波 谱 学 杂 志
第 29 卷第 1 期 Vol. 29 No. 1
2012 年 3 月 Chinese Journal of Magnetic Resonance Sep. 2012

Article: 1000-4556(2012)01-112-07

2D NMR Assignments of an *ent*-Pimarane Diterpenoid from *Euphorbia yinshanica*

ZHANG Ben-yin[1,3], *WANG Huan*[1], *LUO Xiao-dong*[2], *DU zhi-zhi*[2], *SHEN Jian-wei*[1], *ZHANG Xiao-feng*[1*]

(1. Northwest Institute of Plateau Biology, Chinese Academy of Sciences, Xining 810008, China;
2. State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650204, China;
3. Graduate University of the Chinese Academy of Sciences, Beijing 100049, China)

Abstract: One known *ent*-pimarane type diterpenoid, 3α, 19-dihydroxyl-*ent*-pimara-9(11), 15-diene, has been isolated from the roots of *Euphorbia yinshanica* for the first time. Its structure has been confirmed by 2D spectral methods (^{1}H-^{1}H COSY, HMBC, HMQC, ROESY), and its NMR signals have been first completely assigned.

Key words: Euphorbiaceae, *Euphorbia yinshanica*, *ent*-pimarane diterpenoid, 2D NMR
CLC number: O482.53 **Document code**: A

Introduction

Euphorbia yinshanica S. Q (euphorbiaceae) belonging to the family of Euphorbiaceae is distributed in Tianjun, Xunhua, Minhe of Qinghai province[1]. It is a traditional Tibetan medicine for curing furuncle, exanthema and cutaneous anthrax which is similar as Euphorbia kansuensis Proch[2]. The chemical constituents of the plant have never been reported previously. In order to find out its anti-tumor constituents, we have in-

Received date: 2011-03-28; **Revised date**: 2011-04-20

Foundation item: The open project of State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences (P2008-KF05); the project of western doctorate foundation and the knowledge innovation project from the northwest institute of plateau biology, Chinese Academy of Sciences.

Biography: Zhang Ben-yin(1985-), Ph. D., major in Natural product chemistry. * Corresponding author: Zhang Xiao-feng, Tel: 13909715266, E-mail: aryinzhang@163.com

vestigated its ethyl acetate extract fraction, from which we have isolated one known *ent*-pimarane diterpene 1 (3α, 19-dihydroxy-*ent*-pimara-9(11), 15-diene) for the first time. Its ^{1}H NMR data had been assigned previously[3], however, other NMR data were not reported in literatures. In this work, we further elucidated the structure by 2D spectra (^{1}H-^{1}H COSY, HMQC, HMBC, ROSEY) and completely assigned the NMR signals.

1 Experimental

1.1 Apparatus

^{1}H-NMR, ^{1}H-^{1}H COSY, HMBC, HMQC (500.03 MHz, $CDCl_3$) and ^{13}C NMR (125.75 MHz, $CDCl_3$) spectra were performed on a Bruker DRX-500, and ROESY experiment (600.13 MHz) was on AV-600 with $CDCl_3$ as solvent and TMS as internal standard. Spectral width of the ^{1}H NMR and ^{13}C NMR spectra were 7 002.80 Hz and 23 584.91 Hz. Spectral width sampled of ^{1}H-^{1}H COSY, HMBC, HMQC were 4 006.41×4 000.20, 4 006.41×25 141.42 and 4 006.41×20 120.72 Hz, as well as sampling data matrices were 1 024×1 024, 1 024×512 and 1 024×1 024, respectively. Spectral width and sampling data matrix of ROESY were 7 211.54×7 201.59 Hz and 1 024×1 024, respectively.

ESIMS and EIMS were carried out on API QSTAR Pulsar I and VG Autospec-3000 mass spectrometers, respectively. Silica gel (100～200 and 200～300 mesh), silica gel H (Qingdao Marine Chemical Ltd., China), RP-18 silica gel (40 μm～60 μm, Amersham Biosciences, Sweden) and Sephadex LH-20 (Amersham Biosciences, Sweden) were used for column chromatography (CC). MeOH-$CHCl_3$ (1∶1) was used for Sephadex LH-20.

