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A pair of unprecedented cyclohexylethanoid enantiomers containing unusual trioxabicyclo[4.2.1]nonane ring from *Clerodendrum bungei*

Hucheng Zhu [a,†], Lijun Huan [b,†], Chunmei Chen [a], Jing Yang [c], Jiangbo He [d], Yong Chen [b], Guangmin Yao [a], Zengwei Luo [a], Yongbo Xue [a,*], Yonghui Zhang [a,*]

[a] Hubei Key Laboratory of Natural Medicinal Chemistry and Resource Evaluation, School of Pharmacy, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430030, People's Republic of China
[b] College of Life Science, Hubei University, Wuhan 430062, People's Republic of China
[c] Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai 201203, People's Republic of China
[d] State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650204, People's Republic of China

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ABSTRACT

A pair of unprecedented enantiomers (**1a/1b**) of cyclohexylethanoid bearing an unusual trioxabicyclo[4.2.1]nonane ring, along with two known structurally related cyclohexylethanoids, (+)-rengyolone (**2**) and cleroindicin E (**3**), were isolated from the aerial parts of *Clerodendrum bungei*. The structures and absolute configurations of the enantiomers were determined by comprehensive spectroscopic analysis, single-crystal X-ray diffraction, and quantum mechanical calculation of the electronic circular dichroic (ECD) spectra. The postulated biogenetic pathway of **1a/1b** was also discussed.

The genus *Clerodendrum* (family Verbenaceae), with more than 400 species, is distributed widely in tropical and subtropical regions of the world.[1] Plants belonging to this genus are well known for their pesticidal properties.[2] *Clerodendrum bungei*, a small shrub that mainly grows in the central and western regions of China, has been historically used as a folk medicine. The leaves and branches of *C. bungei*, have been used to treat ulcer, furunculosis, hemorrhoids, hypertension, and rheumatism; the roots of this plant have been applied in the treatment of dysentery, mammitis, eczema, toothache, and arthrophlogosis.[3] Previous chemical investigations of the extracts of the plant resulted in the isolation and characterization of several diterpenoids,[4,5] phenylethanoid glycosides,[6] cyclohexylethanoid derivatives, and peroxides.[7]

Our current research into the secondary metabolite of the aerial parts of *C. bungei* has led to the isolation of clerobungin A (**1a/1b**), as a pair of unprecedented enantiomers of cyclohexylethanoid bearing an unusual trioxabicyclo[4.2.1]-nonane ring. Two known structurally related cyclohexylethanoids, (+)-rengyolone (**2**)[8,9] and cleroindicin E (**3**),[10] were also characterized. To the best of our knowledge, this is the first report of natural product containing a trioxabicyclo[4.2.1]nonane scaffold. This Letter describes the isolation, structure elucidation, and the postulated biogenetic pathway of **1a/1b** (Fig. 1).

The dried aerial parts of *C. bungei* (13 kg) were extracted with 95% EtOH. The extract was partitioned with petroleum ether (PE) and then chloroform. The chloroform-soluble extract (45 g) was subjected to a silica gel column chromatography (CC) by gradient elution with PE/acetone to furnish seven fractions (Fr.1–Fr.7). Fr.3 was chromatographed over Sephadex LH-20 (CH_2Cl_2/MeOH, 1:1) and purified by silica gel CC (PE/acetone, 18:1) to give clerobungin A (**1**, 1.2 mg). Fr.4 and Fr. 6 were purified on ODS (MeOH/H_2O) and silica gel CC (PE/isopropyl alcohol and $CHCl_3$/MeOH) to afford compounds **2** (8.0 mg) and **3** (6.5 mg), respectively.

Clerobungin A (**1**)[11] was isolated as a colorless crystal. The molecular formula $C_{10}H_{14}O_4$, was deduced from HRESIMS (*m*/*z* 221.0781 $[M+Na]^+$, calcd 221.0790), which was consistent with four degrees of unsaturation. The IR spectrum indicated the presence of a free carbonyl group (ν_{max} = 1719 cm^{-1}). Apart from one unsaturation degree occupied by a carbonyl group, the remaining three degrees of unsaturation required **1** to contain a tricyclic core ring system. The ^{13}C NMR and DEPT spectroscopic data (Table 1) suggested ten carbon signals in accordance with the molecular formula obtained from HRESIMS, resolving into six methylenes (δ_C 76.6, 69.0, 42.8, 41.4, 33.9, and 31.4), two methines (δ_C 103.1 and

* Corresponding authors. Tel./fax: +86 027 83692311.
E-mail addresses: yongboxue@mail.hust.edu.cn (Y. Xue), zhangyh@mails.tjmu.edu.cn (Y. Zhang).
† These authors contributed equally to this work.

