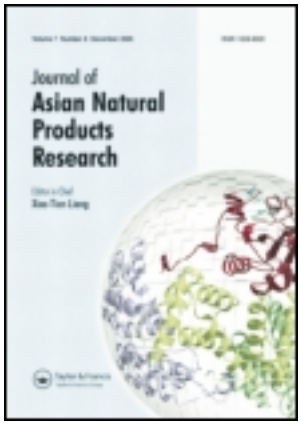

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Constituents of Clerodendrum bungei

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CONSTITUENTS OF *CLERODENDRUM BUNGEI*

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Two new compounds, 5-*O*-ethylcleroindicin D (**1**) and bungein A (**2**), together with 12 known compounds (**3**–**14**), were isolated from the aerial parts of the medicinal plant *Clerodendrum bungei*. The structures of **1** and **2** were elucidated as a perhydrobenzofuran derivative and a peroxide dimer by spectral and chemical evidence. Compounds **3**–**14** have been obtained from this species for the first time.

Keywords: Verbenaceae; *Clerodendrum bungei*; 5-*O*-Ethylcleroindicin D; Bungein A

INTRODUCTION

In a previous paper [1], we reported the isolation and structural elucidation of several perhydrobenzofuran derivatives from *Clerodendrum indicum* (Verbenaceae). Another species of the same genus, *Clerodendrum bungei* Steud., which is distributed widely in China, has long been used in folk medicine to treat headache, dizziness, furuncle, and hysteroptosis [2,3]. A few papers concerning the chemical constituents of this plant have been reported [3–5]. In the present study, a methanol extract of *C. bungei* was seperated to afford two new compounds, 5-*O*-ethylcleroindicin D (**1**) and bungein A (**2**) (Scheme 1), together with 12 known compounds, including acteoside (**3**) [6], betulinic acid (**4**) [7], cleroindicin A (**5**), cleroindicin C (**6**), cleroindicin E (**7**), cleroindicin F (**8**) [1], clerosterol (**9**) [8], clerosterol 3β-*O*-β-D-glucopyranoside (**10**) [8], martinoside (**11**) [9], octadecnoic acid (**12**), *n*-pentacosane (**13**) [10], and 5,7,4′-trihydroxyflavone (**14**) [11]. We report here the isolation and structural elucidation of these compounds.

RESULTS AND DISCUSSION

Compound **1** was obtained as a colorless oil and assigned a molecular formula of $C_{10}H_{16}O_4$ on the basis of its negative HRFABMS ($[M-1]^-$ at *m*/*z* 199.0949). Its IR spectrum

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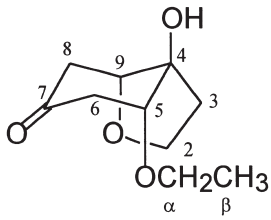
TABLE I NMR data for 5-*O*-ethylcleroindicin D (**1**)*

Position	*^{13}C*	*^{1}H*†	*COSY*	*HMBC*‡
2	66.6 t	3.96, m	3	9
3	39.2 t	2.18, m	2	5, 9
4	79.1 s			2, 6_{ax}, 6_{eq}, 8_{ax}, 8_{eq}
5	79.3 d	3.77, dd (6.7, 3.4)	6_{ax}, 6_{eq}	3, 9, α_a, α_b
6_{ax}	40.6 t	2.86, dd (16.6, 3.4)	5, 6_{eq}	8_{ax}, 8_{eq}
6_{eq}		2.77, dd (16.6, 6.7)	5, 6_{ax}	
7	207.6 s			5, 9
8_{ax}	43.0 t	2.72, dd (16.8, 4.7)	8_{eq}, 9	6_{ax}, 6_{eq}
8_{eq}		3.05, dd (16.8, 4.7)	8_{ax}, 9	
9	83.8 d	4.22, t (4.7)	8_{ax}, 8_{eq}	2, 3, 5
α_a	65.9 t	3.58, m	α_b, β	5
α_b		3.46, m	α_a, β	
β	15.7 q	1.07, t (8.2)	α_a, α_b	

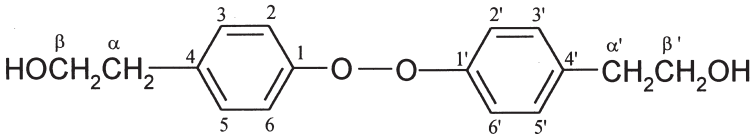
∗ In C_5D_5N, ^1H at 500 MHz, ^{13}C at 125 MHz.
† Couplings in Hz; chemical shifts of overlapped signals determined from 2D NMR data.
‡ Long-range correlation of ^1H signals to ^{13}C, in C_5D_5N, ^1H at 500 MHz, ^{13}C at 125 MHz.

