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BLININ, A NEOCLERODANE DITERPENE FROM *CONYZA BLINII*

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Key Word Index—*Conyza blinii*; Compositae; diterpene; neoclerodane; blinin.

Abstract—A new neoclerodane diterpenelactone, blinin, was isolated from the whole plant of *Conyza blinii*, which is used in folk medicine in the south-west of China. The structure was elucidated by chemical and spectroscopic methods and also confirmed by X-ray crystal analysis.

INTRODUCTION

As a part of our ethnobotanical and ethnopharmaceutical studies of the minorities in Yunnan Province of China, we have investigated the chemical constituents of *Conyza blinii* Lévl. This plant is endemic in the south-west of China and has long been used as a folk medicine in these areas [1]. The whole plant has been used as a sedative, antipyretic, antiinflammatory etc. (Table 1) [1], but no phytochemical investigation of *C. blinii* has yet been undertaken. In this paper, we report the isolation and structure elucidation of blinin (**1**), a new diterpene as the main component from the whole herb of this plant.

RESULTS AND DISCUSSION

The chloroform extract of the whole herb of *C. blinii* was subjected to column chromatography on silica gel to afford a new neoclerodane diterpenoid, blinin (**1**). It was recrystalized from acetone to give colourless needles which had very bitter properties. A molecular ion peak at *m/z* 392 [$M^+(C_{22}H_{32}O_6)$] in FDMS was found for compound **1**. The IR spectrum of **1** showed hydroxyl (3400 cm^{-1}), α,β-unsaturated lactone (1780, 1740 cm^{-1}) and acetyl (1740, 1240 cm^{-1}) absorptions. The presence of a fragment ion at *m/z* 111 ($C_6H_7O_2$) in EIMS of **1** was indicative of a clerodane type skeleton with, a butenolide function in the side chain (Scheme 1) [2, 3]. The ^1H NMR spectrum of **1** showed typical signals for a β-substituted γ-butenolide (a two-proton broad singlet at δ4.75 coupled to a broad singlet at 5.80) as well as signals corresponding to an olefinic proton at 5.86 (*d*, $J=1.5$ Hz) which was coupled with a proton geminal to the secondary hydroxyl group at 4.48 (*t*, $J=4.5$ Hz). In addition, the ^1H NMR spectrum of compound **1** showed signals for secondary and tertiary methyl groups of a clerodane skeleton at δ0.82 (*s*) and 0.81 (*d*, $J=5.4$ Hz), respectively, an acetyl group at 2.02, and two AB systems at 4.09, 4.18 ($J=8.0$ Hz) and 4.05, 4.52 ($J=7.8$ Hz) which can be assigned to the oxymethylene protons of the primary hydroxyl and the primary acetyl groups, respectively.

Comparison of this data with that of closely related neoclerodane lactones previously isolated from other species of Compositae [3–5] suggested the structure **1** for this new diterpenoid.

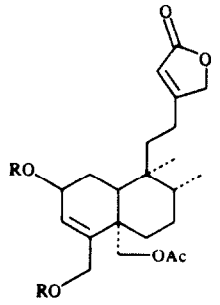
Table 1. Folk uses of *Conyza blinii* by various nationalities of the south-west of China

Nationality	Local name (locality)	Uses
Wa	Pe-Song, Xi-Bi-Sheng	sedative, antipyretic, snake bite
li-Su	Tuo-Bai-Kua (Simao)	antiinflammatory, antipyretic
Yi	Ji-Qiao-Sh (Cuxiong)	stomatitis laryngopharyngitis, bronchitis, nephritis, hepatitis
Na-Xi	Bu-Ka (Lijiang)	dysentery, antiinflammatory
Han	Jin-Hao-Zhi (Yuxi)	sedative, hemostatic, petussis, stomatitis, toothache
	Ku-Hao (Kunming)	antipyretic, acute ear, acute eye, laryngo-pharyngitis
	Xiong-Dan-Cao (Qujing)	hepatitis, tonsillitis, laryngopharygitis
	Jin-Long-Dan (Sichun)	antipyretic, antiinflammatory, tonsillitis

