

A NEW *TRANS*-CLERODANE DITERPENE LACTONE FROM *CONYZA BLINII*

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Abstract—A new *trans*-clerodane lactone, conyzalactone, together with six known compounds, was isolated from the aerial parts of composite *Conyza blinii*. Its structure was elucidated on the basis of extensive NMR analysis and X Ray crystallographic data.

Conyza blinii Levl. (Compositae) is distributed in southwestern districts of China. Its aerial parts are used in traditional Chinese medicine for the treatment of chronic bronchitis and other inflammatory diseases.¹ Preliminary pharmacological and clinical tests showed that it possessed expectorant, antitussive, antiinflammatory and antibacterial effects.² However, chemical study on this plant has not been carried out though plenty of diterpenoids were isolated from other plants of this genus.³⁻⁸ In order to shed light on the chemical entity responsible for its medicinal actions, we conducted a detailed chemical investigation on the

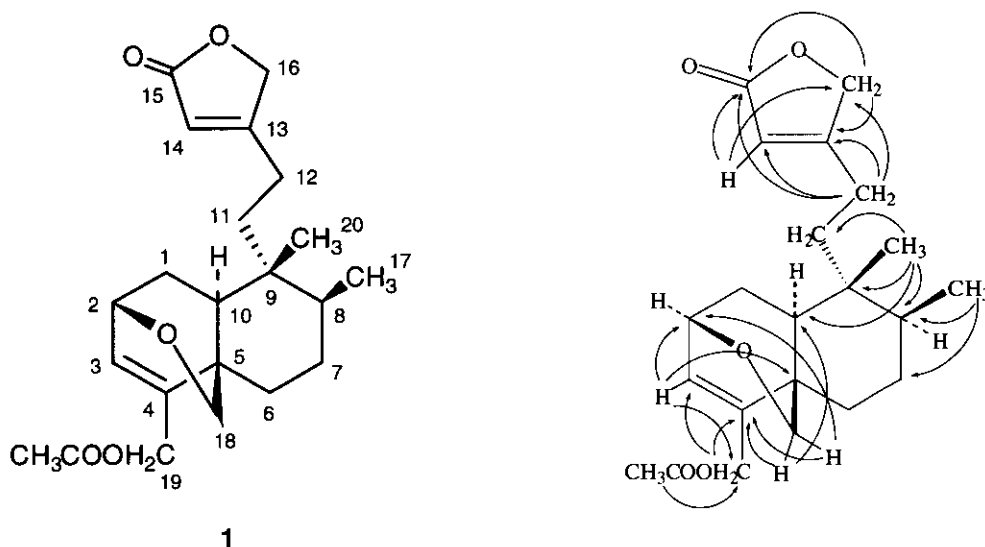


Figure 1. HMBC correlation of conyzalactone (1)

title plant. This paper deals with the isolation and structural elucidation of a new *trans*-clerodane diterpenoid lactone for which we propose the name conyzalactone, from the aerial parts of *C. blinii*.

Conyzalactone (**1**) was a colorless crystal (recrystallized from acetone) with mp 175-176 °C. A molecular formula $C_{22}H_{30}O_5$ was determined from its high resolution EI-MS (m/z 374.2092) and its ^{13}C DEPT NMR spectra. IR spectrum of **1** showed the presence of lactone and acetate functional groups (ν_{max} 1777 and 1740 cm^{-1}). Its spectral feature suggested **1** to be a clerodane type diterpenoid acetate with β -substituted butenolide ring in the side chain. The signals at δ 173.8, 170.4 and 20.9 in ^{13}C NMR spectrum accounted for the carbonyl carbons in butenolide function and acetyl group, respectively. Four signals at δ 170.5, 115.2, 146.3 and 127.9 were assigned to two double bonds. Chemical shifts at δ 72.9, 67.6, 66.5 and 61.8 were attributed to four oxygen-bearing carbon atoms. IR spectrum of **1** did not show any hydroxyl absorptions as it might, coupled with one more remaining degree of unsaturation beyond the three for the butenolide and the three for the decalin system, indicating a fourth oxygen-bridged ring may existed.

