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CASBANE DITERPENOIDS FROM *EUPHORBIA EBRACTEOLATA*†

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Key Word Index—*Euphorbia ebracteolata*; Euphorbiaceae; casbane diterpenoids; yuexiandajisu A and B.

Abstract—From the ethanol extract of the roots *Euphorbia ebracteolata*, two new casbane diterpenoids, named yuexiandajisu A and B, were isolated. Their structures were elucidated by spectral analyses including ^{1}H-^{1}H COSY, ^{13}C-^{1}H COSY, COLOC and NOESY and confirmed by X-ray crystallography. In vitro bioassays showed that yuexiandajisu A exhibited antibacterial activity and yuexiandajisu B inhibited proliferation of B lymphocytes.

INTRODUCTION

"Lang Du" is one of the traditional Chinese medicines. It has been recorded in many ancient and modern Chinese medical monographs. "Lang Du", which in Chinese means extremely toxic, is used for the treatment of pulmonary tuberculosis and chronic tracheitis, and the topical treatment of scabies and psoriasis [1, 2]. According to nationwide market investigation, the original sources of "Lang Du" are the roots of three plants: *Euphorbia ebracteolata*, *E. fischeriana* (Euphorbiaceae) and *Stellera chamaejasme* (Thymelaeaceae). In our search for bioactive components from traditional Chinese medicines, we have studied *E. fischeriana* chemically and reported several new and known terpenoids [3–5]. As a part of our continuing chemical studies of "Lang Du", we have now examined the roots of *E. ebracteolata* Hayata and isolated several terpenoids and sterols, including two new casbane diterpenoids named yuexiandajisu A (**1**) and B (**2**). This paper describes the isolation and structure elucidation of **1** and **2**, and their biological activities.

RESULTS AND DISCUSSION

Yuexiandajisu A (**1**) was assigned the molecular formula $C_{20}H_{30}O_3$ (EIMS, $[M]^+ = m/z$ 318 and NMR). IR absorption at 3380, 1670 and 1626 cm^{-1} suggested the presence of hydroxyl and carboxyl groups and a double bond. The UV spectrum showed a maximum absorption at 250 nm (logε 3.98), indicating the presence of an α, β-unsaturated carboxyl group.

Table 1. ^{13}C NMR data of compounds **1** and **2** ($CDCl_3$)

C	1	2	C	1	2
1	37.3 *d*	36.6 *d*	11	125.5 *d*	125.6 *d*
2	31.9 *d*	32.1 *d*	12	132.9 *s*	132.9 *s*
3	153.5 *d*	150.8 *d*	13	24.1 *t*	24.1 *t*
4	127.0 *s*	125.6 *s*	14	24.1 *t*	24.3 *t*
5	78.0 *d*	33.2 *t*	15	27.9 *s*	26.9 *s*
6	34.7 *t*	25.3 *t*	16	22.0 q^a	22.0 q^b
7	120.6 *d*	125.1 *d*	17	22.9 q^a	23.0 q^b
8	136.0 *s*	134.4 *s*	18	171.8 *s*	173.3 *s*
9	38.6 *t*	38.8 *t*	19	16.2 *q*	15.5 *q*
10	39.1 *t*	39.1 *t*	20	14.8 *q*	14.8 *q*

[a,b] interchangeable assignments.

The molecular formula of **1** required six degrees of unsaturation. The ^{13}C NMR spectrum showed signals for 20 carbons (Table 1), including one carboxyl, six olefinic (three methine and three quaternary), one oxygenated methine carbon together with twelve aliphatic (four methyl, five methylene, two methine and one quaternary) carbons. Three vinyl proton signals at δ 4.88 (H-7), 5.02(H-11) and 5.74(H-3) in ^{1}H NMR spectrum, which equated with six olefinic carbons in ^{13}C NMR, revealed the existence of three trisubstituted double bonds. With all the functionalities known [OH, COOH, (C=CH) × 3], the two remaining degrees of unsaturation were ascribed to two carbocyclic systems.

