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Diterpenes and aromatic compounds from *Euphorbia fischeriana*

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Abstract

From the roots of *Euphorbia fischeriana*, two new diterpene lactones, langduin B and 17-acetoxyjolkinolide A, together with five known diterpenes, jolkinolide A, 17-hydroxyjolkinolide A, jolkinolide B, 17-hydroxyjolkinolide B, and *ent*-11β-hydroxyabieta-8(14),13(15)-dien-16,12β-olide were isolated. The structural determination was accomplished by interpretation of spectral data and in the case of langduin B by X-ray crystallographic analysis. Six aromatic compounds were also obtained during the course of isolation.

Keywords: *Euphorbia fischeriana*; Euphorbiaceae; Diterpene; Langduin B; 17-acetoxyjolkinolide A

1. Introduction

Euphorbia fischeriana Steud (Euphorbiaceae) is a perennial herbaceous plant distributed mainly in North China. The root part of the plant, known as 'Lang Du' in traditional Chinese medicine and classified as a 'toxic drug' due to its high potency and relatively violent pharmacological effects, is used with great care for the treatment of edema, indigestion, cough, asthma and chronic bronchitis (Jiangsu New Medical College, 1977). As part of a continuing program to look for novel chemical constituents from bioactive plant extracts, we reported previously on the isolation of two new tigliane-type diterpenoids, langduin A and 12-deoxyphorbol-13-hexadecanoate, together with prostratin from the roots of *E. fischeriana* (Ma, Liu, Wu, Zhou & Qin, 1997). We now describe the isolation and structure elucidation of two additional new diterpenes, langduin B (**1**) and 17-acetoxyjolkinolide A (**2**), along with five known compounds, jolkinolide A (**3**), 17-hydroxyjolkinolide A (**4**), jolkinolide B (**5**), 17-hydroxyjolkinolide B (**6**), and *ent*-11β-hydroxyabieta-8(14),13(15)-dien-16,12β-olide (**7**). During the course of isolation, six aromatic compounds were also obtained and identified by comparison of their spectral data with published values.

2. Results and discussion

From the petrol-soluble fraction of an EtOH extract of the roots of *E. fischeriana*, seven diterpene compounds (**1**–**7**) were purified by repeated chromatography on silica gel. Work-up of the EtOAc fraction of the same extract also led to the isolation of six aromatic compounds. Each of the isolates was subjected to detailed spectroscopic analyses in order to establish their chemical structures.

Langduin B (**1**) was obtained as colourless crystals. The FAB-MS showed quasi-molecular ion signals at m/z 387 $[M+Na]^+$ and 365 $[M+H]^+$, and its EIMS showed an $[M]^+$ at m/z 364. The ^{13}C NMR and DEPT spectra (Table 1) revealed the presence of 20

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Table 1
^{13}C NMR spectral data of **1**, **2**, **4–6**

Carbon	1	2	4	5	6
1	40.2	40.0	39.3	41.4	41.4
2	18.9	18.4	18.4	18.5	18.5
3	41.8	41.5	41.5	40.0	39.2
4	41.0	33.5	33.5	33.3	33.5
5	46.7	53.5	53.5	53.2	53.6
6	30.7	20.8	20.9	21.2	21.0
7	71.1	33.8	34.0	36.5	36.6
8	155.2	61.3	61.3	60.8	66.9
9	55.3	51.9	51.8	66.6	47.0
10	32.7	41.6	41.4	48.2	39.1
11	70.2	107.6	106.4	61.3	61.6
12	104.3	149.5	147.3	85.4	85.5
13	156.0	147.2	146.5	148.4	150.8
14	115.4	54.3	54.4	55.6	53.6
15	124.0	122.3	127.4	130.1	150.8
16	172.1	170.5	169.2	169.8	168.2
17	55.5	55.4	56.3	8.6	56.5
18	32.9	33.4	33.5	33.2	33.5
19	21.4	21.9	21.9	22.1	21.9
20	14.4	15.0	15.1	15.4	15.6

