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A New Lupane-type Triterpene from the Roots of *Ampelopsis grossedentata*

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Abstract: A previously undescribed lupane-type triterpene **(1)** and five known compounds (**2–6**) were isolated from the roots of *Ampelopsis grossedentata*. The structure of compound **1** was elucidated through comprehensive analysis of spectroscopic data, which included 1D and 2D NMR as well as HRESIMS. The absolute configuration of **1** was resolved by comparing its specific rotation with that of its demethylated derivative 3*β*-*O*-trans-*p*-coumaroylalphitolic acid,and was also supported by comparisons of the experimental and the theoretical ECD spectra. The known compounds were identified as daucosterol (**2**), ellagic acid (**3**) and its trimethylated derivative (**4**), resveratrol (**5**), and dihydrokaempferol (**6**) by comparing their NMR data and optical rotations with those in the literature. Compounds **1–6** were tested for their antimicrobial activity against five different bacterial strains. The results indicated that compounds **3**, **4**, and **6** exhibited weak antibacterial activity, with MIC values ranging from 128 to 256 μg/mL.

Keywords: *Ampelopsis grossedentata*; lupane-type triterpene; antibacterial activity.

1. Introduction

Ampelopsis grossedentata (Miq.) W.T.Wang, often referred to as "Snake Grape," is a climbing plant characterized by its slender vines and is part of the Vitaceae family. This species is extensively distributed across the mountainous and hilly areas of southern China, particularly in Zhejiang, Jiangsu, Anhui, Jiangxi, and Fujian Provinces [1]. The root functions as a traditional Chinese medicinal ingredient that offers various properties, including the ability to clear heat, detoxify, dispel wind, activate meridians, relieve pain, and stop bleeding. It is commonly used in folk medicine for clearing heat and toxins, dispelling wind and dampness, and promoting blood circulation to reduce masses [2].

Studies prior to this, focused on the chemical composition of *A. grossedentata*, resulted in the identification of various constituents, including flavonoids, steroids, terpenoids, polyphenols, and polysaccharides, as well as some well-recognized active ingredients such as dihydromyricetin, gallic acid, catechins, and lupeol [3]. The literature revealed that the extract of *A. grossedentata* and its main components exhibit diverse pharmacological activities, including antibacterial [4, 5], antioxidant [6, [7], anti-tumor [8], anti-inflammatory [9], lipid-lowering [10], antidiabetic [11], antiviral activity [12, [13] , and enhancing immune effects [14].

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2. Materials and Methods

2.1. Plan Material

The roots of *Ampelopsis grossedentata* (Miq.) W.T.Wang were collected in June 2023 in Hubei Province, China, and was authenticated by Prof. Yongshen Ren of School of Pharmaceutical Sciences at Hainan University. A voucher specimen (AMS202306Hnu) was deposited at the School of Pharmaceutical Sciences, Hainan University.

2.2. Extraction and Isolation

In our commitment to discovering new bioactive molecules from natural resources [15, 16], we performed a thorough chemical investigation of the ethyl acetate fraction of the roots of *A. grossedentata*. 4.5 kg roots of *A. grossedentata* were extracted with methanol at room temperature for 3 times. The solution was filtered and concentrated under reduced pressure to obtain a methanol extract (475.6 g). The methanol extract was dissolved in water and subjected to continuous extraction with petroleum ether, ethyl acetate, and *n*-butanol, three times for each solvent. The solution was then concentrated under reduced pressure to obtain the petroleum ether extract (49.88 g), an ethyl acetate extract (85.28 g), and an n-butanol extract (208.79 g), respectively.

The ethyl acetate extract was subjected to silica gel column chromatography, using a gradient of dichloromethane-methanol (1:0 to 5:1) to give eight subfractions (E-1 to E-8). The fraction E-2 was subjected to silica gel column chromatography, using a gradient of dichloromethane -methanol (1:0 to 5:1) to give six subfractions (E-2-1 to E-2-6). An ODS column, eluted with a gradient of methanol/H2O (from 10% to 100%) was used to separate the subfraction of E-2-2, resulting in seven subfractions (E-2-2-1 to E-2-2-7). The subfraction E-2-2-7 was further purified with semi-preparative HPLC using 90% methanol to obtain compound **1** (3.5 mg, t_R = 31 min, 2 mL/min). Compound **4** (2.4 mg) crystallized in fraction E-2-2-4. The fraction E-3 was subjected to silica gel column chromatography, by using a gradient of dichloromethane-methanol (1:0 to 5:1) to give nine subfractions (E-3-1 to E-3-9). Compound **2** (12.9 mg) crystallized in subfraction E-3-7. Compound **3** (9.3 mg) crystallized in fraction E-5. The subfraction E-2-4 was subjected to silica gel column chromatography, by using a gradient of dichloromethane-methanol (1:0 to 5:1) to give five subfractions (E-2-4-1 to E-2-4-5). Subfraction E-2-4-2 was separated using ODS column with a gradient elution of 10→100% methanol to give compound **5** (34.5mg). The subfraction E-2-4-3 was further purified on a semi-preparative HPLC with 52% methanol as solvent to obtain compound **6** (1.5 mg, t_R = 21 min, 2 mL/min).