1.2 Plant material

Euphorbia yinshanica S.Q was collected in Xunhua, Qinghai, 2008, and identified by prof. LIU Shang-wu(the northwest institute of plateau biology, Chinese Academy of Sciences), and a voucher specimen was deposited at the northwest institute of plateau biology, Chinese Academy of Sciences.

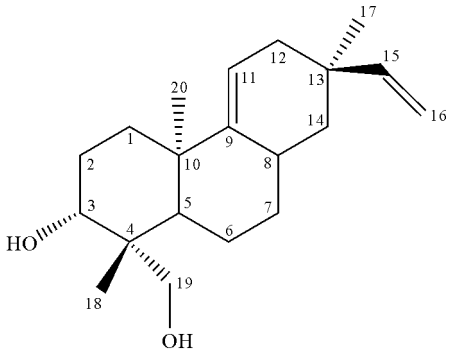
1.3 Extraction and isolation

The air-dried roots of E. yinshanica (8 kg) were extracted with 85% EtOH (25 L) at room temperature for 4 times each 5 days and the extrat was evaporated in vacuo. The residue was suspended in water and was extracted with ethyl acetate. The ethyl acetate extract (60 g) was silica gel column chromatography (Si-gel CC) eluting with Chloroform ($CHCl_3$), $CHCl_3$-acetone (40∶1 to 1∶1) and Methanol (MeOH). Eighteen fractions were obtained according to the difference in composition monitored by TLC (GF254), fraction 7 (1.1 g) was subjected to silica gel CC and eluted with $CHCl_3$-actone (10∶1), fraction 7B was on the reverse-Si-gel(RP-18) CC eluting with MeOH-H_2O (50%～100%) to divide into four subfractions. The subfraction 7B-C eluted with 85% MeOH continued to be on Si-gel CC eluted with $CHCl_3$-actone (25∶1), then

Sephadex LH-20 eluted with $CHCl_3$: MeOH(1 : 1) afforded compound **1** (15 mg).

2 Results and discussion

A combination of column chromatography on silica gel, sephadex LH-20 and RP-18 silica gel of the ether extract of *E. yinshanica* gave one known *ent*-pimarane type diterpenoid.



1

The compound **1** was obtained as white powder, with the molecular formula calculated as $C_{20}H_{32}O_2$, determined by EIMS at m/z 304 $[M]^+$ and ESIMS at m/z 303 $[M-H]^-$. There were three rings deduced from five calculated unsaturated degrees.

The ^{13}C NMR and DEPT spectra exhibited 20 carbon signals, including three methyls, eight methylenes (one hydroxymethyl), five methines (one oxygenated), and four quaternary carbons. The ^1H NMR signals at δ 0.97 (3H, s), 1.24 (3H, s) and 1.03 (3H, s), as well as those at δ 4.88 (1H, dd, J = 10.5, 1.2 Hz) and 4.94 (1H, dd, J = 17.5, 1.2 Hz), were characteristic of a *ent*-pimarane type diterpenoid and were assigned to the methyl groups at C-17, C-18 and C-20 and to the methylene group at C-16, respectively. The ^{13}C NMR spectral data, exhibiting signals for three methyl groups at δ 22.4, 26.0 and 22.0; a methine carbon at δ 150.1; and a methylene at δ 109.1, also provided evidence for **1** being a *ent*-pimarane type diterpenoid. The data of ^1H NMR were in accordance to those of compound 3α, 19-dihydroxyl-*ent*-pimara-9(11), 15-diene which has been reported in limited elucidation methods including ^1H NMR and MS by Jakupovica J[3].

In order to confirm the structure and give the complete NMR assignments, ^1H-^1H COSY, HMQC, HMBC and ROESY spectra of compound **1** had been performed in $CDCl_3$. The ^1H-^1H COSY of **1** confirmed the presence of four partial structures, (1) -CH(OH)-CH_2-CH_2-, (2)-CH-CH_2-CH_2-, (3)-CH-CH_2- and (4)-CH-CH_2-. The connectivity of each partial structure was clarified by the HMBC spectrum (Fig. 1 and Fig. 2).