Table 1 1H and ^{13}C NMR Spectral Data for Conyzalactone in $CDCl_3$ *

	1 (1H)	1 (^{13}C)	brevifloralactone [†]
1	1.37 α , 1.86 β	28.9 t	<u>37.1</u>
2	4.47 m	66.5 d	67.0
3	6.31 d (5.0 Hz)	127.9 d	127.9
4	---	146.3 s	<u>134.8</u>
5	---	39.1 s	39.2
6	1.63 β m, 1.72 α m	27.4 t	<u>19.2</u>
7	1.46 m (2H)	26.7 t	29.3
8	1.46 m	37.7 d	36.8
9	---	39.0 s	39.1
10	1.35 br s	38.5 d	38.6
11	1.51 m (2H)	36.4 t	<u>27.0</u>
12	2.16 m, 2.31 m	22.3 t	<u>27.3</u>
13	---	170.5 s	151.9
14	5.82 br s	115.2 d	143.8
15	---	173.8 s	70.2
16	4.71 s (2H)	72.9 t	174.3
17	0.87 d (2.5 Hz)	15.6 q	15.7
18	2.87 β d (8.0 Hz), 4.11 α d (8.0 Hz)	67.6 t	67.8
19	4.69 d (2.0 Hz, 2H)	61.8 t	60.6
20	1.02 s (3H)	16.3 q	16.4
21	---	170.4 s	
22	2.10 s (3H)	20.9 q	

*The assignment were based upon DEPT, COSY, HOHAHA, HETCOR, HMBC and NOESY.

[†]Data in this column were cited from reference 11. The underlined data need re-examination.

The proton chemical shifts at δ 4.47 for a methine and 2.87, 4.11 (each d, $J = 8.0$ Hz) for a methylene group were in agreement with those protons on oxygen-bearing carbons. The above analyses were confirmed by detailed analysis of COSY, HETCOR and HMBC spectra. COSY and HETCOR furnished most proton and carbon assignments and an HMBC experiment provided a complete long-range coupling network as showed in Figure 1. The orientation of the protons and the relative stereochemistry were established from a phase-sensitive NOESY (Figure 2). The methyl group at C-9 (supposed to be at the β face) showed strong NOE interaction with H-18 β at δ 2.87 d ($J = 8.0$ Hz), suggesting the *trans*-fusion of the A and B rings. This was further confirmed by the NOEs observed between H-10 and H-8, H-10 and H-6 α (Figure 2). The structure of **1** was finally confirmed by X Ray chrystallography except for the absolute configuration (Figure 3). The depicted stereochemistry was deduced from that of the clerodane diterpenoids reported in the literature.^{9,10} However, this needs to be confirmed.

An example of a *trans*-clerodane (named brevifloralactone) with the same C-2 to C-18 ether linkage but with an α -substituted butenolide ring was isolated from *Salvia breviflora*.¹¹ Its structure was established by NMR analysis and further confirmed by X Ray analysis. However, several ^{13}C assignments need to be revised (Table 1). Like in our case the absolute stereochemistry remains to be determined. Another structural closely related compound, amphiacrolide K, was isolated from *Amphiachyris dracunculoids*.¹² It had a *cis*-A/B ring junction and this can be reflected from the chemical shift differences at positions C-10 and 18 when comparing to its counterpart in conyzalactone. The upfield shift of C-18 ($\Delta \delta$ 7.7) in **1** indicated that the ether-bridged ring in conyzalactone was above the plane of A and B ring systems, opposite to that of amphiacrolide K.

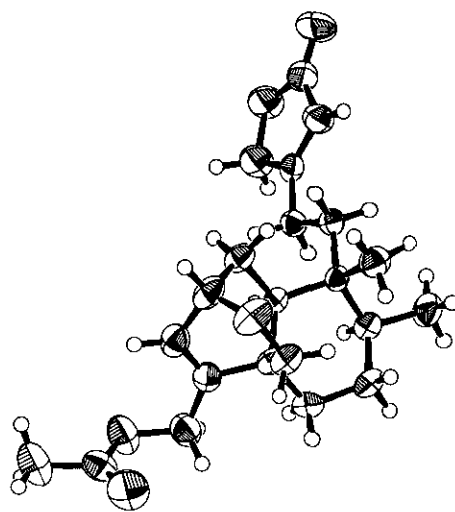
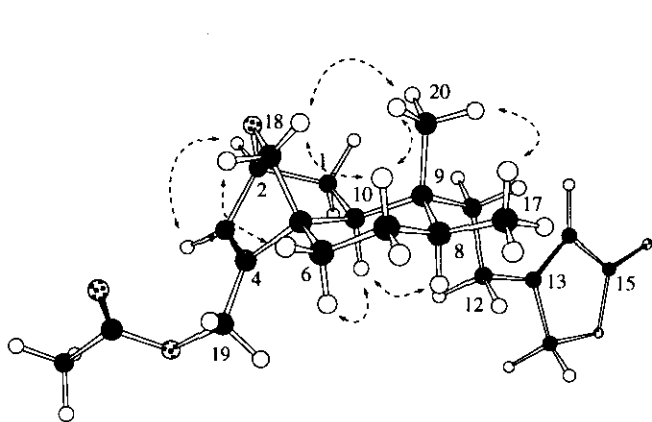


Figure 2. Key nOes relationship for conyzalactone (**1**) from a phase-sensitive NOESY. Figure 3. The X Ray structure of conyzalactone.

