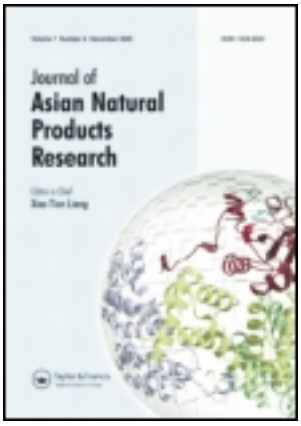

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A new rearranged abietane diterpenoid from *Clerodendrum kaichianum* Hsu

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A new rearranged abietane diterpenoid from *Clerodendrum kaichianum* Hsu

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A new abietane diterpenoid, (3*S*,16*R*)-12,16-epoxy-3,6,11,14,17-pentahydroxy-17(15 → 16)-*abeo*-5,8,11,13-abietatetraen-7-one (**1**), was isolated from the stems of *Clerodendrum kaichianum* Hsu, together with four known diterpenoids. The structures of the isolated compounds were assigned on the basis of their NMR spectra including 2D NMR techniques such as COSY, HMQC, and HMBC experiments, and were compared with those of the literature data. This new compound showed significant cytotoxicity against the HL-60 and A-549 tumor cell lines.

Keywords: Verbenaceae; *Clerodendrum kaichianum* Hsu; abietane diterpenoids; cytotoxicity

1. Introduction

Clerodendrum is a genus of about 400 species in the family Verbenaceae (or Lamiaceae), which mainly grows in the tropical and subtropical regions of the world including Africa and southern Asia. A few species are found in South America, northern Australia, and eastern Asia [1]. There are more than 30 species distributed in China, some of which have been used as traditional Chinese medicine, such as *Clerodendrum inerme* used for skin diseases [2]. Plants of genus *Clerodendrum* have proved to be rich sources of abietane diterpenoids, as well as of iridoids, phenylethanoid glycosides, sterol, triterpenes, and triterpenoid saponins, which have been found to possess many beneficial pharmacological effects, such as antilipid peroxidative, antitumor, and anti-HIV activities [3–9]. In China, the leaves of *Clerodendrum kaichianum* Hsu are used as a traditional medicine for hypertension. But there are no reports on chemical constituents of *C. kaichianum* until now. We initiated a chemical study on *C. kaichianum*, and a new compound (**1**) (Figure 1) with four known abietane diterpenoids teuvincenone G (**2**) [10], teuvincenone H (**3**) [10], 14-deoxycoleon U (**4**) [11], and 12-methylcoleon U (**5**) [12] was isolated from the stems of this plant. The present paper deals with the isolation, structural elucidation, and cytotoxic activity of the new compound.

2. Results and discussion

Compound **1** was isolated as yellowish needles with mp 280–282°C and $[\alpha]_D^{25}$ +31 ($c = 0.1$, MeOH). Its molecular formula was determined as $C_{20}H_{24}O_7$ by HR-ESI-MS at *m/z* 375.1449 $[M - H]^-$, which indicated 9 degrees of unsaturation. The IR spectrum showed the absorption bands for OH (3417 cm^{-1}), α,β-unsaturated ketone (1660 cm^{-1}), and benzene

