SHORT REPORT

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Rec. Nat. Prod. X:X (2025) XX-XX

records of natural products

A New Clerodane-type Diterpenoid from *Conyza blinii* Levl.

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(Received February 20, 2025; Revised April 09, 2025; Accepted April 19, 2025)

Abstract: A new clerodane-type diterpene, conbliate C (**1**), along with three known analogues (**2**−**4**), were isolated from *Conyza blinii* Levl. The structure of the new compound was elucidated on the basis of detailed spectroscopic analysis. The cytotoxic effects of the isolated compounds on two human tumors cell lines (AsPC-1 and HepG-2) were evaluated by the MTT assay.

Keywords: Asteraceae; *Conyza blini*; clerodane diterpenoid; cytotoxicity.

1. Plant Source

The plant was collected from Sichuan Province, the People's Republic of China, in December 2022, and identified as *Conyza blinii* Levl. by Professor Faming Wu at Zunyi Medical University. A voucher specimen with the catalogue No.20220425 was deposited in the Herbarium of the the School of Pharmacy, Zunyi Medical Universty. This specimen has been compared with and verified against specimen NY-5096869-5096872 at The William & Lynda Steere Herbarium (NY).

2. Previous Studies

Terpenoids, flavonoids, saponins, and phenolic compounds have been isolated from *C. blinii* [1-9]. In order to further investigate the chemical constituents of the traditional Chinese medicinal herb *C. blinii* and to expand the medicinal resources of the genus *Conyza* within the Asteraceae family, a new clerodane-type diterpene, conbliate C (**1**), along with three known compounds (**2**−**4**), were isolated from the plant (Figure 1). We report on the structure elucidation of the new clerodane-type diterpene conbliate C (**1**).

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The article was published by ACG Publications
http://www.acgpubs.org/journal/records-of-natural-products Month-Month 202x EISSN:1307-6167
DOI: http://doi.org/10.25135/rnp.511.2502.3442
Available online: April 29, 2025

3. Present Study

Dried and powdered plants of *C. blinii* (9.5 kg) were extracted with MeOH refluxed. The extracts were concentrated to give a residue (1.4 kg), and then dispersed in water, extracted with petroleum ether (PE) (3×5 L), ethyl acetate (EtOAc) (3×5 L), and n-butanol (n-BuOH) (3×5 L), successively. The EtOAc extract (220 g) was subjected to silica gel column chromatography (80 mm×600 mm, 400 g, 300–400 mesh), eluted with a gradient of PE−EtOAc (*v/v* 100:0→4:1→3:2→2:3→1:4→0:100) to yield 6 fractions (Fr.1−Fr.6). Fr.3 was subjected to column chromatography over MCI gel (85 × 100 mm), eluting with MeOH–H_2O (*v/v*, 90:10) to yield Fr.3.1. Fr.3.1 was subjected to silica gel column chromatography (300–400 mesh), eluted with a gradient of PE−EtOAc (*v/v* 4:1→3:2→2:3→1:4) to yield 3 fractions (Fr.1−Fr.3). Fr.3.1.2 was purified by semipreparative HPLC (MeOH−H_2O *v/v*, 60:40, 10.0 mL/min) to give compound **1** (t_R=28.0 min, 15 mg). Fr.3.1.3 was purified by semipreparative HPLC (MeOH−H_2O *v/v*, 45:55, 10.0 mL/min) to give 2 fractions (Fr.3.1.3.1 and Fr.3.1.3.2). Fr.3.1.3.1 was purified by semipreparative HPLC (MeCN−H_2O *v/v*, 55:45, 2.5 mL/min) to yield deacetylconyzalactone (**2**) (t_R=17.2 min, 8.0 mg) [5] and conyzalactone (**3)** (t_R=18.1 min, 7.0 mg) [5]. Fr.3.1.3.2 was purified by semipreparative HPLC (MeCN−H_2O *v/v*, 41:59, 3.0 mL/min) to give blinin (**4**) (t_R=28.0 min, 19 mg) [6].