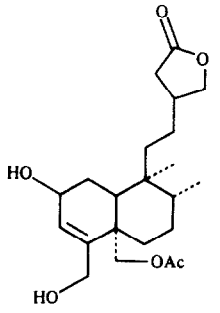
The structure and relative configuration of compound **1** was ascertained by its ^{13}C NMR, ^{1}H–^{1}H and ^{13}C–^{1}H COSY spectra which exhibited chemical shifts for the tertiary and secondary methyl groups at C-9 and C-8, respectively, in agreement with the data of compounds having both of these substitutents as alpha on a *trans*-clerodane skeleton [2, 3]. It was also further confirmed that a secondary hydroxyl group should be located at C-2 with a β-configuration, a double bond of C-3 and C-4, a primary hydroxyl at C-18 and an acetyl group must be located at C-19 [5, 6]. Furthermore, the AB system at δ4.05 and 4.52 was assigned to the protons of a C-19 methyene group which was in turn *W* coupled with an axial H-6 indicating an axial α-configuration of the C-19 methylene group that is characteristic of the *ent*-clerodane skeleton [3]. These results led us to conclude that blinin has the structure **1**.

Some derivatives of **1**, such as a diacetate **2**, a dihydrogenate **3** and an oxidate **4** were obtained by means of chemical methods. The IR spectrum of **4** showed the presence of α,β-unsaturated ketone and aldehyde groups (1675, 1695 cm^{-1}). The ^{13}C NMR spectrum of **4** showed signals of two unsaturated carbonyl carbons at δ199.8 and 193.1, respectively. The assignments of chemical shifts of the ^{1}H NMR and ^{13}C NMR spectra of **2**–**4** also supported the structure of blinin as **1** (see Tables 2 and 3).

The structure of **1** was confirmed by X-ray analysis of single crystals except for the absolute configuration. Circular dichroism showed a strong negative band at 220 nm for **1**. It is believed that **1** belongs to the neoclerodane series as hardwickiic acid and 2β-hydroxyhardwickiic acid, isolated from the same genus [6].



1 R = H
2 R = Ac



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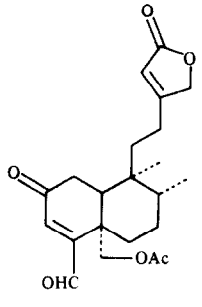

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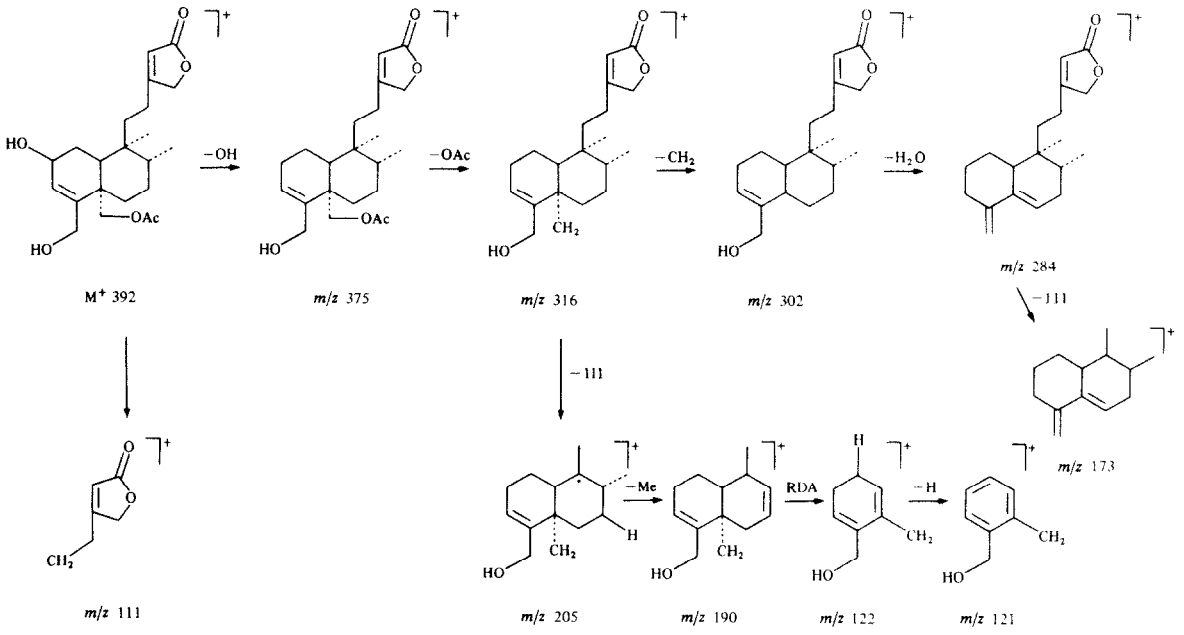
EXPERIMENTAL

Mps: uncorr. ^{1}H and ^{13}C NMR: 400 MHz $CDCl_3$ with TMS as int. standard. IR: KBr pellet.

