







Diterpenoids from the Roots of *Clerodendrum bungei*Dong-Xiang Wu ¹, Jin-Hai Yu ¹, Xiu-Qing Song ¹, Yu-Zhen Ma ²,
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Abstract: Two new rearranged abietane diterpenoids (**1** and **2**), together with eleven previously described analogues (**3–13**), were obtained from the ethanolic extract of a traditional ethnological herb, *Clerodendrum bungei*. The structures with absolute configurations of the new compounds were unambiguously characterized via spectroscopic methods, and that of the formerly reported clerodendrum B (**3**) was corrected in the present work. Biological assessment of these isolates revealed that diterpenoids **2**, **4**, **6** and **12** showed significant inhibition against α -glucosidase enzyme with IC₅₀ values in the range of 17.0–25.7 μ M.

Keywords: *Clerodendrum bungei*; abietane diterpenoid; α -glucosidase. © 2021 ACG Publications. All rights reserved.

1. Introduction

Clerodendrum bungei Steud., a medicinal species from the biggest genus *Clerodendrum* of family Verbenaceae, is a shrub of one to two meters tall with unpleasant odor [1], and it is widely distributed in most areas of China except in the northeast. In addition to serving as an ornamental plant due to its beautiful flowers, roots, stems and leaves of *C. bungei* have also been used as herbal medicine in China to relieve swelling, alleviate pain, remove external heat, and so on. Particularly, the roots of *C. bungei* are a well-known ethnodrug called “Binliang” of the “Dai” people living in Yunnan Province [2]. Besides the rich volatile oil constituents [3] revealed by GC-MS technique, previous phytochemical studies on *C. bungei* also showed the presence of triterpenoids [4] and steroids [5]. Moreover, the most attracting bioactive ingredients from this herb are abietane diterpenoids [6–8] that have exerted interesting anti-complement effect [9], as well as angiotensin converting enzyme (ACE) [10] and α -glucosidase [11] inhibitory activities.

As part of our efforts to explore new bioactive small molecules from “Dai” medicinal plants, the chemical constituents in the roots of *C. bungei* have been systematically investigated in the present work. As a result, two new rearranged abietane diterpenoids (**1** and **2**), as well as 11 known ones (Figure 1), were isolated and characterized. Our bioassay revealed remarkable α -glucosidase

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inhibitory activity for selective diterpenoids. This paper deals with the separation, structure determination and biological evaluation of these compounds.