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† Part 3 in the series *Chemical studies of Lang-Du, a traditional Chinese medicine.*

Table 2. ^1H-^{13}C correlation by COLOC of compound **1** ($CDCl_3$)

C	COLOC (proton)	C	COLOC (proton)
1	H-2, H-16, H-17	11	H-10, H-11, H-20
2	H-1, H-16, H-17	12	H-13, H-20
3	H-3	14	H-13, H-16, H-17
4	H-6	15	H-16, H-17
7	H-9, H-19	18	H-3, H-5
8	H-9, H-19		

The quaternary carbon (C-15, δ 27.6) and the presence of a gem-dimethyl functionality (δ_H1.15 *s*, 1.19 *s*; δ_C22.0 *q*, 22.9 *q*), together with the correlation between two typical cyclopropyl protons (δ 0.75 and 2.06, respectively) in conventional ^1H-^1H COSY NMR indicated the existence of a substituted cyclopropyl ring. Thus, the remaining ring was deduced to be a 14 membered macrocyclic system. The other two methyl groups in **1** were vinylic, as shown by the NMR data (δ_H 1.58 *s*, 3H; 1.57 *s*, 3H and δ_C 16.2 *q*, 14.8 *q*). From all of the above evidence, **1** was considered to be a casbane-type diterpenoid.

The ^1H-^1H COSY and COLOC results (Table 2) of **1**, disclosed a rather lengthy spin-system, beginning with a cyclopropyl proton (δ 0.75, H-1). A correlation between this proton and another cyclopropyl proton (δ 2.06, H-2) led to a cross-peak between H-2 and an olefinic proton (δ 5.74, H-3). The COLOC experiment yielded correlations between C-18 (δ 171.8) and both ends of the H-3 and H-5 (δ 4.15, adjacent to hydroxyl group). Furthermore, the correlation between H-5 and a methylene (δ 2.48, 2.67, H-6) led to the cross-peak between H-6 and a vinylic proton (δ 4.48, H-7). In the COLOC spectrum, correlations between C-7(δ 120.6) and a vinylic methyl (δ 1.58 *s*, 3H, CH_3-19), as well as an olefinic carbon (δ 136.0, C-8) and methyl protons at δ 1.58 (CH_3-19) could be discerned. The remaining unassigned components in **1** could also be determined in this way. Therefore, the structure of yuexiandajisu A was elucidated as shown in **1**. Confirmation of the structure of **1** was obtained by the application of X-ray crystallography. From the results (Fig. 1), **1** was determined to have a 3, 7, 11 all-*E*-triene system. The β-orientated H-1 and α-orientated H-2 indicated the *trans*-substituted cyclopropyl ring of **1**.

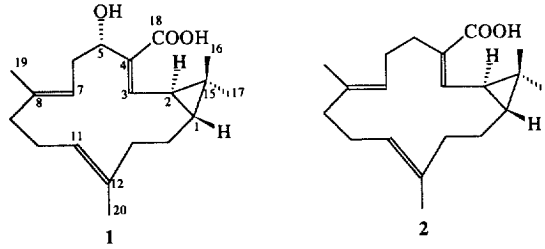

Fig. 1.

Yuexiandjisu B(**2**) was found to have the molecular formula $C_{20}H_{30}O_2$ (MS, $[M]^+ = m/z$ 302, and NMR). Analyses of its UV, IR, ^1H and ^{13}C NMR, and MS data suggested **2** was closely related to **1**. Comparing the ^1H NMR spectrum of **2** to that of **1** revealed the absence of the quartet signal at δ 4.15 (*dd*, 1H). This observation suggested that the 5-hydroxyl group in **1** was missing in **2**, which was confirmed by the C-5 signal being at δ 33.2 *t* in **2** instead of δ 78.0 *d* in **1** and the up-field shift of C-6 to δ 25.3. The COLOC spectrum showed a correlation between H-3 and a carboxyl carbon (δ 173.3, C-18). Thus the carboxyl group was situated in the same position in **1** and **2**. All assignments of ^1H and ^{13}C NMR signals were made by ^{13}C-^1H COSY, and the structure of yuexiandajisu B was determined as shown in **2**.

Casbane-type diterpenoids are rare in the plant kingdom. All those so far reported have *cis*-joined ring with 1*S* or 1*R* configuration [6, 7]. Yuexiandajisu A (**1**) and B (**2**) are the first examples having a *trans*-joined bicyclic system. From *Bertya dimerostigma* (Euphorbiaceae), several lathyrane and berdimerane diterpenoids were isolated, and both classes of compounds were considered to have arisen from a common casbane precursor [8]. The rationale for their origin *via* modification of the casbane skeleton, as proposed in the paper, can also account for the presence of the *trans*-ring fused cyclopropane in some casbane diterpenoids. In preliminary bioassay, **1** exhibited antibacterial activities and **2** inhibited proliferation of B lymphocytes.

