SHORT REPORT

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Calkbisabenacid, A New Bisabolane Sesquiterpenoid from the Leaves of *Callicarpa kwangtungensis*

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Abstract: A new bisabolane sesquiterpenoid, named calkbisabenacid (**1**), was isolated from the leaves of *Callicarpa kwangtungensis*, along with four known compounds: 5-O-coumaroylquinic acid (**2**), kaempferol (**3**), and soyasapogenol B (**4**). The structure of compound **1** was determined by ^{1}H NMR, ^{13}C NMR, COSY, HSQC, and HMBC spectra, in combination with the HRESIMS data. The absolute configuration of C-1 in compound **1** was determined by comparing the experimental ECD spectrum with the calculated ECD spectra of the two possible stereoisomers. The known compounds were identified by comparing their NMR data with those reported in the literature. Compound **4** was isolated from this species for the first time. None of these compounds demonstrated any inhibitory effects against *Staphylococcus aureus* ATCC 25923.

Keywords: Calkbisabenacid; sesquiterpene; *Callicarpa kwangtungensis.* © 2024 ACG Publications. All rights reserved.

1. Plant Source

In June 2021, leaves of *Callicarpa kwangtungensis* were collected from Conghua, Guangdong Province, China. The specimen was identified by Prof. Ying Lu at the First People's Hospital of Linping District in Hangzhou, China. Subsequently, a sample labeled as No. 202106Calkwang was preserved at Hangzhou Vocational & Technical College in China. A voucher specimen was also preserved in the Herbarium of Zhongshan University (Herbarium number:SYS00235653)

2. Previous Studies

C. kwangtungensis Chun, a medicinal plant of the genus *Callicarpa*, is primarily found in Guangdong, Jiangxi, and Hunan Provinces of China. Its stems and leaves exhibit notable effects such as astringency and scattered stasis detumescence [1]. Modern pharmacological research shows that *Callicarpa kwangtungensis* has anti-inflammatory, hemostatic, anticoagulant, antibacterial, antioxidant, and cardioprotective effects [2, 3]. A literature review revealed that the chemical constituents of *C.*

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kwangtungensis include phenylethanol glycosides [4], flavonoids [5], terpenoids [6], sesquiterpenoids [7], and lignans [8].

In our pursuit of discovering new molecules from the leaves of the plant *C. kwangtungensis* [9]. Four compounds, including a new bisabolane sesquiterpenoid (**1**), a phenylpropanoid glycoside (**2**), a flavone (**3**), and a terpenoid (**4**) were obtained. The isolation and structure elucidation are described here.

3. Present Study

The leaves of *C. kwangtungensis* (300 g) were extracted three times using ethanol as the solvent, resulting in the production of a crude extract (35 g). The crude extract was subsequently partitioned with EtOAc/H_2O (100 mL:100 mL) to yield the EtOAc extract (10 g), which was further separated via silica gel column chromatography to give five fractions (Fr. A-Fr. E). Fr. C was separated on an ODS silica gel to yield four fractions: Fr. Ca-Fr. Cd. Fr. Ca was subjected to purification on a Sephadex LH-20 column, with MeOH as the eluting solvent, leading to the isolation of compound **2** (8 mg). Fr. Cb was purified through Sephadex LH-20 using MeOH as eluent and yielded compounds **1** (17 mg) and **3** (21 mg). Fr. D was subjected to Sephadex LH-20 column, with CH_2Cl_2/MeOH (1:1) as the eluent, leading to the isolation of compound **4** (44 mg).

*Calkbisabenacid (**1**)*: Colorless oil; $[\alpha]^{25}_D$ +47 (*c* = 0.1, CH_3OH); UV (MeOH) λ_{max} 242 (3.07) nm; ECD (*c* 0.8 mM, MeOH) λ_{max} ($\Delta\varepsilon$) 212 (–3.1) nm; ^{1}H and ^{13}C NMR data, Table 1; negative HRESIMS *m*/*z* 267.1590 $[M - H]^-$ (calcd for $C_{15}H_{23}O_4^-$, 267.1602).