3. Results and Discussion

3.1. Structure Elucidation

Compound **1** was obtained as a white powder, with its chemical formula assigned as $C_{40}H_{56}O_6$ based on HRESIMS ($[M - H]^-$ *m*/*z* 631.4004, calcd for $C_{40}H_{55}O_6^-$, *m*/*z* 631.4005) (Figure S11) and NMR spectroscopy, suggesting 13 indices of hydrogen deficiency. The resonances of the ^{1}H NMR spectrum (Figure S5 and Table S1) indicate the existence of seven methyl singlets (δ_H 3.67, 1.70, 1.04, 0.98, 0.96, 0.93, 0.88), including a methoxy (δ_H 3.67) and an olefinic methyl singlet (δ_H 1.70). The vinyl protons at δ_H 7.63 (1H, d, *J* = 15.9 Hz) and 6.38 (1H, d, *J* = 15.9 Hz) were characteristic signals for a *trans* double bond. The observation of two sets of doublets, each integrating for two protons, at δ_H 7.47 (1H, d, *J* = 8.6 Hz) and 6.81 (1H, d, *J* = 8.6 Hz) pointed to the presence of a *para*-substituted phenyl group. Two oxygenated protons were observed at δ_H 3.83 and 4.61. The shielded vinyl singlets at δ_H 4.73 and 4.61 are typical resonances for the methylene protons of a terminal double bond. The remaining resonances were attributed to the methine and methylene protons. Utilizing the ^{13}C NMR (Figure S6 and Table S1) spectrum alongside the DEPT135 spectrum, 40 carbon resonances were clarified, which correspond to 10 non-protonated carbons δ_C 178.2 (C-28), 169.7 (C-1′), 161.2 (C-7′), 151.8 (C-20), 127.3 (C-4′), 57.9 (C-17), 43.6 (C-14), 42 (C-8), 40.7 (C-4), 39.5 (C-10), 13 methine carbons δ_C 146.2 (C-3′), 131.1 (C-5′/9′), 116.8 (C-6′/8′), 115.9 (C-2′), 85.6 (C-3), 67.8 (C-2), 56.6 (C-

5), 51.9 (C-9), 50.6 (C-18), 48.6 (C-19), 39.6 (C-13), 10 methylene carbons δ_C 110.3 (C-29), 48.8 (C-1), 37.9 (C-22), 35.4 (C-7), 33.1 (C-16), 31.6 (C-15), 30.8 (C-21), 26.7 (C-12), 22.2 (C-11), 19.4 (C-6), and seven methyl carbons. These carbons could be attributed, with the assistance of the HSQC data, to nine carbons for a *trans-p*-coumaroyl and 31 carbons for a triterpene nucleus. The data mentioned above closely resembled those of the triterpene derivative 3*β*-*O*-*trans-p*-coumaroylalphitolic acid, with the sole difference being the presence of a methoxy group. The HMBC correlation from the methoxy protons at δ_H 3.67 to the carbonyl carbon at 178.2 indicated that **1** was the methyl ester of 3*β*-O-*trans-p*-coumaroylalphitolic acid. The structure of **1** was confirmed by detailed analysis of the 2D NMR data (Figure 2).

