatchana and C. Yenjai (2016). Cytotoxic and antimalarial constituents from the roots of *Toddalia asiatica*, *Phytochem. Lett.* **17**, 242-246.

[10]   F. Wang, Y. Xu and J. K. Liu (2009). New geranyloxycoumarins from *Toddalia asiatica*, *J. Asian Nat. Prod. Res.* **11**, 752-756.

[11]   R. Phatchana and C. Yenjai (2014). Cytotoxic coumarins from *Toddalia asiatica*, *Planta Med.* **80**, 719-722.

[12]   J. Hu, X. D. Shi, J. G. Chen, X. Mao, L. Zhu, L. Yu and J. Y. Shi (2014). Alkaloids from *Toddalia asiatica* and their cytotoxic, antimicrobial and antifungal activities, *Food Chem.* **148**, 437-444.

[13]   S. C. Jain, M. K. Pandey, R. K. Upadhyay, R. Kumar, G. Hundal and M. S. Hundal (2006). Alkaloids from *Toddalia aculeata*, *Phytochemistry* 67, 1005-1010.

[14]   J. Hu, X. D. Shi, X. Mao, J. G. Chen and H. Li (2015). Amides from the roots of *Toddalia asiatica*, *Chem. Nat. Compd.* **51**, 726-729.

[15]   X. C. Liu, H. W. Dong, L. G. Zhou, S. S. Du and Z. L. Liu (2013). Essential oil composition and larvicidal activity of *Toddalia asiatica* roots against the mosquito *Aedes albopictus* (Diptera: Culicidae), *Parasıtol. Res*. **112**, 1197-1203.

[16]   D. -L. Yang, H. Wang, Z. -K. Guo, W. Li, W. -L. Mei and H. -F. Dai (2014). Fragrant agarofuran and eremophilane sesquiterpenes in agarwood 'Qi-Nan' from *Aquilaria sinensis*, *Phytochem. Lett*. **8**, 121-125.

[17]   A. P. K. Nissanka, V. Karunaratne, B. M. R. Bandara, V. Kumar, T. Nakanishi, M. Nishi, A. Inada, L. M. V. Tillekeratne, D. S. A. Wijesundara and A. A. L. Gunatilaka (2001). Antimicrobial alkaloids from *Zanthoxylum tetraspermum* and *caudatum*, *Phytochemistry* **56**, 857-861.

[18]   N. F. de Moura, H. B. Ribeiro, E. C. S. Machado, E. M. Ethur, N. Zanatta and A. F. Morel (1997). Benzophenanthridine alkaloids from *Zanthoxylum rhoifolium*, *Phytochemistry* **46**, 1443-1446.

[19]   M. T. Martin, L. H. Rasoanaivo and A. Raharisololalao (2005). Phenanthridine alkaloids from *Zanthoxylum madagascariense*, *Fitoterapia* **76**, 590-593.

[20] B. K. Nganou, A. T. Mbaveng, S. A. T. Fobofou, A. G. Fankam, G. T. M. Bitchagno, J. D. Simo Mpetga, L. A. Wessjohann, V. Kuete, T. Efferth and P. Tane (2019). Furoquinolines and dihydrooxazole alkaloids with cytotoxic activity from the stem bark of *Araliopsis soyauxii*, *Fitoterapia* **133**, 193-199.

[21] T. -S. Wu, C. -Y. Li, Y.-L. Leu and C. -Q. Hu (1999). Limonoids and alkaloids of the root bark of *Dictamnus angustifolius*, *Phytochemistry* **50**, 509-512.

[22] C. Kamperdick, N. H. Van, T. V. Sung and G. Adam (1999). Bisquinolinone alkaloids from *Melicope ptelefolia*, *Phytochemistry* **50**, 177-181.

[23] Y. D. Min, H. C. Kwon, M. C. Yang, K. H. Lee, S. U. Choi and K. R. Lee (2007). Isolation of limonoids and alkaloids from *Phellodendron amurense* and their multidrug resistance (MDR) reversal activity, *Arch. Pharm. Res.* **30**, 58-63.

[24] Z. Cheng, Y. Li, W. Liu, L. Liu, J. Liu, W. Yuan, Z. Luo, W. Xu and Q. Li (2019). Butenolide derivatives with α-glucosidase ınhibitions from the deep-sea-derived fungus *Aspergillus terreus* YPGA10, *Mar. Drugs* **17**, 332.

[25] S. Jibril, H. M. Sirat and N. Basar (2017). Bioassay-guided ısolation of antioxidants and α-glucosidase ınhibitors from the root of *Cassia sieberiana* DC (Fabaceae), *Rec. Nat. Prod.* **11**, 406-410.