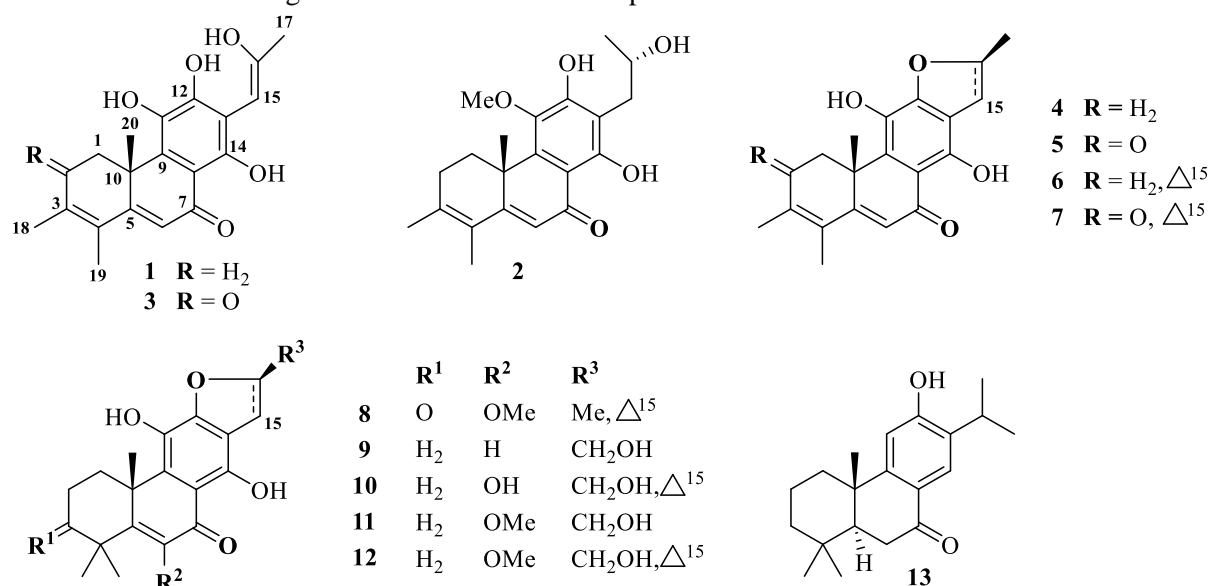


Figure 1. Structures of diterpenoids from *C. bungei*

2. Materials and Methods

2.1. General

Optical rotations were measured on a Rudolph VI polarimeter (Rudolph Research Analytical, Hackettstown, USA) with a 10 cm length cell. ECD spectra were recorded on a Chirascan photospectrometer (Applied Photophysics Ltd., Leatherhead, UK) with a 0.1 cm pathway cell. NMR experiments were carried out on a Bruker Avance DRX600 spectrometer (Bruker BioSpin AG, Fallanden, Switzerland) and referenced to residual solvent peaks (CDCl₃: δ_{H} 7.26, δ_{C} 77.16; CD₃OD: δ_{H} 3.31, δ_{C} 49.00). HR-ESIMS spectra were recorded on an Agilent 6545 Q-TOF mass spectrometer (Agilent Technologies Inc., Waldbronn, Germany). ESIMS analyses were conducted on an Agilent 6460 Triple Quad LC-MS instrument (Agilent Technologies Inc., Waldbronn, Germany). All HPLC separations were performed using an Agilent 1260 series LC instrument (Agilent Technologies Inc., Waldbronn, Germany) coupled with an YMC ODS-pack column (10 × 250 mm, Agilent Technologies Inc., Santa Clara, USA). Column chromatography (CC) was carried out on reversed phase C₁₈ (RP-18) silica gel (Merck KGaA, Darmstadt, Germany), Sephadex LH-20 (GE Healthcare Bio-Sciences AB, Uppsala, Sweden) and silica gel (300–400 mesh; Qingdao Marine Chemical Co. Ltd., Qingdao, China). All solvents used for CC were of analytical grade (Tianjin Fuyu Fine Chemical Co. Ltd., Tianjin, China) and solvents used for HPLC were of HPLC grade (Oceanpak Alexative Chemical Ltd., Goteborg, Sweden). Pre-coated silica gel GF₂₅₄ plates (Qingdao Marine Chemical Co. Ltd., Qingdao, China) were used for thin layer chromatography (TLC) analyses. All solvent mixtures used for HPLC and CC analyses and separations were presented as the ratio of volume to volume, unless otherwise specified.

2.2. Plant Material

The roots of *Clerodendrum bungei* Steud. were collected in October 2018 in Jinghong, Yunnan province, China, and were authenticated by Prof. Guo-hua Ye from Shandong College of Traditional Chinese Medicine. A voucher specimen has been deposited at School of Biological Science and Technology, University of Jinan (Accession number: npmc-039).

2.3. Extraction and Isolation

The air-dried powdered roots (30 kg) of *C. bungei* were extracted with 95% EtOH at room temperature for three times (once for a week), and the extracting solvent was removed under reduced pressure to return a dark residue (1.35 kg). The residue was re-suspended in 1.5 L water and partitioned successively with EtOAc (1.5 L \times 3) and *n*-BuOH (2.0 L \times 3) to afford the EtOAc and *n*-BuOH soluble extracts, respectively. The EtOAc soluble part (110 g) was chromatographed over MCI absorption resin, eluted with MeOH-H₂O (30%, 50%, 80% and 95%), to give four fractions (A, B, C and D). Fraction D (30 g) was subjected to a silica gel column using petroleum ether (PE)-EtOAc (20:1 to 5:1) as eluent, to acquire 11 subfractions (D1–D11). The subfraction D3 (1.2 g) was applied to Sephadex LH-20 CC (CH₂Cl₂-MeOH, 1:1) to afford three fractions (D3-1–D3-3). Fraction D3-1 (350 mg) was purified by semi-preparative HPLC (3.0 mL/min, 85% MeOH-H₂O) to yield compounds **4** (8.7 mg, t_R = 11.6 min) and **1** (2.8 mg, t_R = 12.5 min). Fraction D3-3 (50 mg) was separated by silica gel CC (CH₂Cl₂-MeOH, 40:1) and then purified by HPLC (3.0 mL/min, 85% MeOH-H₂O) to yield compounds **9** (0.8 mg, t_R = 13.3 min) and **13** (1.3 mg, t_R = 16.5 min). Fractions D10 and D11 were combined and fractionated by Sephadex LH-20 CC (CH₂Cl₂-MeOH, 1:1) and HPLC (3.0 mL/min, 85% MeOH-H₂O) to afford compounds **7** (1.3 mg, t_R = 5.7 min), **8** (0.7 mg, t_R = 11.2 min) and **5** (6.4 mg, t_R = 11.8 min). The mother solution of D4 (2.6 g), a portion crystallized in methanol, was separated by Sephadex LH-20 CC (CH₂Cl₂-MeOH, 1:1) to produce two subfractions (D4-1 and D4-2). The fraction D4-1 was separated on RP-18 silica gel CC, eluted with MeOH-H₂O (70% to 100%), to obtain two subfractions, and the first one was subjected to silica gel CC (PE-EtOAc, 1:1) and then purified by HPLC (3.0 mL/min, 80% MeOH-H₂O) to yield compound **2** (10.3 mg, t_R = 10.1 min). The fraction D4-2 was separated by HPLC (3.0 mL/min, 87% MeOH-H₂O) to yield compounds **10** (1.7 mg, t_R = 5.1 min), **12** (1.1 mg, t_R = 11.0 min) and **11** (11.8 mg, t_R = 11.7 min). The fraction C (23 g) was chromatographed using the same procedure as fraction D to produce 11 subfractions (C1–C11). The fifth fraction C5 (1.2 g) was first fractionated by silica gel CC (PE-EtOAc, 20:1 to 1:1) and then purified by HPLC (3.0 mL/min, 80% MeOH-H₂O) to yield compound **3** (4.1 mg, t_R = 13.0 min). The fraction C8 was fractionated by RP-18 CC (MeOH-H₂O, 60% to 100%) to generate two subfractions, and the second one was purified by HPLC (3.0 mL/min, 75% MeOH-H₂O) to yield compound **6** (3.5 mg, t_R = 10.3 min).

