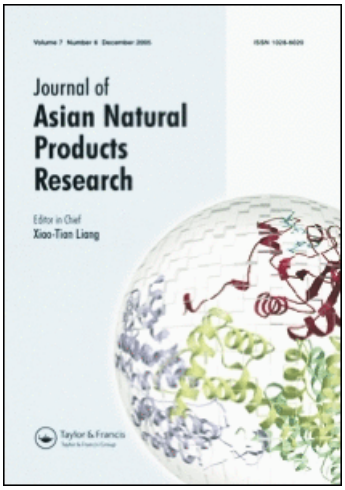

Journal of Asian Natural Products Research
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713454007

An Isopimarane Diterpene from *Euphorbia Ebracteolata* Hayata

Zhi-Hong Xu[a]; Guo-Wei Qin; Ren-Sheng Xu[a]
[a] Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai, China

To cite this Article Xu, Zhi-Hong , Qin, Guo-Wei and Xu, Ren-Sheng(2000) 'An Isopimarane Diterpene from *Euphorbia Ebracteolata* Hayata', Journal of Asian Natural Products Research, 2: 4, 257 — 261
To link to this Article: DOI: 10.1080/10286020008041364
URL: http://dx.doi.org/10.1080/10286020008041364

JAPR, Vol. 2, pp. 257–261
Reprints available directly from the publisher
Photocopying permitted by license only

AN ISOPIMARANE DITERPENE FROM *EUPHORBIA EBRACTEOLATA* HAYATA

ZHI-HONG XU, GUO-WEI QIN* and REN-SHENG XU

Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai 200031, China

(Received 25 October 1999; Revised 1 November 1999; In final form 16 November 1999)

From the ethanolic extract of the roots of *Euphorbia ebracteolata* Hayata four compounds were isolated. They are 24-methylenecycloartanone, tirucallol, procesterol and a new isopimarane diterpene, namely yuexiandajisu C. The structure of yuexiandajisu C was elucidated by spectral analysis. The bioassay *in vitro* showed yuexiandajisu C exhibited immunomodulatory activity.

Keywords: *Euphorbia ebracteolata* Hayata; Euphorbiaceae; Isopimarane diterpene; Yuexiandajisu C

INTRODUCTION

Our continuous study on the roots of the plant *Euphorbia ebracteolata* Hayata, one of the original sources of traditional Chinese medicine "Lang Du" [1–3], resulted in the isolation of four compounds from the chloroform fraction of the ethanolic extracts. They have been elucidated as 24-methylencycloartanone [4], tirucallol [5], procesterol [6] and a new isopimarane diterpene named yuexiandajisu C which showed activity *in vitro* inhibiting proliferation of lymphocyte B. This paper reports the isolation and structural determination of yuexiandajisu C.

* Corresponding author. Tel.: 86-21-64311833. Fax: 86-21-64370269.
E-mail: gwqin@server.shcnc.ac.cn.

RESULTS AND DISCUSSION

Yuexiandajisu C was assigned the molecular formula $C_{20}H_{28}O_3$ (HRMS, $[M^+]=m/z$ 316.2055, calcd: 316.2038). The IR absorption at 3400, 1720, 1670 and 1640 cm^{-1} suggested the presence of hydroxyl and carbonyl groups and double bond. The UV maximum absorption at 267 nm ($\log\varepsilon$ 3.82) indicated the conjugation relationship between carbonyl group and double bond. In the ^{13}C NMR and DEPT spectra of yuexiandajisu C, 20 carbon signals were observed, including one carbonyl, six olefinic carbons (one methylene, three methine and two quaternary, of which one is oxygenated quaternary carbon). While the signals were assigned as four methyl, four methylene, six methine and six quaternary carbons. Six olefinic carbons revealed the existence of three double bonds (one mono-substituted and two tri-substituted).

