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Two new cytotoxic cycloartane triterpenoids from *Aphanamixis polystachya* (Wall.) R. N. Parker

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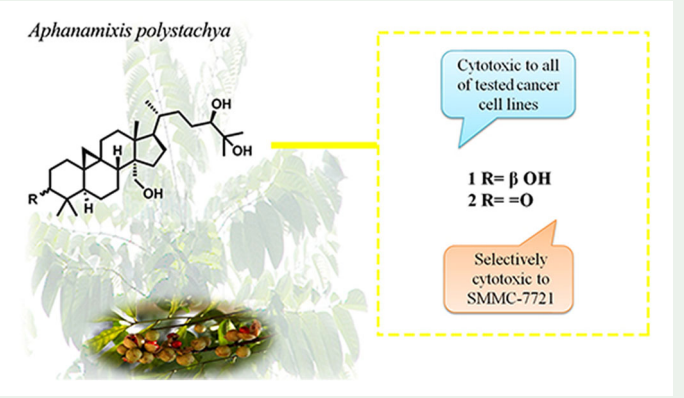
ABSTRACT

Two new cycloartane triterpenoids, (24 *R*)-cycloartane-3β,24,25,30-tetrol (**1**) and (24 *R*)-24,25,30-trihydroxy-9,19-cycloartane-3-one (**2**), along with three known compounds (**3-5**) were isolated from leaves and twigs of *Aphanamixis polystachya*. The new compounds were elucidated based on comprehensive spectroscopic analysis, including 1 D, 2 D NMR and HREIMS. The in vitro cytotoxic activities evaluation of five human cancer cell lines revealed that compound **1** exhibited cytotoxic activity on all of tested human cancer cell lines, while compound **2** only had specific activity on SMMC-7721 cell line.



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1. Introduction

The genus *Aphanamixis* belongs to the Meliaceae family which consists of about 25 species and distributed in India, Indonesia, Malaysia and China (Cai et al. 2012).

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Complex and diverse secondary metabolites remain attractive for the phytochemical research. In previous investigations, series of cycloartane triterpenoids isolated from this genus have been widely studied (Rabi 1996; Liu et al. 2010; Zeng et al. 2012; Wang et al. 2013). Some cycloartane triterpenoids and related glycosides have good bioactivities, such as anti-aging, antibacterial, antiviral and anti-inflammatory effects (Denizli et al. 2014; Fang et al. 2019; Wei et al. 2019; Zhao et al. 2020). And some have particularly potent anti-cancer activity, for example, (21*S*,23*R*)-21,23-diepoxy-21-methoxycycloartan-1,24-diene-3,27-dione (Rahim et al. 2018), 25-*O*-methyl-24-*O*-acetylisodahurinol-3-*O*-β-D-xylopyranoside (Zhu et al. 2016) and 3β,21-dihydroxy-24,31-epoxy-24-methylenecycloartane (Prawat et al. 2013) with obtained IC_{50} values of 6.1 μM (KB-VIN), 2.61 μM (A-549) and 7.82 μM (NCI-H187), respectively. On the purpose of discovering bioactive molecules, a systematic research on the chemical constitutes of *Aphanamixis polystachya* was conducted. Intriguingly, two new cycloartane triterpenoids with anti-cancer activity were harvested. Herein, the purification, structural identification and biological activity of two new compounds are presented.

2. Result and discussion

Compound **1** was obtained as a colorless amorphous powder with molecular formula $C_{30}H_{52}O_4$ according to positive HRESIMS (*m/z* 499.3758 $[M + Na]^+$, calcd 499.3763), which required 5 degrees of unsaturation. The ^{1}H NMR spectra of **1** (Figure S1), exhibited characteristic signals for cyclopropane-methylene protons as an AX system at δ_H 0.39, 0.63 (each 1H, d, $J = 3.3$ Hz, H_2-19), and six tertiary methyl groups at δ_H 1.05 (H-21), 1.15 (H-29), 1.17 (H-18), 1.23 (H-28), 1.53 (H-26) and 1.56 (H-27). The ^{13}C NMR and DEPT spectra of **1** (Figure S2), showed 30 well-resolved resonances including 6 quaternary carbons, 6 methines, 12 methylenes and 6 tertiary methyls. Based on spectroscopic features posed above, **1** was prompted as a typical cycloartane-type triterpenoid. One quaternary carbon (δ_C 73.2, C-25) and two methine carbons (δ_C 78.5, C-3; δ_C 80.4, C-24), along with one methylene carbon (δ_C 63.8, C-30), were ascribed to those bearing an oxygen atom. Comparison of the NMR data of **1** with known compound (24*R*)-cycloartane-3β,24,25-triol (Della Greca et al. 1994) implied that compound **1** possessed a similar skeleton, except for a pair of extra protons at δ_H 4.02 and 4.11 (each 1H, d, $J = 11.3$ Hz, H_2-30) in the ^{1}H NMR data of **1** (Table S1). Furthermore, the molecular weight of **1** was larger than that of (24*R*)-cycloartane-3β,24,25-triol by 16 units, suggesting that **1** was a hydroxyl derivative of (24*R*)-cycloartane-3β,24,2-5-triol. The hydroxyl group located at C-30 was confirmed by HMBC spectra (Figure S5), in which the correlations of H-30 (δ_H 4.02 and 4.11, each 1H, d, $J = 11.3$ Hz) to C-8 (δ_C 49.8), C-14 (δ_C 55.0) and C-15 (δ_C 33.2), as shown in Figure S15. Thus, the planar structure of **1** was established (Figure 1).