(–)-*Croderodendrum A* (**1**): Orange powder; $[\alpha]_D^{25}$ –30.1 (*c* 0.56, MeOH); UV λ (log ϵ) 227 (3.98), 289 (3.68), 332 (3.53) nm; ECD (*c* 0.02, MeOH) λ ($\Delta\epsilon$) 347 (–3.1), 318 (+4.9), 251 (+8.0), 228 (–8.5), 207 (–13.0); ¹H and ¹³C NMR see Table 1; (+)-HR-ESIMS m/z 325.1431 [$M + H - H_2O$]⁺ (calcd for C₂₀H₂₁O₄, 325.1434).

(–)-*Croderodendrum C* (**2**): Yellow powder; $[\alpha]_D^{25}$ –102.5 (*c* 0.13, MeOH); UV (MeOH) λ (log ϵ) 283 (3.95), 327 (3.84) nm; ECD (*c* 0.02, MeOH) λ ($\Delta\epsilon$) 346 (–7.5), 317 (+12.2), 251 (+7.2), 226 (–12.8), 210 (–12.3); ¹H and ¹³C NMR see Table 1; HR-ESIMS m/z 359.1850 [$M + H$]⁺, (calcd for C₂₁H₂₇O₅, 359.1853).

2.4. Preparation of (*S*) and (*R*)-MTPA Esters of compound **2**

To a solution of **2** (1.3 mg) in pyridine (500 μ L) was added (*R*)-(–)- α -methoxy- α -(trifluoromethyl)phenylacetyl chloride (10.0 μ L) and dimethylaminopyridine (DMPA, 2.0 mg). The solution was allowed to stand at room temperature for 6 h. The acquired residue after evaporation of the solvent was applied to semi-preparative HPLC to afford the (*S*)-MTPA ester **2a** (0.4 mg). The (*R*)-MTPA ester **2b** was obtained from (*S*)-(+)- α -methoxy- α -(trifluoromethyl)phenylacetyl chloride using the same protocol.

2.5. ECD Calculations

The theoretical ECD spectra of compound **1** and its enantiomer were acquired by Time-dependent Density Functional Theory (TD-DFT) method as we described formerly [12].

2.6. α -Glucosidase Inhibitory Assay

The α -glucosidase inhibitory activity of all the compounds were evaluated as depicted in a previous report [12]. A single concentration of 100 μ M was chosen for the primary screening, and only compounds showing inhibition ratio above 50% were further submitted for IC₅₀ measurements. Acarbose was used as the positive control (IC₅₀ = 236.0 \pm 14.0 μ M).

3. Results and Discussion

Compound **1** was assigned the molecular formula of C₂₀H₂₂O₅ based on the HR-ESIMS ion peak at m/z 325.1431 (calcd for [M+H–H₂O]⁺, 325.1434). The ¹H NMR data (Table 1) for **1** displayed resonances for a remarkably downfield shifted hydroxy proton (δ_H 14.14), two olefinic protons (δ_H 6.60 and 6.28), two methylene protons (δ_H 3.31, 2.55, 2.24 and 1.64) and four methyl groups (δ_H 2.46, 1.93, 1.90 and 1.54).