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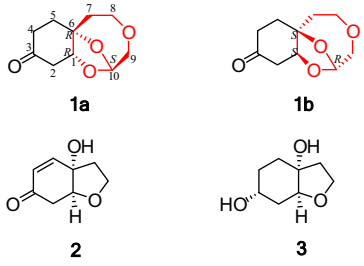


Figure 1. Structures of clerobungin A (**1a/1b**), **2** and **3**.

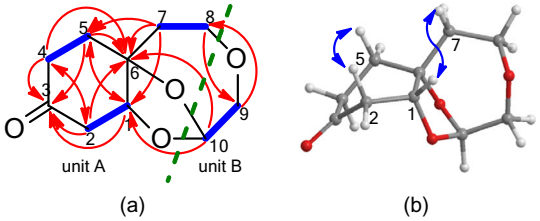


Figure 2. (a) ^{1}H–^{1}H COSY (bold) and the selected HMBC (arrow) correlations of **1**; (b) the key NOESY correlations of **1**.

81.2), and two quaternary carbons (δ_C 209.3 and 79.6). In the ^{1}H NMR spectrum (Table 1), two oxygenated methine protons at δ_H 4.77 and 5.33, and four oxymethylene protons at δ_H 4.13, 3.88, 3.77, and 3.46 were observed. Proton signals attributed to the remaining four methylenes were observed in a relatively high-field region around δ_H 1.94–2.67.

The detailed analyses of the ^{1}H–^{1}H COSY and HMBC spectra (Fig. 2) suggested the presence of two substructures (unit A: C-1–C-8; unit B: C-9–C-10). In unit A, the connectivities of C-1/C-2, C-4/C-5, and C-7/C-8 were elucidated by the cross-peaks from ^{1}H–^{1}H COSY spectrum (H-1/H_2-2, H_2-4/H_2-5, and H_2-7/H_2-8), then, the cyclohexane ring was further disclosed by the HMBC correlations as follows: correlations from H-1 to C-3 and C-5; from H_2-2 to C-3, C-4, and C-6; from H_2-4 to C-3 and C-6; and from H_2-5 to C-1, C-3, and C-6. The linkage of the side chain (–CH_2-CH_2-O–) at C-6 was revealed by HMBC cross-peaks from H_2-7 to C-1, C-5, and C-6, and H_2-8 to C-6. In unit B, the spin system of H_2-9/H-10 in the ^{1}H–^{1}H COSY spectrum indicated the direct connectivity of C-9 and C-10. The obvious down-field chemical shift of C-10 (δ_C 103.1, CH) implied the presence of an acetal carbon. The connectivities of units A and B were assembled by the HMBC correlations from H-10 (δ_H 5.33) to C-1 and C-6, and from H-9/C-9 to C-8/H-8. Thus, the planar structure of clerobungin A (**1**) was elucidated as shown.

The relative configuration of **1** was assigned by the interpretation of the NOESY spectrum (Fig. 2). The NOESY cross-peak between H-1 (δ_H 4.77) and H-7 (δ_H 2.19) suggested that the cyclohexane and dioxolane rings were *cis*-fused, and H-1 and C-7 were arbitrarily assigned as β-oriented. Cross-peak between H-5 (δ_H 2.47) and H-2 (δ_H 1.95) implied that the cyclohexane ring adopted a boat conformation. The absence of a NOESY correlation H-10/H-1 or H-10/H-7 revealed that there is no such spatial relationship, suggesting H-10 is on the opposite side of the dioxolane ring. Finally, suitable crystal for X-ray diffraction experiment was

Table 1
NMR data for clerobungin A (**1a/1b**) (400 MHz for ^{1}H, and 100 MHz for ^{13}C in $CDCl_3$)