The molecular formula of the compound required seven degrees of unsaturation. With all the functionalities (two hydroxyl functions, one carbonyl group and three double bonds), the three remaining sites of unsaturation were ascribed to tricyclic system. Considering the presence of a vinyl group attached to a quaternary carbon (δ 5.17, 1H, dd, $J=10.9, 11.0$ Hz; 5.08, 1H, dd, $J=17.6, 1.0$ Hz; 5.83, 1H, dd, $J=17.6, 10.9$ Hz; δ 114.01 t; 145.61 d) together with the ^{13}C NMR data, the compound was suggested to have an isopimarane skeleton [7]. The proton and carbon signals were assigned from the ^1H-^1H COSY, ^{13}C-^1H COSY and COLOC spectra and the ^{13}C NMR data were shown in Table I.

The α,β-unsaturated carbonyl group as part of ring A and a hydroxyl group at C-2 were determined from ^{13}C-^1H COSY and COLOC spectra. The fact that two singlet methyls at δ 1.16 and 1.21 correlated with two methyl carbons at δ 22.46 and 25.80 in the ^{13}C-^1H COSY as well as these methyl protons also correlated with a quaternary carbon at δ 43.42, a methine carbon at

TABLE I ^{13}C NMR data of yuexiandajisu C ($CDCl_3$)

No.	*C*	*No.*	*C*
1	124.04(d)	11	37.83(t)
2	143.57(s)	12	25.89(t)
3	200.64(s)	13	41.75(s)
4	43.42(s)	14	72.49(d)
5	48.27(d)	15	145.61(d)
6	22.75(t)	16	114.01(t)
7	121.69(d)	17	23.01(q)
8	134.79(s)	18*	22.46(q)
9	42.61(d)	19*	25.80(q)
10	36.00(s)	20	15.72(q)

*Interchangeable assignments.

δ 48.27 and a carbonyl carbon at δ 200.64 in the COLOC spectrum indicating the assignment of C-18, 19, 4, 5 and 3. In addition, the COLOC correlation between carbonyl carbon at C-3 and olefinic proton at δ 6.21, and the latter proton at δ 6.21 and an oxygenated quaternary carbon at δ 143.57 revealed a double bond between C-1 and C-2 with hydroxyl group at C-2. Furthermore, the correlation for C-1 at δ 124.04 and 20-H at δ 1.08 was observed in COLOC spectrum, confirming the ring A assignment. The correlation between C-5 (δ 48.27) and a proton (δ 2.05), one of C_6-2H (2.05, 1.90), was also observed in the COLOC spectrum. The ^{1}H-^{1}H COSY spectrum showed the cross peaks of two H-6 signals and an olefinic proton, H-7 (δ 5.52). This indicated that another tri-substituted double bond was as part of B ring (C-7 and C-8). Thus, the structure moiety from C-1 to C-10 was determined.

The spectral evidence proved that a vinyl group connected with C-13, a quaternary carbon in the skeleton. In the COLOC spectrum a series of correlations for H-16 (δ 5.08, 5.17)/C-13 (δ 41.75), H-17 (δ 0.92)/C-15 (δ 145.61), H-17 (δ 0.92)/C-14 (δ 72.49) and H-11 (δ 1.81, 2.58)/C-8 (δ 134.79) were observed. Besides, H-14 showed as a broad singlet signal in the ^{1}H NMR spectrum. The ^{1}H-^{1}H COSY experiment revealed a weak correlation between H-14 (δ 3.69) and a methylene group H-12 (δ 1.91, 1.66), which was a typical 1,3-position correlation. It is obvious that another hydroxyl group was situated on C-14 of C ring.

The relative and absolute configurations of yuexiandajisu C were determined by the NOESY experiment and CD spectroscopy, respectively. In the NOESY spectrum, the correlation between H-14 (δ 3.69) and H-17 (δ 0.92), H-17 (δ 0.92) and H-11 (δ 1.66), H-11 (δ 1.66) and H-20 (δ 1.08) indicated that H-14 and two methyl groups at C-13 and C-10 were seated on the same side of the structure, while the hydroxyl group at C-14 and the vinyl group at C-13 were seated on the other side. The chirality of C-10 was determined as "S" according to a positive Cotton effect at 328 in the CD spectrum [8]. Thus the structure of yuexiandajisu C was determined as shown in Fig. 1. Preliminary *in vitro* bioassay showed yuexiandajisu C exhibited inhibition for proliferation of lymphocyte B.

