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

Ting-Ting Lin and Gang Chen*

Jiangxi Provincial People's Hospital, Nanchang 330006, People’s Republic of China

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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 IC₅₀ value of 5.14 μM.

Keywords: *Toddalia asiatica*; sesquiterpene; alkaloids; inhibitions toward phosphodiesterase-4. © 2019 ACG Publications. All rights reserved.

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

*Corresponding author: E-Mail: chengang_ye@163.com.*
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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 IC₅₀ 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 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 (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/H₂O, 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 CH₃CN/H₂O (80:20) as eluent to obtain 3 and 4.

![Figure 1. Structures of compounds 1–10 isolated from *T. asiatica*](image-url)
Compound 1: colorless oil; [α]25O +154 (c 0.1, MeOH); UV (MeOH) λmax (log ε) 232 (3.91); ECD (c 4.5 × 10−4 M, MeOH) λmax (Δε) 224 (+8.62), 328 (−0.88), 278 (+0.19) nm; 1H NMR (400 MHz, DMSO-d6) δH 1.15 (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-12α), 2.67 (1H, d, J = 4.4 Hz, H-12β), 3.41 (1H, dd, J = 12.3, 5.7 Hz, H-13α), 3.76 (1H, dd, J = 12.3, 5.9 Hz, H-13β), 1.22 (3H, s, H3-14), 0.86 (3H, d, J = 6.7 Hz, H3-15), 5.05 (1H, br s, 1-ΟΗ), 4.80 (1H, dd, J = 5.9, 5.7 Hz, 13-ΟΗ). 13C NMR (100 MHz, DMSO-d6) δC 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 C13H22O4Na+, 289.1410), 555.2944 [2M + Na]+ (calcd for C30H40O4Na2+, 555.2928).

Compound 1 was obtained as a colorless oil, its molecular formula was determined as C13H22O4 according to its HR-ESI-MS peak at m/z [M + Na]+, suggesting five degrees of unsaturation. The 1H NMR spectrum displayed signals for an olefinic proton [δH 5.67 (3H)], a methyl doublet [δH 0.86 (d, J = 6.7 Hz, 3H)], a methyl singlet [δH 1.22 (3H)], five oxygenated protons (δH 2.49, 2.67, 3.41, 3.76, 4.15), and several alkyl protons. The 13C NMR spectrum resolved 15 carbon signals attributable to one carbonyl (δC 199.2), two olefinic carbons (δC 125.2, 168.5), two methyis (δC 15.1, 17.5), five methylenes (δC 24.7, 32.9, 37.8, 46.1, 63.9), three sp3 methines (δC 42.4, 42.8, 70.7), and two sp3 quaternary carbons (δC 38.3, 59.3). The carbonyl group and the double bond covered two degrees of unsaturation, the remaining three degrees of unsaturation required that I 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 1H-1H COSY correlation from 1-HO (δH 5.05) to H-1 (δH 4.15) in addition to the HMBC correlation from H-9 (δH 5.67) to C-1 (δH 70.7), while the hydroxymethyl group was positioned at C-11 by the HMBC correlations from H-7 (δH 2.90) to C-13 (δC 63.9) and from H2-13 (δH 3.41, 3.76) to C-11 (δC 59.3) and C-12 (δC 46.1). Detailed interpretation of the 2D NMR spectra confirmed the gross structure of 1 (Figure 2).

Figure 2. 1H-1H 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 (δH 4.15, br s). The coupling constant between H-7 and H-6a (JH-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 H3-14 (δH 1.22, s) to H1-15 (δH 0.86, d) and H-7 (δH 2.90, dd, J = 3.8, 14.6 Hz) and between H-6a (δH 1.82)/H-4, H3-15/H-6b (δH 1.90) (Figure 2) determined the same orientation of H3-14, H3-15, and H-7, while H-4 was in an other orientation (axial orientation). The JH-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 (1R, 4S, 5R, 7S-1) and 1b (1S, 4R, 5S, 7R-1). The experimental ECD spectrum of 1 showed a curve with Cotton effects around...
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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 1R, 4S, 5R, 7S configuration.

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 PED4D2 (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.

![Figure 3](image-url)  
*Figure 3. Experimental ECD spectrum of 1 in MeOH and calculated ECD spectra of 1a and 1b*

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Rolipram$^*$ 0.59 ± 0.05

$^*$positive

**Table 1. Inhibitory Effects of all compounds against PDE4D2.**

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Supporting Information

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

ORCID
Tingting Lin: 0000-0001-6953-720X
Gang Chen: 0000-0002-3945-4371

References

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