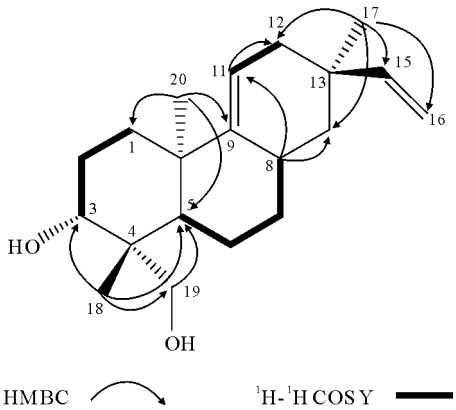


Fig. 1　Selected HMBC and ^{1}H-^{1}H COSY correlations of compound **1**

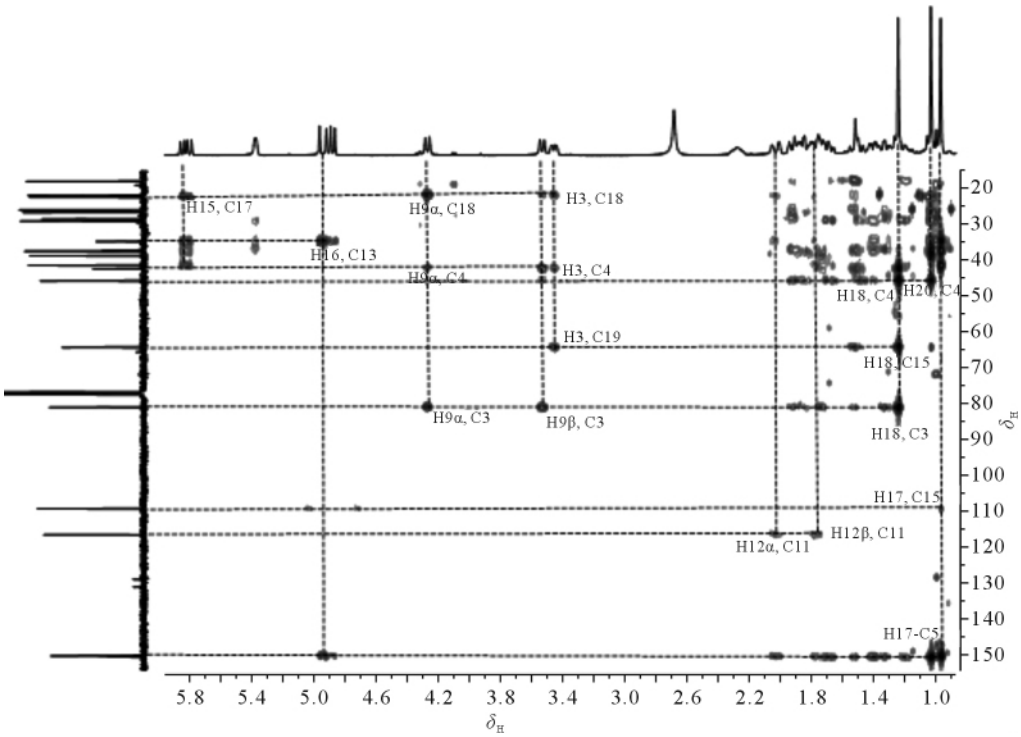


Fig. 2　HMBC spectrum of compound **1**

The relative stereochemistry of the compound was finally determined by ROESY spectrum (Fig. 3). The relative configuration of H-5 and H-20 were assumed to be β and α according to the data of *ent*-pimara type deterpenoids[4]. ROESY correlations observed between H-5 with H-3, H-7β and H-18, H-20 with H-19α, H-1α, H-11, and H-8, H-17 with H-12α and H-8, suggested that the relative configurations of H-3, H-17, H-18 should be β, β and α, respectively. Accordingly, the structure of **1** was determined to be 3α, 19-dihydroxyl-ent-pimara-9(11), 15-diene. ^{1}H NMR and ^{13}C NMR data (Table 1) were completely assigned by the above 2D experiments.