*Methyl ester of 3β-O-trans-p-coumaroylalphitolic acid (**1**)*: White powder; $[\alpha]^{20}_D$ −27 (*c* 0.1, pyridine); UV (MeOH) λ_{max} 313 nm; ^{1}H NMR (400 MHz, CD_3OD): δ_H 7.63 (d, *J* = 15.9, H-3′), 7.47 (d, *J* = 8.6, H-5′/9′), 6.81 (d, *J* = 8.6, H-6′/8′), 6.38 (d, *J* = 15.9, H-2′),4.73 (s, H-29a), 4.61 (s, H-29b), 4.61 (d, *J* = 11.0 Hz, H-3), 3.83 (td, *J* = 11.0, 11.0, 4.8 Hz, H-2), 3.67 (s, OCH_3), 3.0 (m, H-19), 2.26 (m, H-13), 2.24 (m, H-16a), 2.06 (dd, *J* = 11.0, 4.8 Hz, H-1a), 1.87 (m, H-15a), 1.86 (m, H-22a), 1.78 (m, H-21), 1.75 (m, H-12), 1.66 (m, H-18), 1.56(m, H-6a), 1.50 (m, H-6b), 1.49 (m, H-11a), 1.48 (m, H-7a), 1.47 (m, H-22b), 1.46 (m, H-9), 1.44 (m, H-16b), 1.40 (m, H-7b), 1.39 (m, H-15b), 1.32 (m, H-11b), 1.71 (s, H-30), 1.04 (s, H-27), 1.10 (m, H-12), 0.99 (dd, *J* = 11.0, 11.0 Hz, H-1b), 0.98 (s, H-25), 0.96 (m, H-5), 0.96 (s, H-26), 0.93 (s, H-24), 0.88 (s, H-23). ^{13}C NMR (100 MHz, CD_3OD): δ_C 178.2 (C-28), 169.7 (C-1′), 161.2 (C-7′), 151.8 (C-20), 146.2 (C-3′), 131.1 (C-5′/9′), 127.3 (C-4′), 116.8 (C-6′/8′), 115.9 (C-2′), 110.3 (C-29), 85.6 (C-3), 67.8 (C-2), 57.9 (C-17), 56.6 (C-5), 51.9 (C-9), 51.9 (C28-OCH_3), 50.6 (C-18), 48.8 (C-1), 48.6 (C-19), 43.6 (C-14), 42 (C-8), 40.7 (C-4), 39.6 (C-13), 39.5 (C-10), 37.9 (C-22), 35.4 (C-7), 33.1 (C-16), 31.6 (C-15), 30.8 (C-21), 29.1 (C-23), 26.7 (C-12), 22.2 (C-11), 19.5 (C-30), 19.4 (C-6), 18.1 (C-24), 17.9 (C-25), 16.7 (C-26), 15.1 (C-27); HRESIMS *m*/*z* 631.4005 $[M + Na]^+$ (calcd for $C_{40}H_{55}O_6^-$, 631.4004).

The other compounds were identified to be daucosterol (**2**) [17], ellagic acid (**3**) [18], 3,3′,4′-tri-*O*-methylellagic acid (**4**) [19], resveratrol (**5**) [20], and dihydrokaempferol (**6**) [21] by comparisons of the ^{1}H and ^{13}C NMR data, as well as optical rotations of **2**–**6** and compounds bearing identical gross structures in the literature (Figure 1).