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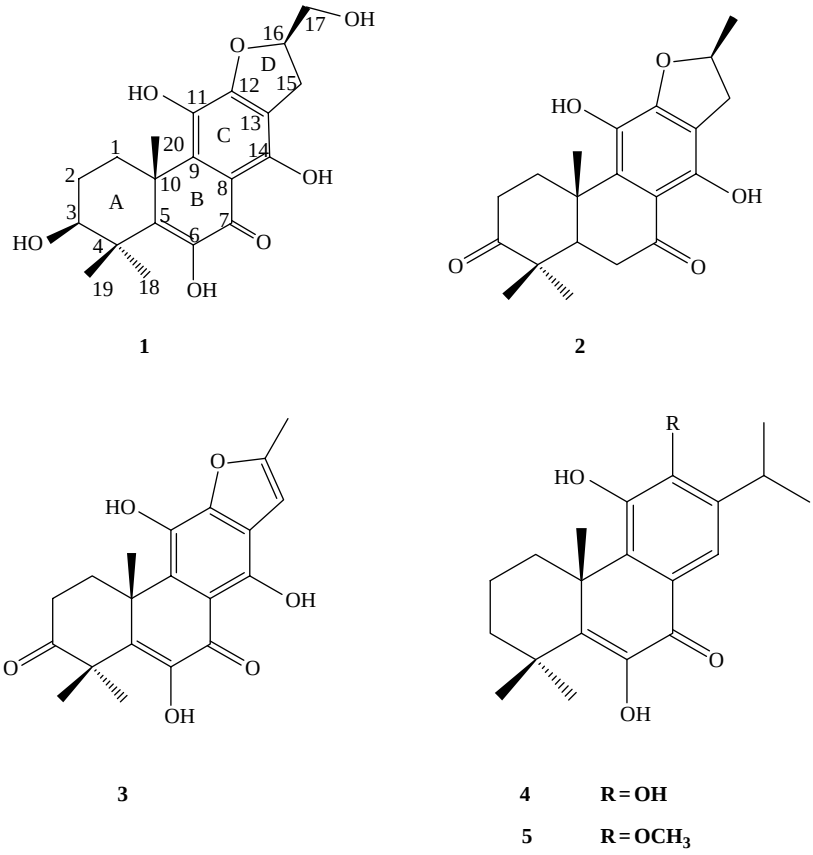

Figure 1. Structures of compounds **1**–**5**.

moieties (3042, 1621, 1585, 1520 cm^{-1}). The absorption maxima in the UV spectrum (265, 290, 338, 381 nm) also exhibited the presence of a benzene ring and a ketone.

The ^1H NMR spectrum (Table 1) showed signals corresponding to three methyl singlets at δ_H 1.41 (3H, s, H-18), 1.55 (3H, s, H-19), and 1.63 (3H, s, H-20) attached to fully substituted carbons. A very deshielded resonance of hydroxyl proton at δ_H 12.50 (1H, br s, OH-14) of compound **1**, together with its slow exchange with D_2O, confirmed the existence of a phenolic hydroxyl group at C-14 position, forming a strong intramolecular hydrogen bond with the C-7 ketone function.

A total of 20 C-atom signals were observed in the ^{13}C NMR and DEPT 135 spectra of **1** (Table 1), which showed one carbonyl [δ_C 182.8], six aromatic quaternary carbons [δ_C 154.5, 153.3, 139.3, 130.6, 110.6, and 106.9] in the low-field region, three methyls [δ_C 17.8, 24.4, and 28.2], four methylenes [δ_C 26.7, 28.7, 28.9, and 64.7], two methines [δ_C 72.6 and 86.4], and two quaternary carbons [δ_C 30.0 and 41.3] in the high-field region (Table 1). These data revealed that **1** was a diterpenoid. The NMR spectra of **1** were very similar to those of teuvincenone B [12], which showed the presence of a 2-hydroxymethyl-2,3-dihydrofuran condensed with the aromatic ring [δ_H 3.32 (1H, dd, $J = 15.5$, 9.6 Hz, H-15α), 3.05 (1H, dd, $J = 15.5$, 7.2 Hz, H-15β), 5.12 (1H, m, H-16), 3.92 (1H, dd, $J = 12.2$, 3.2 Hz, H-17a), and 3.82 (1H, dd,

Table 1. ^{1}H (500 MHz) and ^{13}C NMR (125 MHz) spectral data of compound **1** in $CDCl_3$.