Table 1 NMR data of compounds **1** and **2** in $CDCl_3$ (δ in ppm, *J* in Hz)

No.	**1**[a]		**2**[b]	
	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C
1	2.22, dd (18.3, 4.8); 2.69, dd (18.3, 4.2)	35.1	1.35, m; 1.85, m	29.1
2		199.7	4.47, m	66.7
3	6.22, s	124.8	6.27, m	125.1
4		168.7		151.5
5		42.5		39.1
6	2.02, m; 1.44, m	30.8	1.82, m; 1.65, m	27.1
7	1.49, m	26.6	1.44, m	26.7
8	1.50, m	36.2	1.45, m	36.6
9		38.8		39.0
10	2.04, m	45.8	1.36, m	38.5
11	1.57, m	34.7	1.52, m	36.3
12	2.23, m; 2.10, m	21.8	2.31, m; 2.16, m	22.3
13		170.0		170.1
14	5.81, s	115.3	5.81, s	115.1
15		174.0		174.1
16	4.71, s	73.1	4.71, s	73.0
17	0.85, d (6.0)	15.6	0.86, d (6.0)	15.6
18	4.41, d (17.2); 4.26, d (17.2)	61.8	4.27, d (12.0); 4.23, d (12.0)	60.7
19	4.55, m; 4.23, m	66.4	4.10 d (8.2); 2.83, d (8.2)	67.6
20	0.94, s	17.9	1.02, s	16.3
21		171.0		
22	1.96, s	20.9		

[a] 400/100 MHz; [b] 600/150 MHz

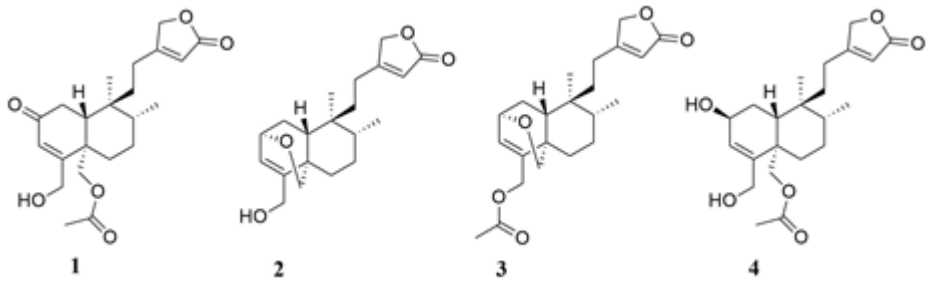

Figure 1. The chemical structures of compounds **1**−**4**

Cytotoxicity Assay: Cytotoxicity assay was carried out as previously described [10,11]. The impacts of the compounds **1**−**4** on the proliferation of AsPC-1 and HepG-2 cell lines were observed at a concentration of 20 μM. None of the compounds exhibited significant cytotoxicity (IC_{50}＞20 μM).

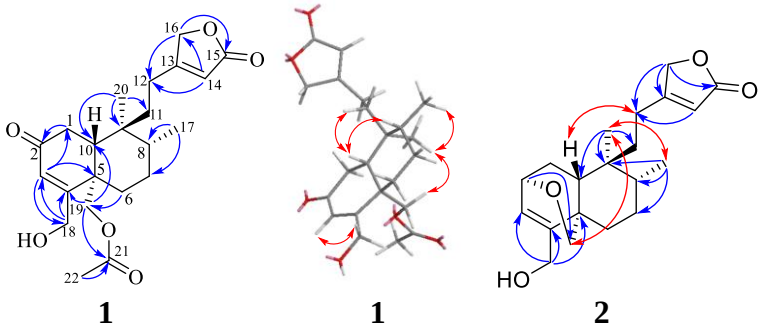

Figure 2. Key HMBC **(**) and NOESY **(**) correlations of compounds **1** and **2**