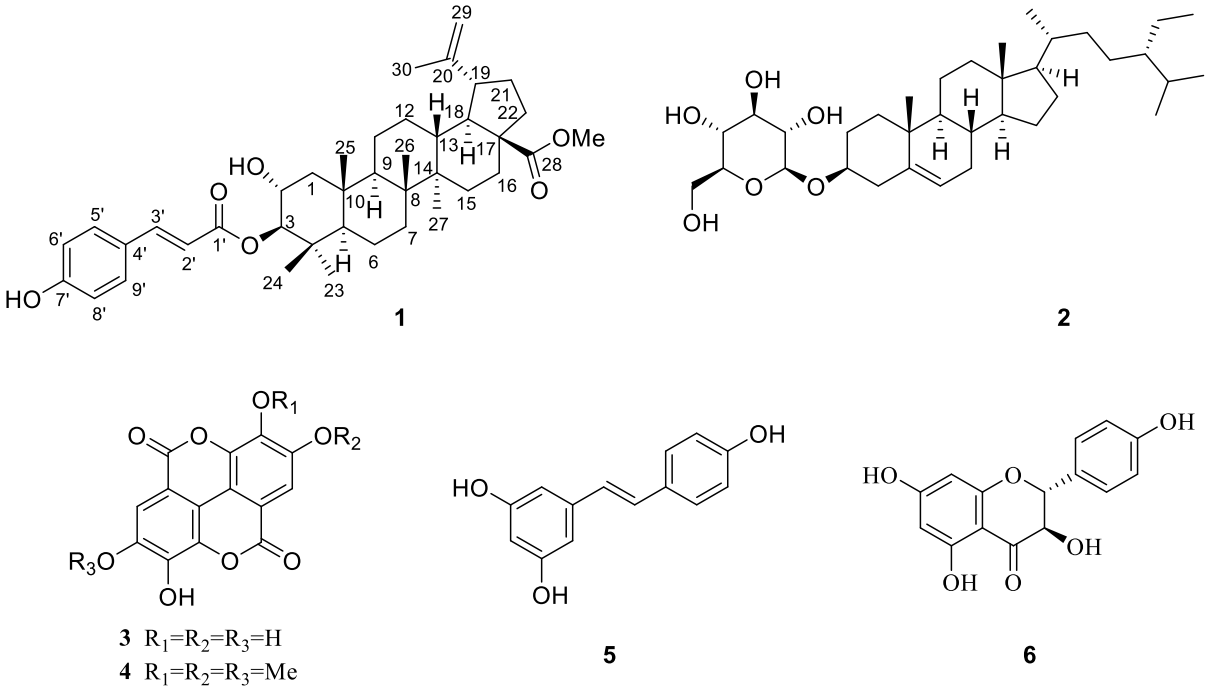

Figure 1. Structures of compounds **1–6** from the roots of *A. grossedentata*

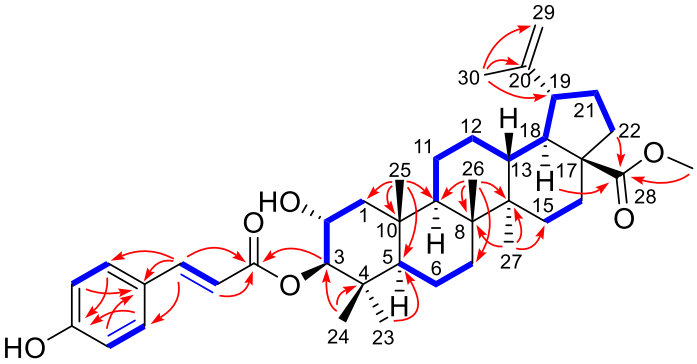

Figure 2. Key COSY (—) and HMBC (→) correlations of 1.

The relative configuration of **1** was determined by the NOESY correlations (Figure 3). Specifically, the NOESY correlations from H_3-25 to H-2, H_3-24, H_3-26, from H-13 to H_3-26, H-19 indicated these protons occupied the same spatial orientation, The NOESY correlations from H-5 to H-3 and H-9, as well as from H_3-27 to H-9 and H-18, suggested that these protons reside on the opposing face. These NOESY correlations indicated the *trans* fusion of the pentacyclic nucleus and the four six-membered rings adopted a chair conformation, the same as that of 3*β*-O-*trans*-*p*-coumaroylalphitolic acid [22, 23].

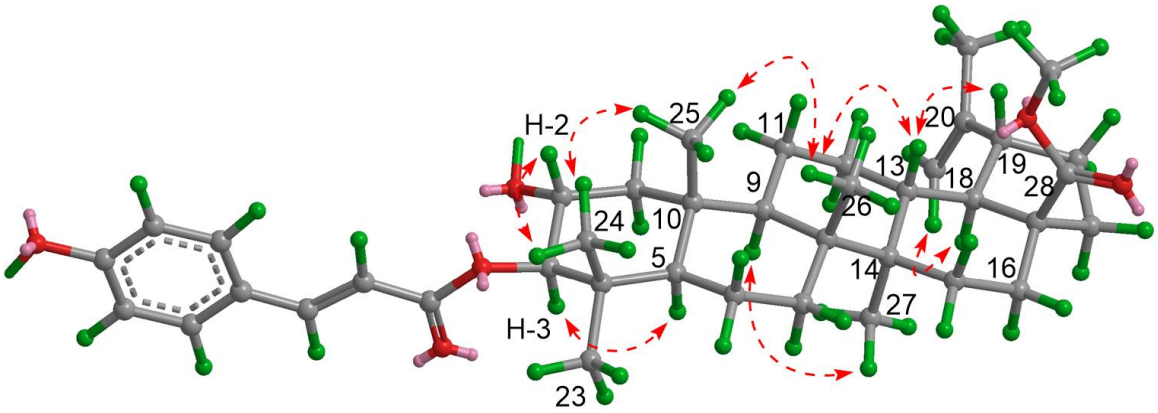

Figure 3. Key NOESY correlations (◄- -►) of **1**.