EXPERIMENTAL

Melting points were measured on a Yanagimoto micromelting point hot stage type apparatus and uncorrected. IR spectra were determined using a JASCO 300E FTIR spectrometer. Optical rotation were measured using a JASCO DIP-370 digital polarimeter. MS were obtained at 70 eV by direct inlet. ^1H NMR and ^{13}C NMR were recorded using a JEOL α -500 (^1H at 500 MHz, ^{13}C at 125 MHz) FT-NMR spectrometer. Chemical shifts were expressed in δ (ppm) downfield from tetramethylsilane as an internal standard, and coupling constants were reported in hertz (Hz).

Extraction and Isolation. The aerial parts of *C. blinii* were collected from Sichuan province in 1996 and the voucher specimen No. 960818 was deposited at the Herbarium of School of Pharmaceutical Sciences, Beijing Medical University. The air dried powdered *C. blinii* (23 kg) were extracted with 95% ethanol (3 times, each 50 L) at 80 °C. The alcoholic extract (1.1 kg) was macerated with water (2 L) and extracted successively with petroleum ether (3 x 2 L), EtOAc (3 x 2 L), and finally with *n*-butanol (3 x 2 L) to yield 42 g, 160 g, 305 g, respectively. A part of the EtOAc extract (50 g) was chromatographed on silica gel, elution being carried out with petroleum ether with increasing concentration of EtOAc. The effluent fractions (each 100 mL) were monitored by TLC (silica gel, petroleum-EtOAc/3:1). The combined fractions (Fr. 65-75) were rechromatographed over silica gel eluting with petroleum-EtOAc (3:1) and fractions 12-16 were combined and recrystallized in acetone to give conyzalactone (58 mg). Repeated chromatography on silica gel with the later fraction from the first column afforded additional six known compounds. The known compounds were identified as α -spinasterol (150 mg),¹³ 5,8-dihydroxy-7,3'4'-trimethoxyflavone (25 mg),¹⁴ friedelinol (67 mg),¹⁵ syringic acid (36 mg),¹⁶ caffeic acid (86 mg), and *n*-triacontanol, respectively by comparison of their IR, NMR and other physiochemical data with that from literature or by direct comparison with authentic samples.

Conyzalactone (1). mp 175-176 °C. $[\alpha]^{20}_{\text{D}} +62.0^\circ$ (*c* 0.90, CHCl_3). CD (6.95×10^{-5} M, MeOH) $[\theta]_{203} +34$ 165, $[\theta]_{389}$ 0, and $[\theta]_{442} -2$ 118. IR ν_{max} 1777 and 1740 (C=O, lactone and acetate), 1672 (C=O), 1468, 1366 and 1236 cm^{-1} . HR EIMS *m/z*: Calcd for $\text{C}_{22}\text{H}_{30}\text{O}_5$ 374.2085. Found 374.2092. EIMS (rel. int.): *m/z* 374 (M^+ , 7), 332 (34), 314 (28), 284 (100), 217 (22), 173 (30), 149 (45), 121 (38). ^1H and ^{13}C NMR data see Table 1.

X Ray analysis. Crystallized from acetone and belonging to orthorhombic space group $P2_12_12_1$ (#19). Lattice constants and intensity data were measured on a Rigaku AFC-7R diffractometer with a device for graphite-monochromated $\text{CuK}\alpha$ radiation. Crystal data: $\text{C}_{22}\text{H}_{30}\text{O}_5$, $a=11.146(2)$, $b=22.900(3)$, $c=7.765(2)$ Å, $Z=4$, $D_{\text{calc}}=1.255$ g/cm^3 , $\text{CuK}\alpha \lambda=1.54178$ Å. A total of 1440 independent reflections with $I>3\sigma(I)$ was used for structure analysis. The structure was solved by the direct method (SHELX86)¹⁷ and expanded using Fourier techniques.¹⁸ The structure was then refined by full-matrix least squares with anisotropic temperature factors for non-hydrogen and isotropic atoms for hydrogen atoms to an *R* factor of 0.033 ($R_w=0.033$) (Figure 3). The final Fourier difference synthesis showed a maximum and minimum of +0.11 and -0.13 $\text{e}/\text{\AA}^3$. Atomic coordinates, bond lengths, bond angles, thermal parameters, and structure factors have been entered into the Cambridge Crystallographic Center.

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