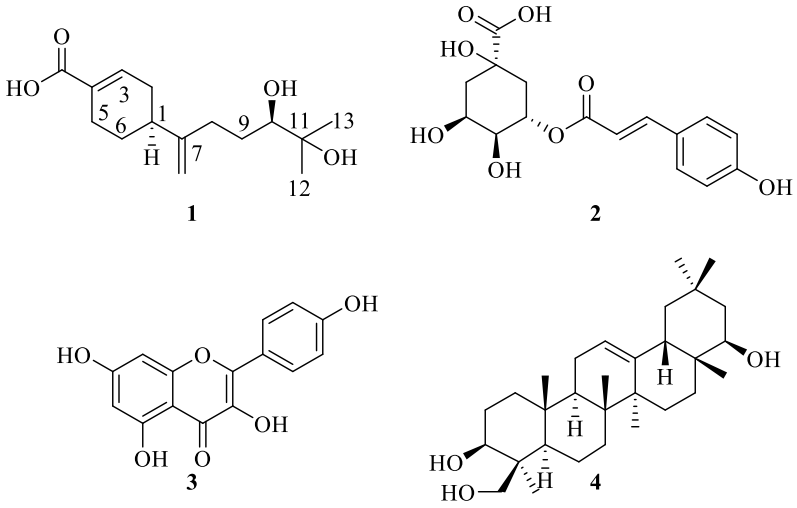

Figure 1. Structures of compounds **1**–**4** from *C. kwangtungensis*

The molecular formula of compound **1** was determined to be $C_{15}H_{24}O_4$ by HRESIMS, requiring four degrees of unsaturation. The ^{1}H NMR spectrum (Table 1) included resonances for two methyl singlets (δ_H 1.17, 1.13), an oxygenated proton (δ_H 3.27), two olefinic protons (δ_H 4.80, 4.83) for a terminal double bond, a deshielded olefinic proton at δ_H 6.98, and several methylene and methine protons. The ^{13}C NMR spectrum of **1** (Table 1) indicated the presence of 15 carbons, which corresponded to the calculated molecular formula. The ^{13}C NMR and HSQC spectra showed signals for an α,β-unsaturated carboxylic acid (δ_C 171.3, 140.1, 131.8), two olefinic carbons for a terminal double bond (δ_C 154.5, 108.5), five sp^3 methylene carbons, two sp^3 methine carbons (δ_C 73.7, 40.1) including an oxygenated carbon, two methyl cabons (δ_C 25.6, 24.7), and a sp^3 oxygenated non-protonated carbon at δC 79.0.

Table 1. ^{1}H (400 MHz) and ^{13}C NMR (100 MHz) data of **1** and gxsespene (**1a**) in Methanol-d_4 (δ in ppm)

No.	**1**		**1a**	
	δ_H, Mult. (*J* in Hz)	δ_C	δ_H, Mult. (*J* in Hz)	δ_C
1	2.19, m	40.1, CH	2.70, m	35.9
2	2.37, m; 2.17, m	32.6, CH_2	2.20, m; 2.10, m	30.9
3	6.98, br s	140.1, CH	7.10, br d	140.9
4		131.8, C		131.4
5	2.41, m; 2.20, m	25.8, CH_2	2.45, dd (15.8, 4.1) 2.20, m	25.8
6	1.92, m; 1.49, m	28.8, CH_2	1.72, m; 1.56, m	27.8
7		154.5, C		140.3
8	2.37, m; 2.10, m	32.9, CH_2		124.6
9	1.77, m; 1.41, m	30.8, CH_2	2.37, m; 2.02, m	30.3
10	3.27, dd (10.0,1.5)	73.7, CH	3.25, td (10.1, 2.4)	73.8
11		79.0, C		80.2
12	1.13, s	24.7, CH_3	1.13, s	24.7
13	1.17, s	25.8, CH_3	1.17, s	25.9
14	4.80, br s; 4.83, br s	108.5, CH2	1.66, s	19.4
15		171.3, C		

Three of the four degrees of unsaturation could be explained by the α,β-unsaturated carboxylic acid and terminal double bond, the remaining one indicated that compound **1** was monocyclic.

