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Unusual 28, 29-nor-9, 19 cycloartane triterpenoids from Chinese medical plant *Streptocaulon griffithii* Hook

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ABSTRACT

Phytochemical investigation of the root of *Streptocaulon griffithii* Hook (Asclepiadaceae) led to the isolation of three unusual novel triterpenoids, 28, 29-nor-3*β*, 4*β*-dihydroxyl-9, 19-cycloartan-26-acid (**1**), 28, 29-nor-3*β*, 4*β*-dihydroxyl-9, 19-cycloartan- 26-acid methylester (**2**), a 30-nor-lupeol derivative 30-nor-3*β*-acetoxy-lupan-20-one (**3**) and five known compounds **4–8**. Their structures were established on the basis of physical and spectroscopic analysis, including 1D and 2D NMR (1H, ^{13}C, COSY, HSQC, and HMBC), ESI mass spectrometry, and by comparison with the NMR data published in the literature. The cytotoxicities of the isolated compounds against a panel of cultured tumor cell lines (Hela, PC3, SMMC7721, CNE) were evaluated. The new compounds **1** and **2** showed moderate activities with IC_{50} values of 11.76 ~ 26.52 μg/mL, respectively. The result showed that triterpenoids are typical compounds of *Streptocaulon* genus, which could be useful as characteristic markers in chemotaxonomic research and might helpful for explaining the use of *S. griffithii* in traditional medicine.

1. Introduction

Triterpenoids are interesting for industrial medicinal chemistry because of their various chemical structures and biological activities, such as antitumor, anti-inflammatory, antibacterial, antimicrobial, antimalarial, and antiviral activities. Therefore, triterpenoids are attracted more and more attentions of researcher for discovering novel analogues with different biological properties (Prachayasittikul et al., 2010; Srisurichan et al., 2017; Wang et al., 2006; Wei et al., 2017).

Streptocaulon griffithii (Asclepiadaceae), distributed in southern of China such as Guizhou, Guangxi and Yunnan province, has been used in traditional Chinese medicine (TCM) for the treatment of colds and fevers, dysentery, hronic nephritis, and snakebite [4], especially for the Dai ethnic living in Guangxi Province. But only few reports on its constituents and their biological activities (Ueda et al., 2003; Zhang et al., 2006, 2007, 2008). During the continue research work on discovering novel natural bioactive compounds (Wang et al., 2006, 2016a, 2016b; Li et al., 2016), we found that the MeOH extract of *S. griffithii* strongly inhibited proliferation of the human Hela tumor cell line. Then the chemical constituents of *S. griffithii* were studied systematically. Eight triterpeniods, including two unusual novel 28, 29-nor-9, 19 cycloartane triterpenoids and a novel 30-nor-lupeol derivative were obtained from this medical plant (Fig. 1). This paper reported the isolation, chemical structures identification, antitumor activities of all isolated compounds.

2. Material and methods

2.1. General

Melting points were measured on a BUCHI M565 instrument. 1D and 2D NMR (^{1}H NMR (500 MHz), ^{13}C NMR (126 MHz), ^{1}H-^{1}H COSY, HSQC, and HMBC) spectra were recorded on a *Bruker AVANCE III* 500 MHz spectrometer in $CDCl_3$ or CD_3OD using standard Bruker microprograms and TMS as internal standard. ESI–MS mass spectra were performed on a Thermo LCQ Fleet ion trap mass spectrometer and high resolution mass spectrometry were measured on Agilent 6210 TOF-MS spectrometer equipped with an ESI source. Column chromatography was carried out on silica-gel (100–200, 200–300, and 300–400 mesh, Qingdao Haiyang Chemical Factory) and/or Sephadex LH-20. Analytical TLC was carried out on silica-gel plates (GF_{254}, Qingdao Haiyang Chemical Factory). Semi-preparative HPLC was performed on Waters 600 series, with a SunFire Prep C18 (250 × 19 mm, 10 μm) column.