displayed absorption bands for hydroxyl (3383 cm^{-1}) and carbonyl (1698 cm^{-1}) groups. A comparison of the MS and NMR spectral data of **1** with those of cleroindicin D [1] indicated that the two compounds were structurally related, except for the presence of an oxymethylene (δ 65.9, C-α) and a methyl group (δ 15.7, C-β) in **1**. Analysis of the 2-dimensional NMR spectra of **1** (Table I) clearly suggested that **1** possessed the same carbon skeleton as cleroindicin D (perhydrobenzofuran), and an ethoxyl group was assignable to the C-5 (δ 79.3, d) position based on the observation of long-range carbon–proton correlation (HMBC) between C-α and H-5. Consequently, the structure of **1** was determined as 5-*O*-ethylcleroindicin D.

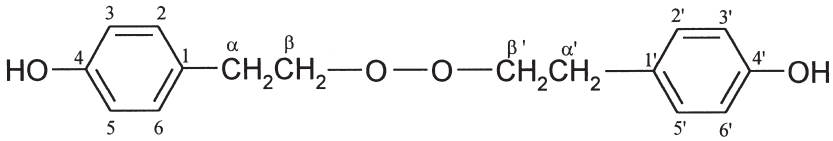
Bungein A (**2**) was obtained as an optically inactive colorless wax. Its molecular formula ($C_{16}H_{18}O_4$) was deduced from the negative HRFABMS ($[M - 1]^-$ at *m/z* 273.1218). The IR absorption bands revealed the presence of hydroxyl (3147–3391 cm^{-1}) and aromatic (1599, 1512 and 819 cm^{-1}) functional groups. The UV spectrum of **2** displayed absorption maxima at 224 (4.16), 278.5 (4.09) and 281.5 (4.37) nm, suggesting the presence of an aromatic structure. Interestingly, in its ^{13}C NMR spectrum only six carbon signals, namely, two quaternary carbons (δ 156.5 and 130.8), two methines (δ 130.7 and 115.8), and two methylenes (δ 64.2 and 39.3) were observed. Such a simplified spectrum led to the assumption that **2** has a symmetrical structure. Indeed, the ^1H NMR spectrum exhibited four groups of proton signals, i.e. aromatic protons at δ 7.03 (4H, dd, $J = 8.4$, 2.9 Hz, H-3, 5, 3′, 5′) and 6.73 (4H, dd, $J = 8.4$, 2.9 Hz, H-2, 6, 2′, 6′), oxymethylenes at δ 3.69 (4H, t, $J = 7.2$ Hz, H-β, β′), and methylenes at δ2.70 (4H, t, $J = 7.2$ Hz, H-α, α′). Analysis of the proton coupling patterns of **2** led to the conclusion that the molecule was composed of two units of 2-phenylethanol derivative. Additionally, Compound **2** produced a positive reaction with the Farbentwickler 3 reagent (Merck), whereas with the above reagent after treatment with triphenyl phosphine it showed a negative reaction. Such experimental results indicated that **2** was a peroxide [12]. This was further confirmed by a downfield shift of the aromatic carbon signals at δ 156.5 (s, C-1 and C-1′) due to the effect of peroxide bridge. From the above discussion, two possible Structures **2** and **A** (Scheme 1) could be suggested for the compound. The EIMS data {*m/z* 274 $[M]^+$ (27), 256 $[M - H_2O]^+$ (4.5), 243 $[M - CH_2OH]^+$ (45), and 225 [*m/z* 256 − CH_2OH or *m/z* 243 − $H_2O]^+$ (23)} excluded the possibility of structure A, since it would not produce the fragmentation patterns as those listed above. Hence, all available evidence suggested the structure of bungein A as depicted in **2**.



1



2



A

SCHEME 1

In addition to the two new constituents, Compounds **3**–**14** were purified from the plant extract. These constituents are reported from this species for the first time. Their structures were identified by direct comparison with reference samples, or by comparing their physical and spectral properties with those reported in the literature.

EXPERIMENTAL SECTION

General Experimental Procedures

Optical rotations were measured on a JASCO-20C digital polarimeter. UV spectra were determined on a UV 210A spectrometer and IR spectra on Bio-Rad FTS-135 spectrometer. 1D- and 2D-NMR spectra were taken on a DRX-500 instrument with TMS as internal reference. MS were recorded on a VG Auto Spec-3000 mass spectrometer.

Plant Material

The plant material was collected in Meishan County, Sichuan Province, China, in August 1997, and identified as *Clerodendrum bungei* Steud. by Prof. Ri-Zheng Fang (Kunming Institute of Botany). A voucher specimen (KIB 97-8-16 Fang) was deposited in the Herbarium of the Department of Taxonomy, Kunming Institute of Botany, Chinese Academy of Sciences.