Plant material. Whole plants of *C. blinii* were collected in Dali county, Yunnan province of China. The plant was identified by Prof. C. Y. Wu and a voucher specimen has been deposited in the Herbarium of Kunming Institute of Botany.

Extraction and isolation. The whole plants (3.5 kg) were extracted with $CHCl_3$ under reflux. The $CHCl_3$ extract (50 g) was chromatographed on a silica gel column by eluting with C_6H_6–Me_2CO to afford four fractions. Fr. 2 was recrystallized with Me_2CO to give colourless needles, blinin (**1**): mp 111–112°; $[\alpha]_D^{18}$ −81.19° (MeOH; *c* 0.505); CD nm (MeOH): 350 (0), 220 (−19.5), 209 (+2.1), 206 (−2.3), 203 (−5.1); UV λ_{max}^{MeOH} nm (log ε): 210 (3.19); IR ν_{max}^{KBr} cm^{-1}: 3400, 3250, 1780, 1740 (*br*), 1635, 1240,



Scheme 1.

Table 2. ^{1}H NMR chemical shifts of compounds **1**–**4** (400 MHz in $CDCl_3$)

H	1	2	3	4
1	1.35 (1H, *m*)	1.29 (1H, *m*)	1.37 (1H, *m*)	1.43 (1H, *t*, *J* = 10.8)
	1.95 (1H, *m*)	2.18 (1H, *m*)	1.93 (1H, *m*)	2.75 (1H, *dd*, *J* = 10.8, 3.4)
2	4.48 (1H, *t*, *J* = 4.5)	5.39 (1H, *dd*, *J* = 5.8, 1.5)	4.20 (1H, *br*)	
3	5.86 (1H, *d*, *J* = 1.5)	5.84 (1H, *d*, *J* = 1.5)	5.51 (1H, *d*, *J* = 1.5)	6.54 (1H, *s*)
6	1.67 (1H, *dd*, *J* = 6.6, 1.8)	1.65 (1H, *m*, *J* = 7.0)	1.48 (1H, *m*)	1.66–1.45 (2H, *m*)
	1.95 (1*H*, *m*)	1.97 (1H, *m*, *J* = 3.7)	1.96 (1H, *m*)	
7	1.46 (2H, *m*)	1.45 (2H, *m*)	1.45 (2H, *m*, *J* = 9.6)	1.66–1.45 (2H, *m*)
8	1.56 (1H, *m*)	1.52 (1H, *m*, *J* = 7.3)	1.15 (1H, *d*, *J* = 9.6)	1.66–1.45 (1H, *m*)
10	1.79 (1H, *dd*, *J* = 6.6, 1.8)	1.79 (1H, *dd*, *J* = 6.6, 1.8)	1.70 (1H, *m*)	1.66–1.45 (1H, *m*)
11	1.66 (2H, *m*)	1.66 (2H, *m*)	1.61 (2H, *dd*, *J* = 8.0, 1.3)	1.66–1.45 (2H, *m*)
12	2.25 (1H, *m*)	2.32 (1H, *m*)	2.45 (1H, *m*)	2.12 (1H, *m*)
	2.50 (1H, *m*)	2.12 (1H, *m*)	2.60 (1H, *dd*, *J* = 8.0, 2.6)	2.36 (1H, *m*)
13			1.25 (1H, *m*)	
14	5.80 (1H, *br*, *s*)	5.85 (1H, *d*, *J* = 1.5)	2.20 (2H, *m*)	5.85 (1H, *s*)
16	4.75 (2H, *br*, *s*)	4.73 (2H, *s*)	3.92 (2H, *dd*, *J* = 5.0, 1.3)	4.76 (2H, *s*)
17	0.82 (3H, *s*)	0.89 (3H, *s*)	0.80 (3H, *s*)	0.90 (3H, *s*)
18	4.09 (1H, *d*, *J* = 8.0)	4.12 (1H, *d*, *J* = 8.0)	4.07 (1H, *d*, *J* = 8.0)	9.78 (1H, *s*)
	4.18 (1H, *d*, *J* = 8.0)	4.49 (1H, *d*, *J* = 8.0)	4.43 (1H, *d*, *J* = 8.0)	
19	4.05 (1H, *d*, *J* = 7.8)	4.12 (1H, *d*, *J* = 7.8)	3.96 (1H, *d*, *J* = 7.8)	4.39 (1H, *d*, *J* = 7.8)
	4.52 (1H, *d*, *J* = 7.8)	4.72 (1H, *d*, *J* = 7.8)	4.42 (1H, *d*, *J* = 7.8)	4.59 (1H, *d*, *J* = 7.8)
20	0.81 (3H, *d*, *J* = 5.4)	0.85 (3H, *d*, *J* = 5.4)	0.82 (3H, *d*, *J* = 5.4)	0.89 (1H, *d*, *J* = 5.4)
OAc	2.02 (3H, *s*)	2.05 (3H, *s*)	2.02 (3H, *s*)	
		2.10 (3H, *s*)		
		2.12(3H, *s*)		