2.2. Plant material

The root of plant *S. griffithii* Hook was collected on Jul. 2015 in Nanning, Guangxi Province, P. R. China. The voucher specimen (SG-20150901) was identified by Prof. Bin Wu of Zhejiang University, and

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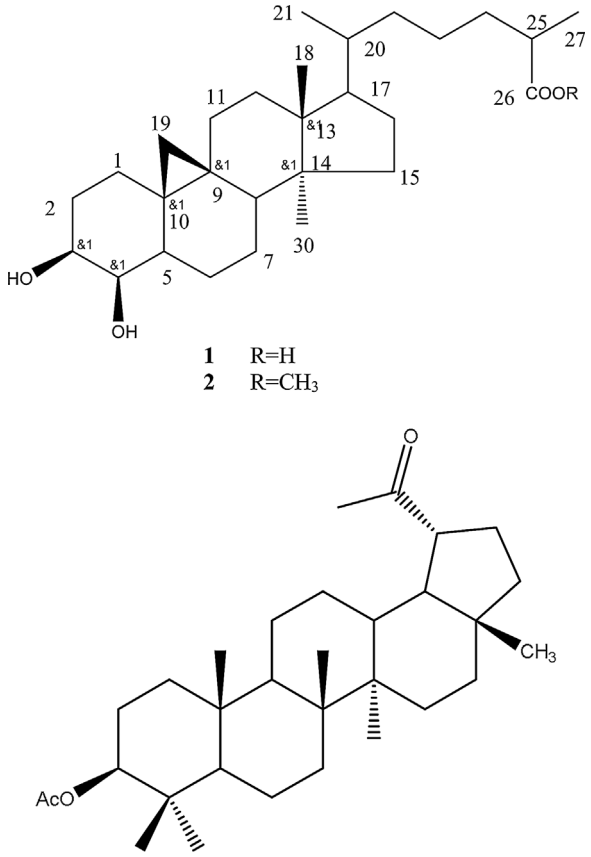

Fig. 1. Chemical structures of triterpenoids **1**–**3** from *Streptocaulon griffithii* Hook.

deposited in the School of Food Science and Biotechnology, Zhejiang Gongshang University, Hangzhou, P. R. China.

2.3. Isolation of the compounds

The air-dried samples of *S. griffithii* Hook (23 kg) were powdered and extracted 3 times by 95% ethanol for 7 days each time. The extract were pooled and evaporated *in vacuo* until no alcohol. The obtained gummy residue were partitioned with ethyl acetate (EtOAc, 4 L × 3) and *n*-butanol (BuOH, 4 L × 3) successively, and give 620 g and 423 g extracts after evaporating of the solvent under reduced pressure, respectively. The EtOAc extract was then subjected to silica-gel column chromarography (CC, 90 × 1000 mm, 200–300 mesh) with a gradient solvent of petroleum ether (PE)-EtOAc (6:1, 3:1, 2:1, 1:1, 1:2, 1:3, 1:6; 8 L for each step) to give 40 fractions. The fractions were detected by TLC and combined to twelve main fractions (*Fr.* 1- *Fr.* 12). *Fr.* 1 was further-separated on silica gel column (45 × 600 mm, 200–300 mesh), eluted with PE-EtOAc (20:1-10:1) to give compounds **4**, **6**, and **7**. *Fr.* 2 was re-purified on silica gel column (45 × 600 mm, 200–300 mesh), eluted with PE-EtOAc (8:1-6:1) to give compounds **3** and **8.** Compound **5** obtained from *Fr.* 5 by CC (25 × 500 mm) eluting with PE/Acetone (4:1-2:1). *Fr.* 7 was passed throught another silica gel column (25 × 500 mm, 200–300 mesh), eluted with PE/Acetone (3:1-1:1) to give compound **2**. *Fr.* 8 was separated repeatedly on silica gel column (25 × 500 mm, 200–300 mesh), eluted with CH_2Cl_2/CH_3OH (25:1-10:1) and followed by Pre-HPLC (CH_3OH/H_2O–60:40) to give compound **1**.

28, 29-nor-3β, 4β-dihydroxyl-9, 19-cycloartan-26-acid (**1**). White amorphous powder, m.p. 256–258 °C, ESI–MS *m/z*: 445 $[M-H]^-$, 447 $[M + H]^+$, HR-ESI–MS at *m/z* 447.3461 $[M + H]^+$ (calcd for $C_{28}H_{47}O_4^+$, 447.3469). ^1H and ^{13}C NMR data see Table 1.

