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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.

Keywords: *Toddalia asiatica*; sesquiterpene; alkaloids; inhibitions toward phosphodiesterase-4.

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

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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_3CN/H_2O (80:20) as eluent to obtain **3** and **4**.

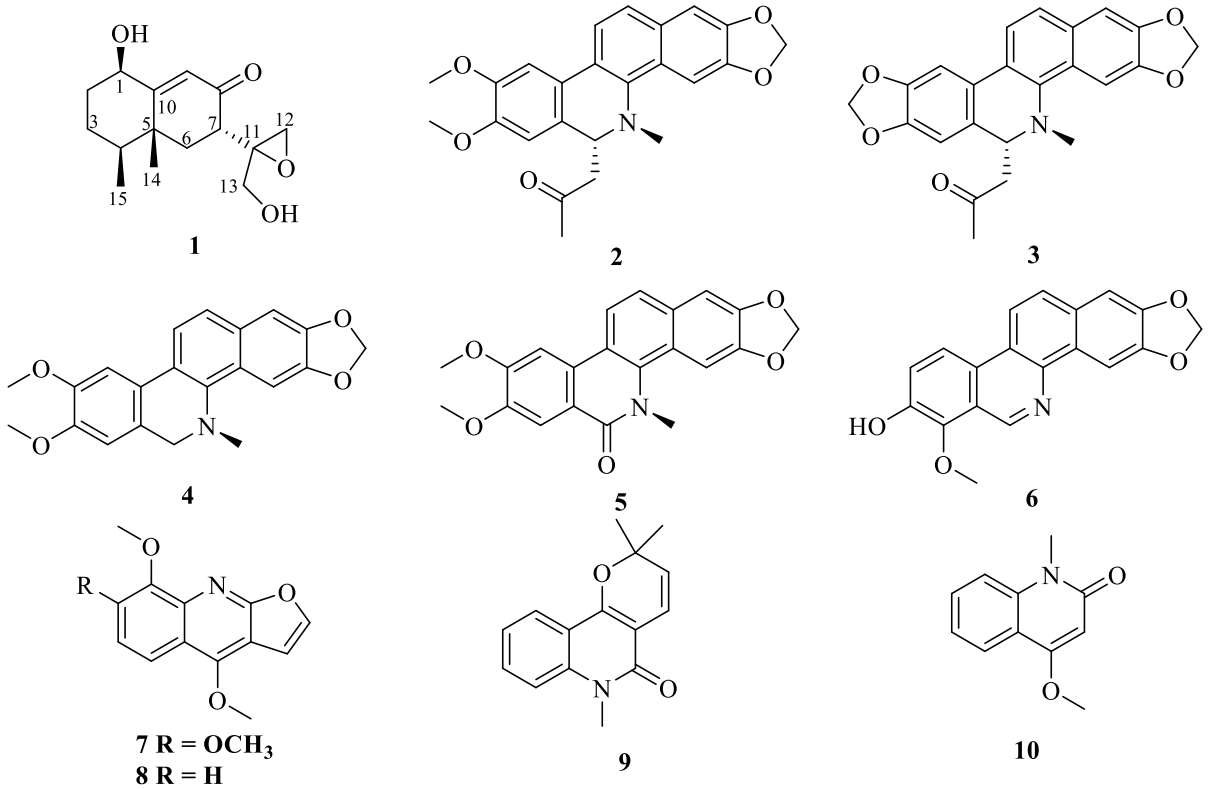

Figure 1. Structures of compounds **1**–**10** isolated from *T. asiatica*

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).

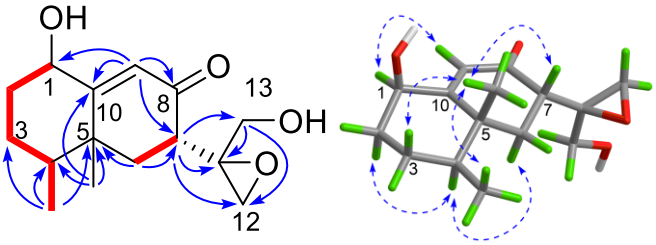

Figure 2. ^{1}H-^{1}H COSY (—), HMBC (→), and NOESY (⇢) correlations of **1**

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 ($J_{H\text{-}7/H\text{-}6a}$ = 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 $J_{H\text{-}3/H\text{-}4}$ 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.

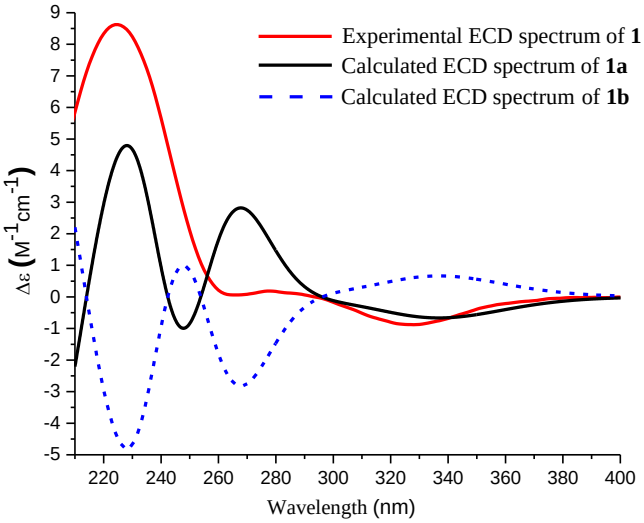

Figure 3. Experimental ECD spectrum of **1** in MeOH and calculated ECD spectra of **1a** and **1b**

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 μM.

Table 1. Inhibitory Effects of all compounds against PDE4D2.

NO.	Inhibitory rate (%, 10 μM)	IC_{50} (μM)	Inhibitory rate (%, 10 μM)	IC_{50} (μM)
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

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Supporting Information

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

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