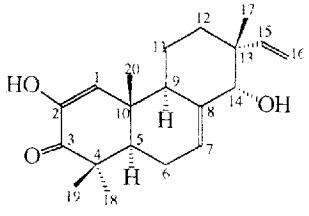

FIGURE 1 The structure of yuexiandajisu C.

EXPERIMENTAL SECTION

General Experimental Procedures

Melting points were determined on a Kofler micromelting point apparatus and are uncorrected. Optical rotations were measured with a JASCO DIP-181 polarimeter. The IR spectra were run on a Perkin-Elmer 599B spectrometer and UV spectra obtained on a Shimitzu UV-250 spectrometer. ^{1}H and ^{13}C NMR were recorded on Bruker AM-300 and 400 instrument in $CDCl_3$. MS were performed with a Finnigan MAT-711 instrument.

Plant Material

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 Isolation

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 column chromatography over silica gel, using petroleum-ether mixtures of increasing polarity as eluent. 24-Methylenecycloartan-3-one (17 mg) was obtained in petroleum–ether (9 : 1) elution. Yuexiandajisu C (20 mg), tirucallol (32 mg) and procesterol (7 mg) were obtained from fractions eluted by petroleum–ether (4 : 1).

Yuexiandajisu C. Brown oil $[\alpha]_D^{30} - 8.63$(c 0.67, EtOH), *IR* ν_{max}^{KBr} cm^{-1}: 3400, 1720, 1670, 1640, 760; *UV* ν_{max}^{EtOH} nm (log) : 267 (3.82); ^{1}H NMR ($CDCl_3$): 0.92 (3H, s, H-17), 1.08 (3H, s, H-20), 1.16 (3H, s, H-18*), 1.21 (3H, s, H-19*), 1.90 (1H, m, H-5), 1.81, 2.58 (2H, d, $J = 13.9$ Hz, H-11), 1.91, 1.66 (2H, td, $J = 13.6, 2.7$ Hz, H-12), 2.05, 1.90 (2H, m, H-6), 2.35 (1H, dd, br, H-9), 3.69 (1H, s, br, H-14), 5.08 (1H, dd, $J = 17.6, 1.0$ Hz, H-16), 5.17 (1H, dd, $J = 10.9, 1.0$ Hz, H-16), 5.52 (1H, dd, $J = 5.5, 2.1$Hz, H-7), 5.83 (1H, dd, $J = 17.6, 10.9$ Hz, H-15), 6.21 (1H, s, H-1); ^{13}C NMR ($CDCl_3$): see Table I. HRMS *m/z*: 316.2055 (M^+), calcd: 316.2038. EI-MS: 55, 83, 124, 131, 152 (base), 185, 255, 283, 298, 316. *Interchangeable.

References

[1] Jiangsu College of New Medicine, *Dictionary of Chinese Materia Medica*. Shanghai Science and Technology Press, 1986, pp. 1898–1900.
[2] X.Z. Guo, *Dictionary of Chinese Poisonous Herbs*. Tianjin Science and Technology Translation Press, 1992, p. 446.
[3] Z.H. Xu, J. Sun, R.S. Xu and G.W. Qin, *Phytochemistry*, 1998. **49**(1): 149–151.
[4] R.C. Cambie, A.R. Lal, P.S. Rutledge and Woodgate, *Phytochemistry*, 1991, **30**(1): 287 292.
[5] D.H.R. Barton and E. Seoane, *J. Chem. Soc.*, 1956, 4150–4157.
[6] A.Q. Khan and A. Malik, *Phytochemistry*, 1989, **28**: 2859–2861.
[7] M. Taran, B.A.S. Miguel, B. Delmond, M. Petraud and B. Barbe, *J. Magn. Reson. Chem.*, 1987. **25**: 1090–1093.
[8] B. Kagan Henri, *Stereochemistry, Dipole Moments, CD or ORD*. Vol. 2. George Thieme Publishers, Stuttgart, 1977, pp. 123 124.