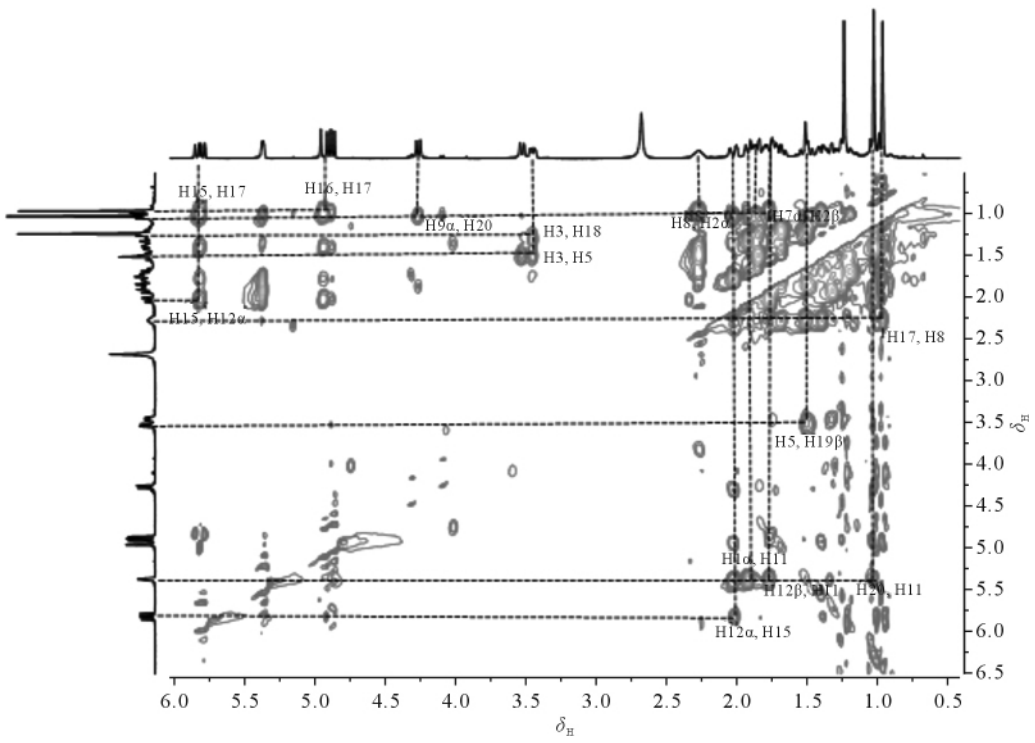


Fig. 3 ROESY spectrum of compound **1**

Table 1 1D NMR and 2D NMR data of the compound 1 in $CDCl_3$

Position	δ_H (J in Hz)	δ_C	^1H–^1H COSY	HMBC
1α	1.92(d, J=3.5 Hz, 1H)	38.8(t)	H1β, H2α, H2β	C-2, C-3, C-5, C-10, C-20
1β	1.33(m, 1H)		H1α, H2α, H2β	
2α	1.87(m, 1H)	18.1(t)	H1α, H2β, H3	C-1, C-3, C-4
2β	1.52(brs, 1H)		H1α, H1β, H2α, H3	
3	3.46(dd, J=11.5, 3.6 Hz, 1H)	81.0(d)	H2α, H2β	C-4, C-5, C-18, C-19
4		42.4(s)		
5	1.52^{overlapped}	45.7(d)	H6α, H6β	C-3, C-6, C-10, C-18, C-20
6α	1.68(m, 1H)	26.7(t)	H6β, H7α, H7β, H5	C-5, C-7, C-8
6β	1.20(m, 1H)		H6α, H7α, H7β, H5	
7α	1.86(m, 1H)	28.6(t)	H6α, H7β, H8	C-5, C-6, C-8, C-14
7β	1.75(m, 1H)		H6α, H6β, H7α, H8	
8	2.27(brs, 1H)	29.1(d)	H7α, H7β, H14α, H14β	C-7, C-9, C-13, C-14
9		150.4(s)		
10		37.3(s)		
11	5.37(t, J=2.6 Hz, 1H)	116.4(d)	H12α, H12β	C-8, C-12
12α	2.03(d, J=17.4 Hz, 1H)	37.6(t)	H11, H12β	C-9, C-11, C-13, C-17