Position	δ_C	δ_H (J, Hz)
1	28.9	1.75 m
		3.18 m
2	26.7	2.11 m
		2.25 m
3	72.6	4.25 dd (10.8, 5.6)
4	30.0	–
5	143.2	–
6	141.7	–
7	182.8	–
8	106.9	–
9	139.3	–
10	41.3	–
11	130.6	–
12	153.3	–
13	110.6	–
14	154.5	–
15	28.7	3.32 dd (15.5, 9.6)
		3.05 dd (15.5, 7.2)
16	86.4	5.12 m
17	64.7	3.92 dd (12.2, 3.2)
		3.82 dd (12.2, 3.1)
18	17.8	1.41 s
19	24.4	1.55 s
20	28.2	1.63 s
6-OH		7.02 s
14-OH		12.50 s

$J = 12.2$, 3.1 Hz, H-17b); δ_C 28.7, 86.4, 64.7] instead of the 2,3-dihydro-2-methylbenzofuran moiety [δ_H 3.40 (1H, dd, $J = 15.2$, 9.1 Hz, H-15α), 2.88 (1H, dd, $J = 15.2$, 7.3 Hz, H-15β), 5.14 (1H, dd q, $J = 15.2$, 9.1, 7.3 Hz, H-16), 1.53 (3H, d, $J = 6.6$ Hz, H-17); δ_C 34.3, 83.5, 22.0] in teuvincenone B.

In the HMBC spectrum, the ^{1}H, ^{13}C long-range correlations from H-1 to C-2 at δ 26.7, C-3 at δ 72.6, and C-10 at δ 41.3, H-20 to C-1 at δ 28.9, C-5 at δ 143.2, and C-9 at δ 139.3, H-15 to C-13 at δ 110.6, C-16 at δ 86.4, and C-17 at δ 64.7, H-16 to C-13 at δ 110.6, C-15 at δ 28.7, and C-17 at δ 64.7 suggested the abietane diterpenoid framework with a CH_2OH group on C-16 position. Furthermore, other key correlations of H-18 at δ 1.41 and H-19 at δ 1.55 to C-3 at δ 72.6, C-4 at δ 30.0, and C-5 at δ 143.2 further proved the skeleton of compound **1**.

The absolute configuration of C-16 was not ascertained, however, on biogenetic grounds, it is reasonable to assume that it possesses the same absolute stereochemistry as the known abietane diterpenoids [12] that were isolated from the same plant materials. Furthermore, the configuration at position 3 was determined according to Mosher's method [13]; the (*S*)- and (*R*)-MTPA esters of **1** were prepared using the corresponding (*S*)-(+) and (*R*)-(−)-MTPA chloride, respectively. The Δ_δ values ($\delta_S - \delta_R$) of H-1α, H-1β, H-2α, and H-2β (left unit) were −0.02, −0.03, −0.02, and −0.15 ppm, and those of H-18 and H-19 (right unit) were +0.13 and +0.04 ppm, respectively, implying that the absolute configuration of C-3 was *S*. Therefore, the structure of **1** was elucidated as (3*S*,16*R*)-12,16-epoxy-3,6,11,14,17-pentahydroxy-17(15 → 16)-*abeo*-5,8,11,13-abietatetraen-7-one.

The cytotoxic activities of compound **1** against HL-60 ($IC_{50} = 8.7$ μM) and A-549 ($IC_{50} = 13.7$ μM) human cancer cell lines were measured by MTT method using cisplatin, against HL-60 ($IC_{50} = 4.8$ μM) and A-549 ($IC_{50} = 8.6$ μM), as the positive control. The results suggested that compound **1** could inhibit effectively the proliferation of human HL-60 and A-549 cell lines.

3. Experimental

3.1 General experimental procedures

Melting points were determined on an X-4 apparatus and are uncorrected. Optical rotations were measured with a Perkin-Elmer 241 automatic polarimeter. UV spectra were obtained with a Shimadzu UV-2550 spectrometer. IR spectra were obtained with a Nicolet 380 FT-IR spectrophotometer using KBr pellets. NMR spectroscopic data were recorded on Brüker AVANCE III 500 spectrometers with tetramethylsilane as the internal