Table 1. ¹H and ¹³C NMR data of **1** and **2** (δ in ppm, J in Hz)

No.	1 ^a		2 ^a		2 ^b	
	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
1	29.9	3.31, ddd (13.4, 5.8, 1.4) 1.64, ddd (13.4, 11.9, 5.9)	29.3	3.23, ddd (13.3, 5.7, 1.3) 1.54, ddd (13.3, 11.9, 5.9)	30.4	3.43 ddd (13.3, 5.7, 1.4) 1.48, m
2	30.5	2.55, m 2.24, dd (19.1, 5.8)	30.4	2.52, m 2.22, dd (19.3, 5.7)	31.2	2.55, m 2.24, dd (19.2, 5.7)
3	141.0		141.6		143.1	
4	125.5		125.5		126.3	
5	166.1		166.9		168.8	
6	118.7	6.28, br s	118.6	6.27, s	119.0	6.22, s
7	191.5		190.7		192.1	
8	109.6		111.7		112.1	
9	129.8		134.0		136.1	
10	39.7		39.5		40.9	
11	131.4		139.2		141.6	
12	151.7		151.7		154.9	
13	117.2		118.3		119.6	
14	148.7		155.6		156.4	
15	101.7	6.60, q (1.2)	33.4	2.91, dd (13.9, 8.2) 2.85, dd (13.9, 3.6)	34.2	2.89, dd (12.9, 5.7) 2.78, dd (12.9, 6.6)
16	154.9		68.3	4.17, m	68.1	4.14, m
17	14.1	2.46, d (1.2)	23.9	1.29, d (6.2)	23.2	1.15, d (6.2)
18	15.2	1.93, br s	15.1	1.91, br s	15.1	1.91, br s
19	20.9	1.90, br s	20.9	1.89, br s	20.7	1.94, br s
20	22.7	1.54, s	22.2	1.49, s	22.3	1.52, s
11-OMe			61.8	3.84, s	61.3	3.80, s
14-OH		14.14, s		13.92, s		

^a Measured in CDCl₃; ^b Measured in CD₃OD.

The ¹³C NMR data (Table 1) of **1** showed 20 signals corresponding to the aforementioned proton-bearing carbons, as well as 12 quaternary carbons including a conjugated carbonyl (δ_C 191.5), a sp³ one (δ_C 39.7) and 10 aromatic/olefinic carbons (δ_C 166.1–109.6). These NMR data were highly consistent with those of crolerodendrum A, isolated from the roots of *Clerodendrum philipinum* [13] which was further confirmed by analysis of 2D NMR data (Figures S3–S6, Supporting information) that compound **1** had the same planar structure and relative configuration as crolerodendrum A. Interestingly, the specific optical rotation of **1** showed opposite sign to that of crolerodendrum A, indicating an antipodal enantiomer of the latter. Compound **1** was thus identified as (–)-crolerodendrum A, and its absolute configuration was determined to be *S* by comparing its experimental ECD curve with the calculated one (Figure 2A). Thus, the absolute configuration of the formerly reported crolerodendrum A was determined for the first time to be *R*.