EXPERIMENTAL

Mps: ^1H and ^{13}C NMR: Bruker AM-300 instrument in $CDCl_3$; EI-MS Finnigan MAT-711 instrument; X-ray data: Rigaku AFC7R diffractometer. The roots of *Euphorbia ebracteolata* Hayata were collected in Anhui Province in June, 1994, and identified by Mr. Xu Lei. A voucher specimen (No. 19940601) was deposited in the Herbarium of Shanghai Institute of Materia Medica, Chinese Academy of Sciences.

Extraction and separation

The air-dried ground plant materials (5 kg) were extracted with hot EtOH and the extracts after concentration were subsequently partitioned against petrol and $CHCl_3$. The $CHCl_3$ extract (228 g) was found to exhibit activity against cultured P-388 cells. A portion of the extract (220 g) was subjected to repeated CC over silica gel, using petrol-Me_2CO and petrol-Et_2O mixtures of increasing polarity as eluents. **2** (17 mg) was obtained in fractions eluted by petrol-Et_2O(4:1). The petrol-Et_2O(2:1) fractions were combined and were further fractionated by RP-8 chromatography, using 65% EtOH as eluent, to yield **1** (21 mg).

Yuexiandajisu A (1). Orthorhombic crystals, mp 162–164°, $[\alpha]_D^{30}+172°$ (c 0.78, EtOH. IR ν_{max}^{KBr} cm^{-1}: 3380, 1670, 1626; UV λ_{max}^{EtOH} nm (log ε): 250(3.98); EI-MS: 318$[M]^+$; 300, 257, 250, 232, 217, 189, 149, 135, 121(base), 107; ^1H NMR ($CDCl_3$): δ 0.75(1H, *m*, H-1), 1.15(3H, *s*, H-16), 1.19(3H, *s*, H-17), 1.19(1H, *m*, H-14), 1.57(3H, *s*, H-20), 1.58(3H, *s*, H-19), 1.95(1H, *m*, H-14), 2.04(2H, *m*, H-9), 2.06(1H, *m*, H-2), 2.12(2H, *m*, H-10), 2.14(2H, *m*, H-13), 2.48(1H, *m*, H-6), 2.67(1H, *m*, H-6), 4.15(1H, *dd*, *J* = 5.3, 11.0 Hz, H-5), 4.88(1H, *dd*, *J* = 5.2, 7.2 Hz, H-7), 5.02(1H, *t*, *br*, H-11), 5.74(1H, *d*, *J* = 11.1 Hz, H-3); ^{13}C NMR: Table 1; COLOC correlation: Table 2.

Yuexiandajisu B (2). Needles, mp 102–104°, $[\alpha]_D^9$ +102° (c 0.59, EtOH). IR ν_{max}^{KBr} cm^{-1}: 1680, 1620. UV λ_{max}^{EtOH} nm (log ε): 250(3.89); EI-MS *m/z* 302$[M]^+$; 287, 259, 231, 219, 191, 164, 151, 137, 121(base), 107; ^1H NMR($CDCl_3$): δ 0.67(1H, *m*, H-1), 1.01(1H, *m*. H-14), 1.12(6H, *s*, H-16, 17), 1.54(3H, *s*, H-20*), 1.58(3H, *s*, H-19*), 1.92(1H, *m*, H-14), 2.10-2.13(8H, *m*), 2.36(2H, *m*, H-6), 2.72(1H, *m*, H-5), 4.92(1H, *t*, *br*, H-7), 5.01(1H, *t*, *br*, H-11), 5.57(1H, *d*, *J* = 10.8 Hz, H-3); ^{13}C NMR: Table 1; COLOC correlation: δ 173.3(C-18)/δ 5.57(H-3), δ 134.4(C-8)/δ 1.54(H-19), δ 132.9(C-12)/δ 1.58(H-20), δ 26.9(C-15)/δ 1.12(H-16, 17). *interchangeable.

X-ray diffraction data for 1. $C_{20}H_{30}O_3$, orthorhombic, space group $P2_12_12_1$, $a = 11.951(3)$ Å, $b = 17.233(2)$ Å, $c = 9.295(2)$ Å, $V = 1914.5(6)$ Å^3, $Z = 4$, Dc = 1.105g/cm^3, $\lambda = 0.71069$ Å (MoKα). The data were collected at a temp. of 20 ± 1° using the ω-2θ scan technique to a maximum 2θ value of 50.0°. Of the 2153 reflections which were collected, 1953 were unique (Rint = 14.857), $R = 0.043$, $R_\omega = 0.051$. The structure was solved by the direct method (SHELXS 86) and expanded using fourier techniques (DIFDIF 92).

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