Based on the ROESY correlations (Figure S6), the relative configuration of ring A-D was identical to that of (24*R*)-cycloartane-3β,24,25-triol, but the configuration of C-24 was still unclear for the free rotation of single bond between C-23/C-24/C-25. The differences of ^{13}C NMR data between 24*R*- and 24*S*- stereomers of cycloartane triterpenoids mainly appeared in the chemical shifts of C-24. However, the chemical shift of **1** at δ_C 80.4 (C-24) was essentially the same as that of (24*R*)-cycloartane-3β,24,25-triol at

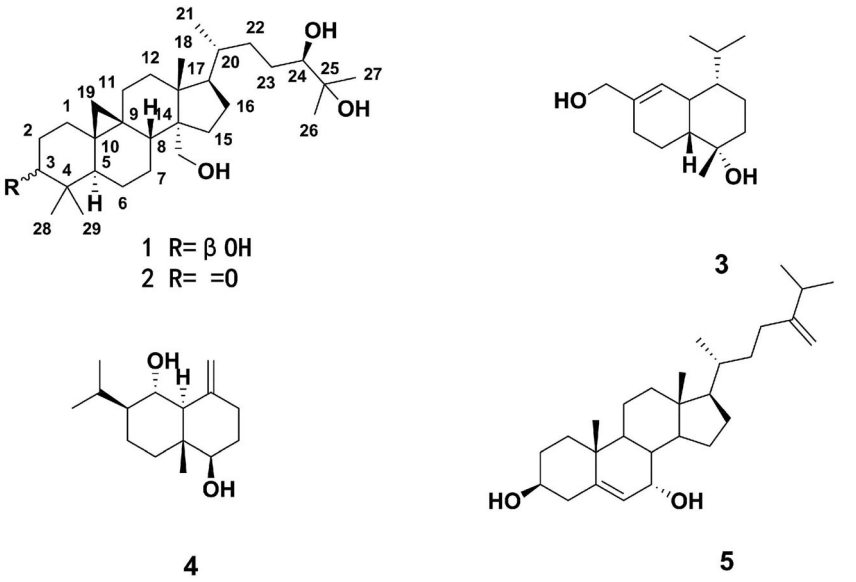

Figure 1. Molecular structures of **1–5** isolated from *Aphanamixis polystachya*.

δ_C 79.7 and different from that of the 24*S* isomer at δ_C 78.7 (Inada et al. 1995, 1997). Then, it could be identified that the configuration of C-24 was *R*. Furthermore, the H-3 appeared at δ_H 3.53 as a double doublet ($J = 11.0$, 3.4 Hz) was similar to (24 *R*)-cycloartane-3β,24,25-triol (dd, $J = 10.1$, 4.4 Hz), rather than (24 *R*)-cycloartane-3α,24,25-triol (t, $J = 2.6$ Hz), established the β-orientation of 3-OH (Inada et al. 1997). Therefore, the structure of compound **1** was defined as (24 *R*)-cycloartane-3β,24,25,30-tetrol.

Compound **2** was obtained as a colorless amorphous powder. Its molecular formula was established as $C_{30}H_{50}O_4$ by positive HRESIMS (*m/z* 497.3601 $[M+ Na]^+$, calcd 497.3619), suggesting 6 degrees of unsaturation. The ^{13}C NMR and DEPT spectra (Figure S9) displayed 30 carbon resonances ascribed to 6 methyls, 12 methylenes, 5 methines and 7 quaternary carbons. The similarity of ^{13}C NMR pattern between compound **2** and **1** established that they shared the same skeleton. The major difference was that compound **2** had a keto carbon at δ_C 216.6 (C-3), instead of a hydroxymethine carbon (compound **1** at δ_C 78.5). Thus, molecular weight of compound **2** was smaller than **1** by two units. The HMBC correlations of H_2-2 (δ_H 2.72, td, $J = 13.9$, 6.4 Hz, 1H; δ_H 2.32, ddd, $J = 14.1$, 4.1, 2.6 Hz, 1H), H-28 (δ_H 1.06, s, 3H) and H-29 (δ_H 1.10, s, 3H) to C-3 (δ_C 216.6) indicated that the carbonyl group was located at C-3, as shown in Figure S15. The structure of compound **2** was concluded as (24 *R*)-24,25,30-trihydroxy-9,19-cycloartane-3-one.

The cytotoxicity of two new cycloartane triterpenoids against five human cancer cell lines, HL-60, A-549, SMMC-7721, MCF-7 and SW480 were evaluated. As a result, two compounds exhibited moderate inhibitory activity. Compound **1** showed cytotoxic activity on all of tested human cancer cell lines, with IC_{50} values of 3.16, 14.02, 2.59, 17.26 and 30.17 μM, respectively (Table S4). However, compound **2** only exhibited activity on hepatocellular carcinoma SMMC-7721 cell line with the IC_{50} value of 28.13 μM.