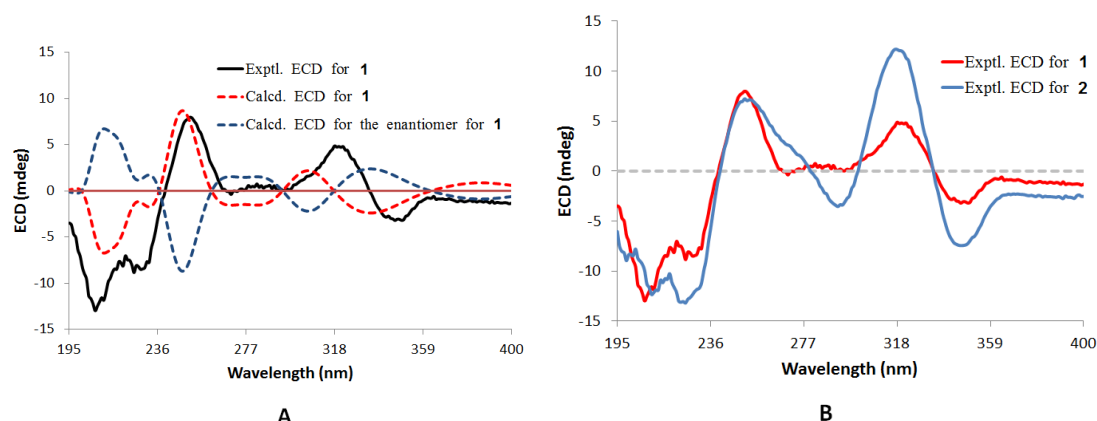


Figure 2. Experimental and calculated ECD spectra for **1** and **2**

Compound **2** was assigned the molecular formula of $C_{21}H_{26}O_5$ by HR-ESIMS analysis at m/z 359.1850 (calcd for $[M+H]^+$, 359.1853). Analysis of the NMR data (Table 1) for **2** revealed identical structural features with those of 11-methoxyl-12,14-dihydroxy-13-(2-hydroxypropyl)-3,5,8,11,13-abietapentaen-7-one isolated from *Clerodendrum kaichianum*, the latter being reported with only planar structure [14]. This assignment was further corroborated by detailed examination of 2D NMR data (Figures S11–S14, Supporting information). Interestingly, the specific optical rotation of **2** showed opposite sign to that of the above-mentioned known analogue, indicating an antipodal analogue (enantiomer or diastereoisomer). The absolute configuration at C-16 of **2** was assigned to be *S* by Mosher's method (Fig. 3), while its C-10 absolute configuration was also established to be *S* on the basis of its consistent ECD curve with that of **1** (Fig. 2B). The structure of **2** was thus unequivocally elucidated.

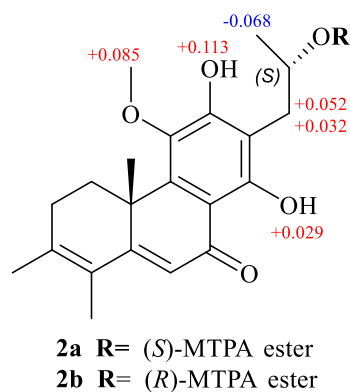


Figure 3. $\Delta\delta$ ($\delta_S - \delta_R$) values in ppm for MTPA esters of **2**

Compound **3** was identified to be clerodendrum B, originally reported from *Clerodendrum inerme* [15], and its Δ^{15} double bond was initially assigned an *E*-geometry. However, based on the NOESY correlation of H-15 with H₃-17 (Figure S26, Supporting information), Δ^{15} in **3** should be re-assigned to be *Z*-configured. Moreover, the ^{13}C NMR data for both (*E*)- Δ^{15} and (*Z*)- Δ^{15} isomers of compound **3** were theoretically calculated, and the (*Z*)-isomer showed a much better linear correlation coefficient ($R^2 = 0.9949$) than that ($R^2 = 0.9891$) of the (*E*)-isomer (Figure S27, Supporting information), which further corroborated the (15*Z*)-configuration of **3**.

The remaining known diterpenoid analogues were identified as uncinatone (**4**) [16], teuvincenone E (**5**) [17], 12,16-epoxy-17(15→16),18(4→3)-diabeo-abieta-3,5,8,12,15-pentaene-7,11,14-trione (**6**) [18], teuvincenone F (**7**) [19], 12,16-epoxy-11,14-dihydroxy-6-methoxy-17(15→16)-abeo-abieta-5,8,11,13,15-pentaene-3,7-dione (**8**) [20], 17-hydroxymandarone B (**9**) [21], 12,16-epoxy-6,11,14,17-

tetrahydroxy-17(15→16)-abeo-5,8,11,13,15-abietapentaen-7-one (**10**) [22], 6-methoxyvillosin C (**11**) [23], 15-dehydro-17-hydroxycyrtophyllone A (**12**) [19], sugiol (**13**) [24], on the basis of spectroscopic analyses and comparison with those reported in the literature.

All the isolates were evaluated for their inhibitory effect against α -glucosidase in an *in vitro* bioassay model. As shown in Table 2, compounds **2**, **4**, **6** and **12** displayed much stronger inhibitory activity than the control drug acarbose, with IC_{50} values in the range of 17.0–25.7 μ M.

Table S3. α -Glucosidase inhibitory activity of diterpenoids **1–13** (IC_{50} in μ M)

Compounds	IC_{50}	Compounds	IC_{50}
1	NT	8	NT
2	17.0 \pm 2.3	9	NT
3	NT	10	NT
4	11.9 \pm 3.6	11	NT
5	NT	12	19.0 \pm 1.6
6	25.7 \pm 1.8	13	NT
7	NT	Acarbose	236.0 \pm 14.0

NT: IC_{50} not tested

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Supporting Information

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