No.	δ_H (J in Hz)	δ_C	DEPT	HMBC
1	4.77 dd (4.2, 2.4)	81.2	CH	3, 5, 7
2	2.67 dd (16.5, 2.3)	42.8	CH_2	3, 4, 6
	2.47 dd (16.5, 4.2)			1, 3
3		209.3	C	
4	2.54 ddd (18.9, 13.6, 5.0)	33.9	CH_2	3, 5, 6
	2.27 ddd (18.9, 4.4, 2.5)			3, 5, 6
5	2.13 ddd (14.7, 5.1, 2.5)	31.4	CH_2	1, 3, 4, 6
	1.95 m			1, 3, 4, 6
6		79.6	C	
7	2.19 ddd (11.5, 7.0, 3.9)	41.4	CH_2	1, 8,
	1.94 m			5, 6
8	4.13 ddd (12.4, 6.7, 1.1)	69.0	CH_2	6, 9
	3.77 ddd (12.4, 11.5, 4.9)			6, 7, 9
9	3.88 d (12.8)	76.6	CH_2	8, 10
	3.46 dd (12.8, 1.0)			8, 10
10	5.33 s	103.1	CH	1, 6, 9

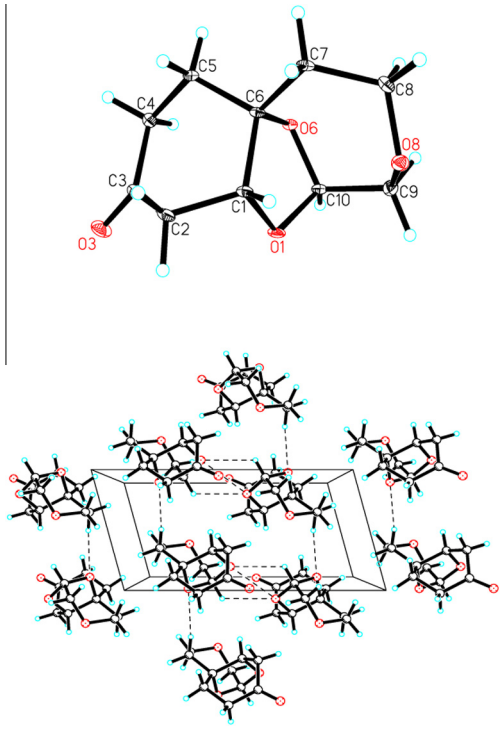


Figure 3. X-ray crystal structure of clerobungin A.

obtained from Me_2CO–MeOH (20:1) after careful recrystallization, which enabled us to determine the structure and relative stereochemistry of **1** (Fig. 3).

It is interesting that the crystals of **1** had the space group *P2*(1)/*n*, indicating a racemic nature in the crystal.[12–14] However, compound **1** was most likely a mixture of two enantiomers (**1a/1b**) in unequal amounts (see discussion in Supporting information), because clerobungin A (**1**) was found to be optically active, with the optical rotation $[\alpha]_D^{20}$ +36.2 (*c* 0.07, $CHCl_3$). This was supported by HPLC analysis of **1** over an analytical chiral column, displaying two peaks with an integration of about 1.2:1 ratio (**1a**:**1b**, Fig. 4). Although the separation of **1a** and **1b** had not yet been achieved due to the limitation in the amount of the stereoisomers, the CD spectrum of the mixture was also analyzed. Generally, enantiomers share the same NMR data but opposite optical rotation and Cotton effect (CE).[15,16] The CD spectrum exhibited an apparent positive CE at 290 nm, which should be in accordance with the major stereoisomer **1a**. This elucidation was clarified by the quantum mechanical calculation of the electronic circular dichroic (ECD) spectrum for clerobungin A (**1a**: 1*R*, 6*R*, 10*S*; **1b**: 1*S*, 6*S*, 10*R*), with the time-dependent density function theory (TD-DFT) method at the B3LYP-PCM/aug-cc-pVDZ//B3LYP/6-31G(d,p) level in CH_3CN (Fig. 5). Finally, the absolute configurations for (+)-clerobungin A and (−)-clerobungin A were assigned as 1*R*, 6*R*, 10*S* and 1*S*, 6*S*, 10*R*, respectively.