Continuation of the Table 1

Position	δ_H (J in Hz)	δ_C	^1H-^1H COSY	HMBC
12β	1.78(m,1H)		H11,H12α	
13		34.8(s)		
14α	1.41(m,1H)	41.4(t)	H8,H14β	C-8,C-13,C-17
14β	1.03^{overlapped}		H8,H14α	
15	5.82(dd,J=17.5,10.5 Hz,1H)	150.1(d)	H16α,H16β	C-13,C-14,C-16
16α	4.94(dd,J=17.5,1.5 Hz,1H)	109.1(t)	H15,H16β	C-13,C-15
16β	4.88(dd,J=10.5,1.5 Hz,1H)		H15,H16α	
17	0.97(s,3H)	22.4(q)		C-12,C-13,C-14,C-15
18	1.24(s,3H)	22.0(q)		C-3,C-4,C-5,C-19
19α	4.27(d,J=11.1 Hz,1H)	64.2(t)	H19β	C-3,C-4,C-5,C-18
19β	3.53(d,J=11.1 Hz,1H)		H19α	
20	1.03(s,3H)	26.0(q)		C-1,C-5,C-9,C-10

3 Identification

3α, 19-dihydroxyl-*ent*-pimara-9(11),15-diene (**1**): $C_{20}H_{32}O_2$, white powder; ^1H and ^{13}C NMR ($CDCl_3$) spectral data (Table 1). ESIMS m/z 303 $[M-H]^-$; EI/MS m/z 304 $[M]^+$(36), 286(44), 271(67), 255(57), 236(28), 213(32), 201(35), 187(54), 159(58), 149(100), 133(71), 119(69), 105(91), 95(73), 91(78), 81(68), 67(54), 55(78).

References:

[1] Liu Shang-wu(**刘尚武**). Flora of Qinghai(**青海植物志**)[M]. Xining(**西宁**): Qinghai People Press(**青海人民出版社**), 1999. 300.

[2] Zhao Z L, Zhao R N. Plant of origin of the Tibetan medicine "chuanbu"[J]. Chin Pharm J, 1992, 27: 269.

[3] Jakupovica J, Kuhnkea J, Schustera A, *et al*. Phloroglucinol derivatives and other constituents from South African Helichrysum species[J]. Phytochemistry, 1986, 25(5): 1 133-1 142.

[4] Braulio M F, Pedro G, Melchor G, *et al*. Microbial transformation of 18-Hydroxy-9,13-*epi*-*ent*-pimara-7,15-diene by *Gibberella fujikuroi*[J]. J Nat Prod, 2003, 66: 392-397.

阴山大戟中一个对映海松烷型二萜 2D NMR 全归属

张本印[1,3]，王 环[1]，罗晓东[2]，杜芝芝[2]，沈建伟[1]，张晓峰[1*]

（1. 中国科学院 西北高原生物研究所，青海 西宁 810008；
2. 中国科学院 昆明植物研究所 植物化学与西部植物资源持续利用国家重点实验室，云南 昆明 650204；
3. 中国科学院 研究生院，北京 100049）

摘 要：从阴山大戟的根部首次分到一个已知的对映-海松烷型二萜，3α，19-dihydroxyl-*ent*-pimara-9，15-diene，通过二维波谱方法（^{1}H-^{1}H COSY，HMBC，HMQC，ROESY）确定了其结构，并且首次此化合物的 H 谱和 C 谱数据进行了全归属.

关键词：大戟科；阴山大戟；对映-海松烷型二萜；2D NMR 谱

* 通讯联系人：张晓峰，电话：13909715266，E-mail：aryinzhang@163.com.