standard. Chemical shifts are given in ppm; *J* values are given in Hz. High resolution electrospray ionization mass spectrometric (HR-ESI-MS) analyses were performed with an Agilent 6210 TOF-MS spectrometer. A Prep JAIGEL-ODS-BP column (30 × 250 mm, 10 μm; Japan Analytical Industry Co., Ltd., Tokyo, Japan) was used for recycling preparative HPLC. Column chromatography (CC) was carried out on silica gel (200–300 mesh; Qingdao Marine Chemical Company, Qingdao, China) and Sephadex LH-20 (Amersham Pharmacia Biotech, Chalfont, UK). Analytical thin-layer chromatography (TLC) was performed on Merck RP-C_{18} (Merck, Darmstadt, Germany).

3.2 Plant material

The stems of *C. kaichianum* were collected on the mountains of Lin'an County, Zhejiang Province, China, in September of 2009, and identified by Dr Chunhui Dai (Zhejiang Academy of Traditional Chinese Medicine, Hangzhou, China). A voucher specimen (No. 20090913) has been deposited in the lab of Zhejiang Gongshang University, Hangzhou, China.

3.3 Extraction and isolation

The air-dried powder of the stems of *C. kaichianum* (11.6 kg) was extracted three times with 75% EtOH at 75°C (3× 30 L). The EtOH extract was combined and evaporated to dryness to afford a gummy residue (325 g), which was suspended in H_2O and extracted with petroleum ether (60–90°C), EtOAc, and *n*-BuOH, successively. Part of EtOAc extract (90 g) was subjected to a CC over silica gel (2 kg, 100–200 mesh), eluting with petroleum ether–EtOAc (10:1, 8:2, 6:4, 1:1, 1:2, 2:8, and 1:10) to afford 10 fractions (1–10) on the basis of TLC analysis.

The major fraction 4 (9 g) was further subjected to CC eluted with petroleum ether–acetone (5:1–1:1) and separated into six fractions (4A–4F). Fraction 4B (2.8 g) was subjected to silica gel column eluted with petroleum ether–acetone (5:1 and 3:1) to give three major fractions (B1–B3). Fraction B2 (0.8 g) was further purified by Sephadex LH-20 (MeOH), and by preparative HPLC (MeOH–H_2O, 60:40) to give compounds **1** (7 mg), **2** (30 mg), and **3** (15 mg). Fraction 4F (1.1 g) was subjected to CC eluted with petroleum ether–EtOAc (from 3:1 to 1:1) to yield compounds **4** (60 mg) and **5** (10 mg).

3.3.1 *(3S,16R)-12,16-Epoxy-3,6,11,14,17-pentahydroxy-17(15 → 16)-abeo-5,8,11,13-abietatetraen-7-one*

Yellowish needles; mp 280–282°C; $[\alpha]_D^{25}$ +31 ($c = 0.1$, MeOH); UV λ_{max} (nm): 265, 290, 338, 381; IR (KBr) ν_{max}: 3417, 3402, 1660, 1621, 1585, 1458, 1378, 1315, 1222, 1207 cm^{-1}; ^1H and ^{13}C NMR ($CDCl_3$) spectral data, see Table 1; HR-ESI-MS: *m/z* 375.1449 $[M - H]^-$ (calcd for $C_{20}H_{23}O_7$, 375.1442).

3.3.2 *Antiproliferative activity*

The percentage of growth inhibition was determined using a MTT assay to measure viable cells with minor modification [14]. A total of 5000–10,000 exponential phase cells per well were seeded onto a 96-well plate for 24 h, treated with compound **1** at different concentrations for 72 h using cisplatin as a positive control. Briefly, 100 μl of a MTT working solution (1 mg/ml) were added into each well and incubated at 37°C for 4 h and then the medium was removed. The converted dye formazan was solubilized with 150 μl acidic isopropanol (0.04 M HCl in absolute isopropanol), and each concentration was tested in triplicate. The absorbance was then measured at a wavelength of 570 nm using a microplate reader. The dose resulting in 50% inhibition of cell growth (IC_{50}) was calculated by the NDST software.

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