The COSY spectrum (Figure 2) revealed two spin systems. One spin system was assigned via correlations from H-3 (δ_H 6.98) to H_2-2 (δ_H 2.37, 2.17), from H_2-2 to H-1 (δ_H 2.19), from H-1 to H_2-6 (δ_H 1.92, 1.49), and from H_2-6 to H_2-5 (δ_H 2.41, 2.20). The other spin system was assigned by correlations from H_2-8 (δ_H 2.37, 2.10) to H_2-9 (δ_H 1.77, 1.41) and from H_2-9 to H-10 (δ_H 3.27). The HMBC correlations from H-3 and H_2-5 to C-15 (δ_C 171.3) and C-4 (δ_C 131.8) assigned a cyclohex-1-ene-1-carboxylic acid. The two methyl groups, CH_3-12 (δ_H 1.17) and CH_3-13 (δ_H 1.13), were found to be geminal based on shared HMBC correlations to C-11 (δ_C 79.0) and C-10 (δ_C 73.7). A six-carbon unit consisting of the spin system H_2-8/H_2-9/H-10 and the geminal methyls was thus assigned. Additional HMBC correlations from the olefinic protons H_2-14 (δ_H 4.83, 4.80) to C-7 (δ_C 154.5), C-1 (δ_C 40.1), and C-8 (δ_C 32.9) connected the aforementioned moieties via the carbon-carbon bonds C-7–C-8 and C-7–C-1. The planar structure of **1** was thereby established as depicted.

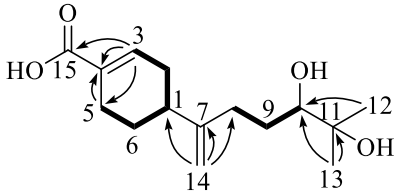

Figure 2. Key HMBC and COSY Correlations of Compound **1**

In order to assign the absolute configuration of C-1, the theoretical ECD data were calculated at the B3LYP/6-31+G(d,p) level using the TDDFT method. The calculated ECD spectrum of 1*S*-**1** showed ECD curve with negative Cotton effect at 214 nm, comparable to the Cotton effect observed at 212 nm in the experimental spectrum of **1**. Thus, the absolute configuration of C-1 was determined to be *S*. The absolute configuration of C-10 was determined to be *R* by comparing the chemical shifts of CH-10, C-11, CH_3-12, and CH_3-13 with the known analog gxsespene (**1a**) [10]. Compound **1** was named calkbisabenacid.

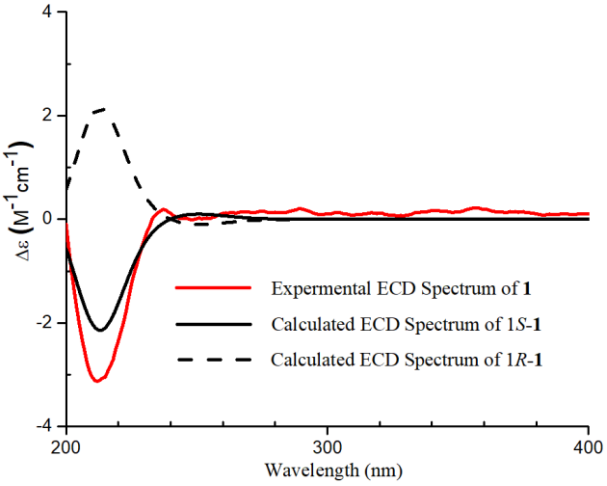

Figure 3. Experimental ECD spectrum of **1** in MeOH and the calculated ECD spectra of 1*S*-**1** and 1*R*-**1** at B3LYP/6-31+G(d,p) level in MeOH

Compounds **2**–**4** were identified to be 5-O-coumaroylquinic acid (**2**) [11, 12], kaempferol (**3**) [13], and soyasapogenol B (**4**) [14] by comparing their ^{1}H NMR data and optical rotations with those reported in the literature. Compound **4** was isolated from this species for the first time. The isolated compounds were evaluated for their antibacterial activity against *Staphylococcus aureus* ATCC 25923 at an initial concentration of 128 μg/mL, however, none of them exhibited any inhibitory activity.

Supporting Information

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

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