Compound **1** was obtained as a colorless gum. HR-ESIMS provided a quasi-molecular ion peak at *m/z* 389.1979 $[M–H]^-$ (calcd for $C_{22}H_{29}O_6^-$, *m/z* 389.1970), confirming its molecular formula as $C_{22}H_{30}O_6$ with eight degrees of unsaturation. The ^{1}H NMR (Table 1) and HSQC spectra revealed the presence of three methyl signals at δ_H 1.96 (3H, s), and δ_H 0.94 (3H, s), and δ_H 0.85 (3H, d, *J* = 6.0 Hz); ten methylene signals at δ_H 2.22 (1H, dd, *J* = 18.3, 4.8 Hz) and δ_H 2.69 (1H, dd, *J* = 18.3, 4.2 Hz), δ_H 2.02 and 1.44 (each 1H, m), δ_H 1.49 (2H, m), δ_H 2.10 and 2.23 (each 1H, m), and δ_H 1.57 (2H, m); six oxygenated methylene signals at δ_H 4.41 and 4.26 (each 1H, d, *J* = 17.2 Hz), δ_H 4.55 and 4.23 (each 1H, m), and δ_H 4.71 (2H, br.s); two methine signals at δ_H 2.04 (1H, m) and δ_H 1.50 (1H, m); and two olefinic signals at δ_H 6.22 (1H, s) and δ_H 5.81 (1H, s). The ^{13}C NMR data combined with HSQC spectra displayed 22 carbon signals, including three methyl carbons at δ_C 15.6, 17.9, and 20.9; five methylene carbons at δ_C 35.1, 30.8, 26.6, 34.7, and 21.8; three oxygenated methylene carbons at δ_C 61.8, 66.4, and 73.1; two methine carbons at δ_C 45.8 and 36.2; two quaternary carbons at δ_C 38.8 and 42.5; and seven sp^2 hybridized carbons at δ_C 124.8, 168.7, 115.3, 170.0, 174.0, 176.0, and 199.7. Analysis of the NMR data suggested that compound **1** is a clerodane-type diterpenoid featuring a five-membered lactone ring and an acetoxy substitution [8]. A careful comparison of the NMR data of compound **1** with that of known compound, and analyze it in conjunction with the mass spectrometry data, indicated that compound **1** is structurally similar to blinin [6], except that the hydroxyl group at C-2 is replaced by a carbonyl group. In the HMBC spectrum (Figure 2), correlations were observed between H-18 (δ_H 4.26 and 4.41) and C-3 (δ_C 124.8), C-4 (δ_C 168.7), C-5 (δ_C 42.5); H-3 (δ_H 6.22) and C-1 (δ_C 35.1), C-4 (δ_C 168.7), C-5 (δ_C 42.5), C-18 (δ_C 61.8); H-1 (δ_H 2.69 and 2.22) and C-2 (δ_C 199.7), C-5 (δ_C 42.5), C-9 (δ_C 38.8), C-10 (δ_C 45.8), confirming the presence of a hydroxyl group at C-18 and a carbonyl group at C-2, with a double bond located between C-3 and C-4, forming an α,β-unsaturated carbonyl system. In the HMBC spectrum, correlations between H-19 (δ_H 4.55 and 4.23) and C-4 (δ_C 168.7), C-6 (δ_C 30.8), C-10 (δ_C 45.8), C-21 (δ_C 171.0), as well as between the methyl hydrogen (δ_H 6.22) and C-21 (δ_C 171.0), confirmed the acetoxy group at C-19. Additionally,

correlations in the HMBC spectrum between H-16 (δ_H 4.71) and C-12 (δ_C 21.8), C-13 (δ_C 170.0), C-14 (δ_C 115.3), C-15 (δ_C 174.0), and between H-14 (δ_H 5.81) and C-12 (δ_C 21.8), C-13 (δ_C 170.0), C-15 (δ_C 174.0), C-16 (δ_C 73.1), established the furan lactone ring connected to C-12 via C-13. In the NOESY spectrum (Figure 2), correlations between H-19 and CH_3-20, and between CH_3-20 and CH_3-17, indicated that H-19, CH_3-20, and CH_3-17 are on the same face. Correlations between H-10 and H-11, and between H-10 and H-8, suggested that H-10, H-11, and H-8 are on the opposite face. To further determine the absolute configuration of compound **1**, density functional theory (DFT) calculations at the [B3LYP/6-311G(d,p), MeOH] level were performed to compute its electronic circular dichroism (ECD). The results showed that the theoretical ECD spectrum of (5*R*,8*R*,9*S*,10*R*) for compound **1** matched the experimental ECD spectrum (Figure 3). Therefore, the structure of compound **1** was elucidated and named conbliate C (**1**).