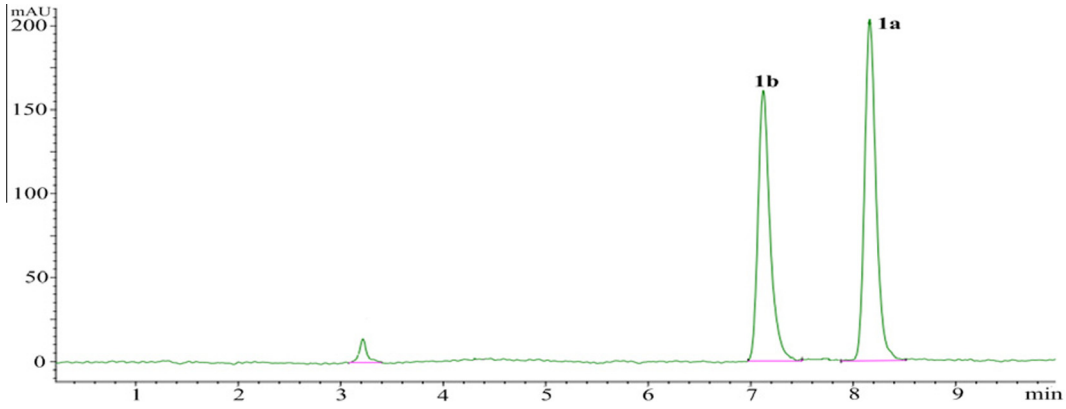


Figure 4. HPLC profile of separation of **1a**/**1b** on chiral column.

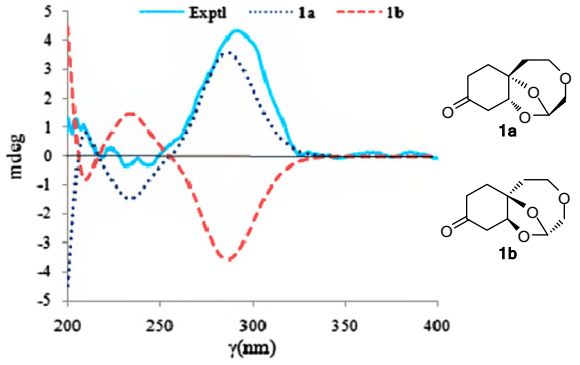
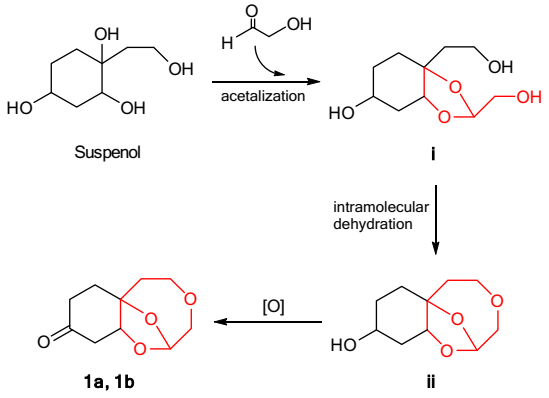


Figure 5. Experimental CD (in CH_3CN) and calculated ECD spectra of **1a** (1*R*, 6*R*, 10*S*) and **1b** (1*S*, 6*S*, 10*R*) at the B3LYP-PCM/aug-cc-pVDZ//B3LYP/6-31G(d,p) level in CH_3CN.



Scheme 1. Proposed biosynthesis of clerobungin A (**1a/1b**).

This is the first report of natural products possessing such a trioxabicyclo[4.2.1]nonane scaffold, which interested us to propose the biogenetic route for **1** (Scheme 1). Glycolaldehyde (GA, could be derived from glucose), which was reported to be the biosynthetic precursor of vitamin B_6,[17,18] was also presumed to be the precursor in the biosynthetic process leading to clerobungin A. In this way, acetalization of GA and a cyclohexylethanoid suspenol (or the hypothetical enantiomer of suspenol) followed by dehydration would give rise to the formation of the trioxabicyclo[4.2.1]nonane ring in the intermediate **ii**. Oxidation of **ii** would then yield **1a** and **1b**.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tetlet.2014.02.088.

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