28, 29-nor-3β, 4β-dihydroxyl-9, 19-cycloartan-26-acid methylester (**2**). White amorphous powder, m.p. 155–156 °C, ESI–MS *m/z*: 459 $[M-H]^-$, 461 $[M + H]^+$, HR-ESI–MS at *m/z* 461.3630 $[M + H]^+$ (calcd for $C_{29}H_{49}O_4^+$, 461.3625). ^1H and ^{13}C NMR data see Table 1.

30-nor-3β-acetoxy-lupan-20-one (**3**). Coreless prism crystal. m. p. 263–264 °C, ESI–MS *m/z*: 471 $[M + H]^+$, 493 $[M + Na]^+$. ^1H and ^{13}C NMR data see Table 1.

2.4. Antitumor assay

The cytotoxicity of the new and known compounds **1**-**3** against cultured human tumor cell lines [human acute promyelocytic leukemia cell (HL-60), mouse leukemia cell (P388), human hepatic carcinoma cell (SMMC7721), human breast cancer cell (Bcap37)] were evaluated by the MTT assay previously described (Wang et al., 2007). Briefly, the tumor cells were cultured at 37 °C under a humidified atmosphere of 5% CO_2 in RPMI-1640 medium supplemented with 10% fetal calf serum, and dispersed in replicate 96-well plates (5×10^4 cells/well) for 48 h. Compounds **1**-**3** (2.5–100 mg/ml) or Cisplatin (positive control) were then added. After 72 h of exposure to the toxins, the cell viability was determined by the MTT by recording the absorbance at λ_{max} 570 nm with an ELISA reader. Each test was performed in triplicate (n = 3).

2.5. Statistical analysis

The data were expressed as mean ± standard deviation (S.D.) and examined for their statistical significance of difference with ANOVA and a Tukey post hoc test. P-values of less than 0.05 were considered to be statistically significant. All the analyses were carried out by using SPSS v22.0 software.

3. Results and discussion

3.1. Structure identification of bioactive pentacyclic triterpenes

Compound **1** was obtained as a white amorphous powder. Its molecular formula was determined as $C_{28}H_{46}O_4$ by HR-ESI–MS at *m/z* 447.3461 $[M + H]^+$ (calcd. for $C_{28}H_{47}O_4^+$, 447.3469) and supported by the analysis of 1D and 2D NMR data. The ^1H NMR spectrum of **1** showed signals due to two tertiary methyl groups at δ_H 1.02 (3H, Me-18) and 0.96 (3H, Me-30), two secondary methyl group at δ_H 0.89 (3H, Me-21) and 1.14 (3H, Me-27), two oxygenated methine signals at δ_H 4.83 (1H, H-3) and 4.53 (1H, H-4), respectively. The ^1H NMR spectrum also showed the presence of two cyclopropane methylene proton signals at δ_H 0.29 (1H, H-19b) and 0.75 (1H, H-19a), indicating the presence of the usual 9, 19-cycloartane ring junction (Mohamed et al., 2016; Zhu et al., 2016).

The ^{13}C NMR, DEPT and HSQC spectra of **1** displayed 28 carbon signals, which assignned to four methyls, twelve methylenes (including a typical cyclopropane methylene carbon resonances, corresponding to the methylene carbon of the cyclopropane ring at C-19 (δ_C 30.0, δ_H 0.29 and 0.76), seven methines (including two oxygenated methine carbons at δ_C 80.9 (C-3) and 76.5 (C-4), respectively), and five quaternary carbons among a carbonyl group (δ_C 181.0 (C-26) (Table 1). These NMR and MS data suggest that compound **1** is a nor-9, 19-cycloartane type triterpenoid acid with two hydroxyls and one carboxyl substituents (Mohamed et al., 2016; Zhu et al., 2016).

The connectivity of the hydroxyls and carbonyl groups were assigned by detailed study of the ^1H-^1H COSY, HSQC and the HMBC spectra. The correlation of the two oxygenated methine hydrogen signals at δ_H 4.83 and 4.53 in ^1H-^1H COSY spectrum suggesting that the two OH groups at adjacent position. The HMBC correlations (Fig. 2) from δ_H 4.83 to C-1 (δ_C 28.0), C-5 (δ_C 38.8), and the correlations from δ_H 4.53 to C-2 (δ_C 27.4), C-6 (δ_C 26.1), C-10 (δ_C 26.2) supported the two OH groups substitute at C-3 and C-4, repectively. So the nor-methyl groups are Me-28 and Me-29. The HMBC spectrum also showed