Table 3. ^{13}C NMR chemical shifts of compounds **1**, **2**, **3** and **4** (400 MHz in $CDCl_3$)

C	1	2	3	4
1	31.5	31.7	32.5	34.8
2	64.0	67.8	64.9	199.9
3	125.1	122.7	125.4	138.1
4	147.6	145.2	145.2	158.0
5	41.1	41.1	41.4	45.8
6	27.9	25.2	28.1	31.5
7	26.9	26.9	27.3	26.7
8	36.2	36.3	36.3	36.1
9	38.3	38.5	38.2	38.8
10	41.3	42.2	41.4	41.8
11	35.0	35.3	35.8	35.3
12	21.9	21.1	20.0	20.7
13	170.9	170.7	26.0	170.2
14	114.0	115.5	34.6	115.2
15	174.9	174.8	177.4	174.0
16	73.0	73.0	73.6	73.1
17	15.6	15.8	15.7	15.6
18	62.7	63.6	62.9	193.1
19	67.0	66.6	66.9	66.1
20	18.2	18.3	18.4	17.6
OAc	21.0	21.0	20.0	20.7
		21.1		
		21.2		
	172.0	171.0	170.7	170.6
		172.0		
		172.0		

1020; FDMS *m/z*: 392 $[M]^+$ ($C_{22}H_{32}O_6$), 391 $[M-H]^+$, 390 $[M-2H]^+$, 374 $[M-H_2O]^+$ (base peak); FABMS *m/z*: 374 $[M-H_2O]^+$, 315 $[M-H_2O-OAc]^+$, 284, 241, 189, 121 (base peak); CIMS *m/z*: 375 $[M-OH]^+$, 316 $[M-OH-OAc]^+$ (base peak), 285; EIMS *m/z*: 375 $[M-OH]^+$, 316 $[M-OH-OAc]^+$, 205, 190, 121 (base peak), 111. For ^{1}H and ^{13}C NMR data see Tables 2 and 3.

Acetylation of **1**. Compound **1** was treated with Ac_2O–pyridine (1:1) in the usual way to afford an acetate **2**. $[\alpha]_D^{25}$ −101.3° ($CHCl_3$; *c* 0.93). ^{1}H and ^{13}C NMR data (see Tables 2 and 3).

Hydrogenation of **1**. A soln of **1** (50 mg), MeOH (2 ml) and Pd-C powder was stirred at room temp under an atmosphere of H_2 for 6 hr. The soln was filtered to remove the Pd-C and the filtrate concd to dryness to give **3** as a white solid. For ^{1}H and ^{13}C NMR data see Tables 2 and 3.

Oxidation of **1**[7]. Compound **1** (100 mg) was gradually added to a suspended soln of pyridinium chlorochromate (PCC) (165 mg), dry NaOAc (6.3 mg) and 2 ml MeOH under stirring at room temp. After reacting for 2 hr, the reaction mixture was poured into Et_2O and filtered. The filtrate was concd to dryness to give a residue. The residue was chromatographed on a silica gel column, eluting with C_6H_6–Me_2CO (5:2) to afford **4** as a white solid. UV λ_{max}^{EtOH} nm (log ε): 244 (4.71), 211 (5.18); IR ν_{max}^{KBr} cm^{-1}: 3450, 1779, 1744, 1695, 1675, 1636, 1230, 1035; For ^{1}H and ^{13}C NMR data see Tables 2 and 3.

X-ray analysis. X-ray determination of **1** was performed on a sample crystallized from Me_2CO, space group C_2, with *a* = 20.306 (7), *b* = 7.753 (2), *c* = 20.226 (7) Å, β = 132.25 (2)°, *V* = 2357,00 Å^3, with calcd density 1.14 g/cm^3, using CuK_α radiation from 1146 reflexions. The structure was solved by a SHELXTL system. Full crystallographic information is deposi-

ted at the Institute of Materia Medica, Chinese Academy of Medical Science.

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