Compound **2** was obtained as a white powder. HR-ESIMS provided its quasi-molecular ion peak at *m/z* 333.2036 $[M+H]^+$ (calculated for $C_{20}H_{29}O_4^+$, *m/z* 333.2060), confirming its molecular formula as $C_{20}H_{28}O_4$ with seven degrees of unsaturation. The ^{1}H NMR (Table 1) and HSQC spectra revealed the presence of two methyl signals at δ_H 1.02 (3H, s) and δ_H 0.86 (3H, d, J = 6.0 Hz); ten methylene signals at δ_H 1.35 and 1.85 (each 1H, m), δ_H 1.82 and 1.65 (each 1H, m), δ_H 1.44 (2H, m), δ_H 1.52 (2H, m), δ_H 2.31 and 2.16 (each 1H, m); three oxygenated methylene signals at δ_H 4.27 and 4.23 (each 1H, d, J = 12.0 Hz), δ_H 4.10 and 2.83 (each 1H, d, J = 8.2 Hz), δ_H 4.71 (2H, br.s); two methine signals at δ_H 1.45 (1H, m) and δ_H 1.36 (1H, m); one oxygenated methine signal at δ_H 4.47 (1H, m); and two olefinic signals at δ_H 6.27 (1H, m) and δ_H 5.81 (1H, s). The ^{13}C NMR combined with HSQC spectra displayed 20 carbon signals, including two methyl carbons; five methylene carbons; three oxygenated methylene carbons; two methine carbons; two quaternary carbons; one oxygenated methine carbon; and five sp^2 hybridized carbons. Detailed NMR data analysis suggested that the structure of compound **2** was very similar to that of 19-deacetylconyzalactone [7], although the absolute configuration of the compound was not determined in the literature. In the HMBC spectrum (Figure 2), correlations between H-2 (δ_H 4.47) and C-19 (δ_C 67.6) confirmed that C-2 and C-19 are connected via an ether bond. The relative configuration of compound **2** was determined through NOESY spectrum (Figure 2) correlations between H-19/CH_3-20, CH_3-20/CH_3-17, H-10/H-12, and H-10/H-8. The absolute configuration of compound **2**, (2*S*,5*R*,8*S*,9*S*, 10*R*), was established by comparing the calculated ECD spectrum with the experimental ECD spectrum (Figure 3). In conclusion, compound **2** was identified as (2*S*,5*R*,8*S*,9*S*,10*R*)-19-deacetylconyzalactone.

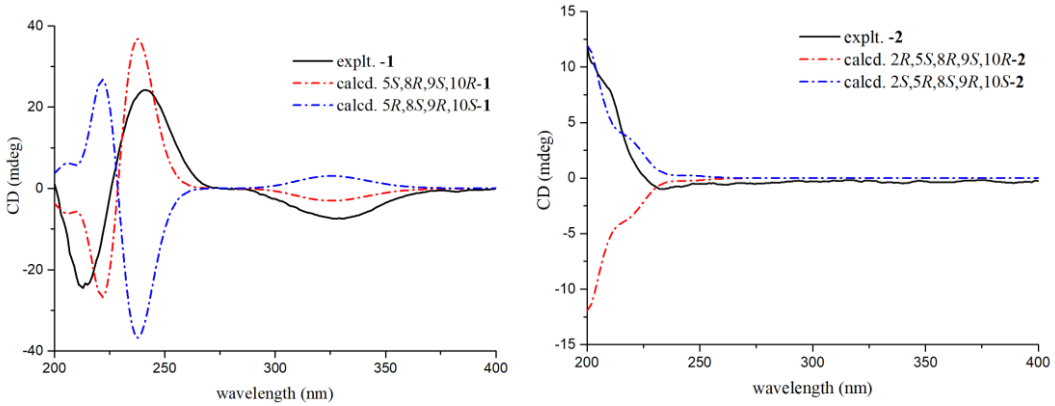

Figure 3. Experimental and calculated ECD spectra of compounds **1** and **2**

Acknowledgments

This work was financially supported by the National Natural Sciences Foundation of China (No. 82060706), the Science and Technology Department Foundation of Guizhou Province (QKHJC-ZK[2024]331).

Supporting Information

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

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