Extraction and Isolation

The air-dried and powdered aerial parts of *C. bungei* (6.0 kg) were extracted with MeOH (3 × 20 l) under reflux to afford a crude extract (600.5 g). The extract was dissolved in 90% MeOH and defatted with petroleum-ether (60–90°C). After removal of excessive MeOH, the residue was partitioned with EtOAc (6 × 1.5 l) and *n*-BuOH (4 × 1.5 l), successively. The EtOAc extract (50.0 g) was subjected to chromatography over silica gel (1.3 kg, 200–300 mesh), eluting with $CHCl_3$–Me_2CO (1:0, 9:1. 8:2, 7:3, 6:4, 3:7, and 0:1) and MeOH, to afford nine fractions (A–I).

From fraction A, *n*-pentacosane (109 mg) was obtained by column chromatography on silica gel eluting with petroleum ether–$CHCl_3$ (7:3). Fraction C was subjected to medium-pressure column chromatography on silica gel, developing with $CHCl_3$–MeOH (90:1 and 30:1) and petroleum ether–Et_2O (5:5, 6:4, 8:2 and 2:8) to yield 5-*O*-ethylcleroindicin D (1,134 mg), cleroindicin C (356 mg), cleroindicin F (116 mg), bungein A (**2**, 12 mg), 5,7,4′-trihydroxyflavone (17 mg), betulinic acid (7 mg), and octadecnoic acid (16 mg). Fraction F was purified by silica gel column chromatography eluted with $CHCl_3$–MeOH (9:1) and petroleum ether–*i*-PrOH (8:2) to afford compounds cleroindicin E (42 mg) and clerosterol (31 mg). From fractions G and H, cleroindicin A (40 mg), clerosterol 3β-*O*-β-D-glucopyranoside (8 mg), martinoside (565 mg), and acteoside (5.4 g) were obtained following repeated chromatography on silica gel, reversed-phase silica gel (RP-18) and MCI-gel CHP-20P [eluents: $CHCl_3$–MeOH (9:1), $CHCl_3$–MeOH–H_2O (9:2:0.1) and MeOH–H_2O (3:7 and 4:6)].

*5-O-ethylcleroindicin D (**1**).* $C_{10}H_{16}O_4$, colorless oil, $[\alpha]_D^{13}$ −0.27 (c 0.93, $CHCl_3$). IR (film) ν_{max} 3383, 2975, 2907, 2869, 1698, 1411, 1352, 1286, 1189, 1127, 1110, 1060, 996 cm^{-1}; ^{1}H- and ^{13}C-NMR, see Table I. EIMS (70 eV) *m/z* 200 $[M]^+$ (26), 182 $[M - H_2O]^+$ (10), 154 $[M - EtOH]^+$ (21), 138 (18), 127 (29), 115 (95), 107 (23), 99 (45), 86 (100), 73 (37), 55 (56); negative HRFABMS *m/z* 199.0949 $[M - 1]^-$ (Calcd for $C_{10}H_{15}O_4$: 199.0970).

*Bungein A (**2**).* $C_{16}H_{18}O_4$, colorless wax, UV (MeOH) λ_{max} (log ε) 281.5 (4.37), 278.5 (4.09), 224 (4.16), 202 (4.15) nm; IR (film) ν_{max} 3391, 3147, 3024, 2955, 2928, 2881, 2702, 2679, 1884, 1599, 1512, 1453, 1364, 1233, 1054, 819, 555 cm^{-1}; ^{1}H-NMR (CD_3COCD_3, 400 MHz) δ 7.03 (4H, dd, *J* = 8.4, 2.9 Hz, H-3, 5, 3′, 5′), 6.73 (4H, dd, *J* = 8.4, 2.9 Hz, H-2, 6, 2′, 6′), 3.69 (4H, t, *J* = 7.2 Hz, H-β, β′), 2.70 (4H, t, *J* = 7.2 Hz, H-α, α′); ^{13}C-NMR (CD_3COCD_3, 100 MHz) δ 156.5 (s, C-1, 1′), 130.8 (s, C-4, 4′), 130.6 (d, C-3, 5, 3′, 5′), 115.8 (d, C-2, 6, 2′, 6′), 64.2 (t, C-β, β′), 39.3 (t, C-α, α′); EIMS (70 eV) *m/z* 274 $[M]^+$ (27), 256 $[M - H_2O]^+$ (4.5), 243 $[M - CH_2OH]^+$ (45), 225 [*m/z* 256 − CH_2OH or *m/z* 243 − $H_2O]^+$ (23), 168 (65), 153 (41), 138 (19), 121 (29), 107 (100), 97 (44), 77 (34); negative HRFABMS *m/z* 273.1218 $[M - 1]^-$ (Calcd for $C_{16}H_{17}O_4$: 273.1204).

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