3. Experimental

3.1. General experimental procedures

The 1 D and 2 D NMR spectra were recorded on the Bruker 500 and 600 MHz spectrometers with TMS as the internal standard. HRESIMS were acquired by Agilent 6500 Q-TOF mass spectrometer. Semi-preparative HPLC was carried out on an Agilent 1100 series LC with a Waters X-Bridge Prep Shield RP18 (10 mm × 150 mm) column. Silica gel (100-200 mesh, 300-400 mesh, Qingdao Marine Chemical, Inc, PR China), C18 reversed-phase silica gel (40-63 μm, Merck, Darmstadt, Germany), MCI gel 20 P (75-150 μm, Mitsubishi Chemical Corporation, Tokyo, Japan) and Sephadex LH-20 (40-70 μm, Amerisham PharmaciaBiotech AB, Uppsala, Sweden) were used for column chromatography (CC).

3.2. Plant material

Leaves and twigs of *Aphanamixis polystachya* were collected at Lancang in Yunnan Province, People's Republic of China, in August 2011. The sample was identified by Mr. Yu Cheng, Kunming Institute of Botany, Chinese Academy Sciences (CAS). A voucher specimen (H20110821) was deposited at the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, CAS (Figure 1).

3.3. Extraction and isolation

The leaves and twigs of *Aphanamixis polystachya* (27 kg) were air-dried, powdered, and extracted three times with 90% ethanol. The combined organic solvents were evaporated under reduced pressure to give the crude residue, which was suspended in water and then extracted successively with petroleum ether and ethyl acetate (EtOAc). The EtOAc extract (500 g) was subjected to a silica gel column, which was eluted with mixtures of petroleum ether/acetone at a 2-50% gradient and of $CHCl_3$/MeOH at a 10-100% gradient, providing seven major pooled fractions. Fraction 4 (50 g) was separated on a column of MCI gel with a 30-100% MeOH/H_2O gradient, affording six fractions (Fr.4A-4F), and then fraction 4 C was separated by a C18 reversed-phase silica gel column with a 50-100% MeOH/H_2O gradient, yielding nine subfractions (Fr.4C1-4C9). Fraction 4C2 was partitioned on a C18 column (eluted with 50-100% MeOH/H_2O), and then the fraction eluted with 60% MeOH (fraction 4C2A) was chromatographed by columns of Sephadex LH-20 (eluted with $CHCl_3$/MeOH) and silica gel (eluted with 5% petroleum ether/EtOAc), to afford **1** (7.3 mg). Fraction 4C3 was separated by a C18 column (eluted with 50-100% MeOH/H_2O), and then the fraction eluted with 65% MeOH (fraction 4C3A) was fractionated on columns of Sephadex LH-20 (eluted with $CHCl_3$/MeOH) and silica gel (eluted with 5% petroleum ether/EtOAc), to afford two fractions, with each being further purified by semi-preparative HPLC (eluted with 39-42% MeCN/H_2O) to afford **2** (4.2 mg), **3** (10 mg), **4** (6.8 mg). With the same purification procedures, **5** (9.5 mg) was obtained from Fr.4D.

3.4. Cytotoxicity assay

Five human cancer cell lines, Human myeloid leukemia HL-60, lung cancer A-549, hepatocellular carcinoma SMMC-7721, breast cancer MCF-7, and colon cancer SW480 cells were used in the cytotoxic assay. All the cells were cultured in DMEM medium (Hyclone, USA), supplemented with 10% fetal bovine serum (Hyclone, USA) in 5% CO_2 at 37 °C. The cytotoxicity assay was performed according to the MTS (3-(4,5-dimethylthiazol-2-yl)-5(3-carboxymethoxyphenyl)-2-(4-sulfopheny)-2H-tetrazolium) method in 96-well microplates. Medium (100 μL) containing 3000 to 15000 cells was seeded into each well of the 96-well cell culture plates and allowed to adhere for 12 to 24 h before the addition of the test compound, while suspended cells were seeded just before the addition of the test compound with an initial density of 1×10^5 cells/mL. Each tumor cell line was exposed to the different concentrations of compounds (0.064, 0.32, 1.6, 8 and 40 μM) for 48 h in triplicate. Cisplatin (Sigma, USA) was used as the positive control. After compound treatment, cell viability was measured and a cell growth curve was plotted. IC_{50} values were calculated by Reed and Muench's method.

4. Conclusion

In summary, this research reported two new cycloartane triterpenoids that isolated from *Aphanamixis polystachya*. Their structures were elucidated by spectroscopic analysis. In addition, these compounds were assessed for their effects on five human cancer cell lines and compound **1** could inhibited the proliferation of all tested cell lines, whereas compound **2** only exhibited cytotoxic activity on hepatocellular carcinoma SMMC-7721 cell line.

Disclosure statement

No potential conflict of interest was reported by the authors.

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