Thus, the structure of **1** was determined to be methyl ester of 3*β*-O-*trans*-*p*-coumaroylalphitolic acid. The absolute configuration of **1** was determined to be the same as that of 3*β*-O-*trans*-*p*-coumaroylalphitolic acid by comparing their specific rotation (**1**: $[\alpha]^{D}_{25}$: –27 (*c* 0.1, pyridine); 3*β*-O-*trans*-*p*-coumaroylalphitolic acid: $[\alpha]^{D}_{25}$ –33.1 (*c* 0.8, pyridine). Meanwhile, the theoretical ECD data of the model molecule 2*R*, 3*R*, 5*R*, 8*R*, 9*R*, 10*R*, 13*R*, 14*R*, 17*S*, 18*R*, 19*R*-**1** was calculated using the TDDFT methodology at the b3lyp/6-31G* level using the solvation model density (SMD) model in MeOH (Figure 4). The calculated spectrum displayed similar curve to the experimental spectrum, with obvious negative Cotton effects around 217 nm. Thus, the absolute configuration of the chiral centers of 1 was assigned as 2*R*, 3*R*, 5*R*, 8*R*, 9*R*, 10*R*, 13*R*, 14*R*, 17*S*, 18*R*, and 19*R*.

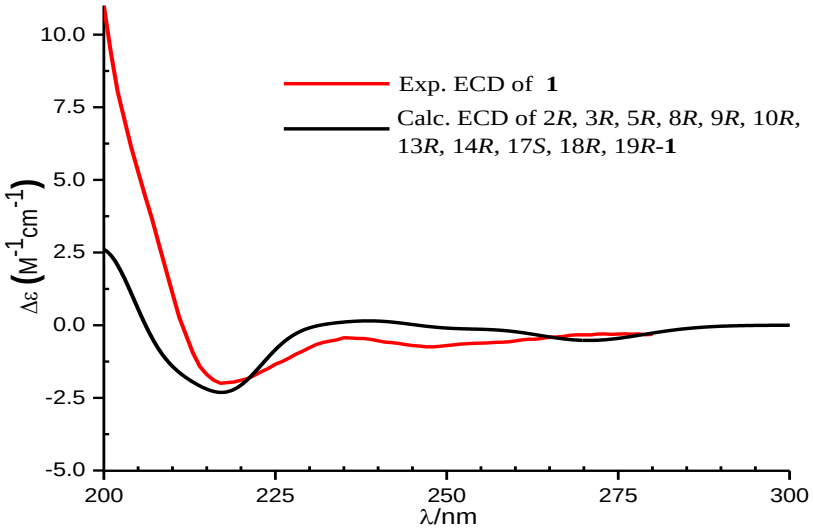

Figure 4. Experimental ECD spectrum of **1** in MeOH and the calculated ECD spectrum of the model molecule 2*R*, 3*R*, 5*R*, 8*R*, 9*R*, 10*R*, 13*R*, 14*R*, 17*S*, 18*R*, and 19*R*-**1** at the B3LYP/6-31G* in MeOH.

3.2. Biological Activity

The antimicrobial activity of compounds **1**–**6** towards five bacterial strains was evaluated: *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923, *Micrococcus luteus* ATCC 49732, *Staphylococcus epidermidis* ATCC 12228, and *Pseudomona aeruginosa* ATCC 27853. The Minimum Inhibitory Concentration (MIC) were determined by broth microdilution method according to the Clinical and Laboratory Standards Institute (CLSI) 2015 guidelines. The commercial antibiotics amoxicillin and levofloxacin was used as the references. As indicated by the results (Table 1), compounds **3** displayed weak inhibition against *M. luteus* and *S. epidermidis*, with an MIC value of 256 μg/mL. Compound **4** exhibited weak inhibition across all five strains, with an identical MIC value of 128 μg/mL for both *Staphylococcus aureus* and *Escherichia coli*. Additionally, compound **6** had an MIC value of 128 μg/mL against *S. epidermidis*.

Table 1. Antibacterial activity (MIC, μg/mL) of the active compounds

Compd.	*E. coli*	*S. aureus*	*M. luteus*	*S. epidermidis*	*P. aeruginosa*
3	–	–	256	256	–
4	128	128	256	256	128
6	–	–	–	128	–
Amoxicillin	4	4	4	4	–
Levofloxacin	–	–	–	–	1

[a]MIC values > 256 μg/mL

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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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