carbons. From the above data, the molecular formula of **1** was deduced as $C_{20}H_{28}O_6$. The IR spectrum of **1** indicated the presence of an OH group (3400 cm^{-1}), an α, β-unsaturated γ-lactone (1760 cm^{-1}), and a conjugated double bond (1640 cm^{-1}). The ^1H and ^{13}C NMR spectra (Table 1) displayed signals for three Me groups (δ_H 1.00, 1.01, 1.11, each 3H, *s*; δ_C 32.9 *s*, 21.4 *s*, 14.4 *s*), one hydroxymethylene (δ_H 5.04, 5.07, each 1 H, AB *q*, J = 14.7 Hz; δ_C 55.5 *t*), two hydroxymethines (δ_H 4.58, *d*, J = 6.6, Hz; 4.86 *br s*; δ_C 71.1 *d*, 70.2 *d*) and an α, β-unsaturated γ-lactone (δ_C 172.1 *s*, 124.0 *s*, 156.0 *s*) which further conjugates with a trisubstituted double bond (δ_H 7.31, *d*, J = 1.6 Hz; δ_C 115.4 *d*, 155.2 *s*), as shown by the UV absorption at λ max 276 (log ε 5.96). The compound also contained a quaternary carbon connected with two oxygen atoms, the signal of which appeared at δ_C 104.32 in the ^{13}C NMR spectrum. Comparison of the spectral features of **1** with those of known diterpenes in *Euphorbia* species (Uemura & Hirata, 1972; Lal, Cambie, Rutledge & Woodgate, 1990) led to the conclusion that **1** was a derivative of *ent*-17-hydroxy-abieta-8(14),13(15)-dien-16,12-olide bearing three additional hydroxyl groups.

In the course of our study, a series of *ent*-abietane diterpenes (**3–7**) were isolated. When compared to these compounds, compound **1** showed the following distinct features based on its NMR characteristics: (a) The 8β,14β-epoxy group was absent. In place of the epoxy function, a double bond between C-8 (δ_C 155.2) and C-14 (δ_C 115.4) was present in **1**. Similar olefinic carbon signals were also observed in **7**. (b) The 11β,12β-epoxy ring (present in **5** and **6**) was replaced

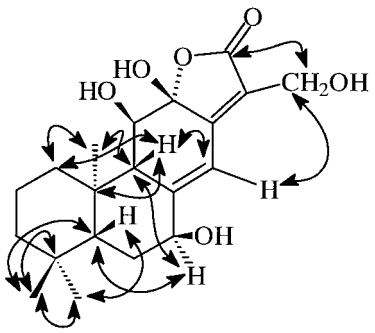


Fig. 1. HMBC results of compound **1**.

by two hydroxyl groups at C-11 and C-12, identifiable by the downfield shifts of C-11 (δ_C 70.2 in **1** compared to 61.3 in **5** and 61.6 in **6**) and C-12 (δ_C 104.3 in **1** compared to 85.4 in **5** and 85.5 in **6**). (c) An additional hydroxymethine group could be assigned based on a proton signal at δ_H 4.86, which displayed NOE correlation with H-14 (at δ_H 7.31) (Fig. 2), suggesting the presence of a hydroxyl group at C-7. On the basis of these results, **1** was therefore a 7,11,12,17-tetrahydroxy derivative of an abieta-8(14),13(15)-dien-16,12-olide. The long-range coupling data revealed by an HMBC experiment were in good agreement with the above structural assignments (Fig. 1).

The stereochemistry of **1** was then determined partially by a NOESY experiment (Fig. 2). Thus, H-12 exhibited NOE with 20-Me and was therefore α-oriented. H-7 was suggested to be equatorial (α-orientation) based on its NOE with H-14. Finally, a single crystal X-ray structural analysis was carried out to confirm the molecular structure (Fig. 3). The X-ray structure of **1** demonstrated an *ent*-abietadienolide skeleton as well as the locations of the hydroxyl functional groups on it. It also confirmed the relative stereostructure, showing that the hydroxyls on C-11 and C-12 are both on the β face, while the lactone oxygen is oriented on the α side. The latter was consistent with the jolkinolide derivatives **5** and **6**. Thus langduin B (**1**) was elucidated as *ent*-7β,11β,12β,17-tetrahydroxy-abieta-8(14),13(15)-dien-16,12α-olide.

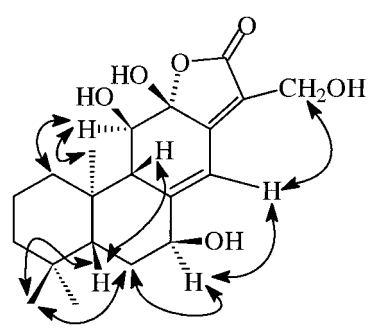


Fig. 2. NOESY results of compound **1**.

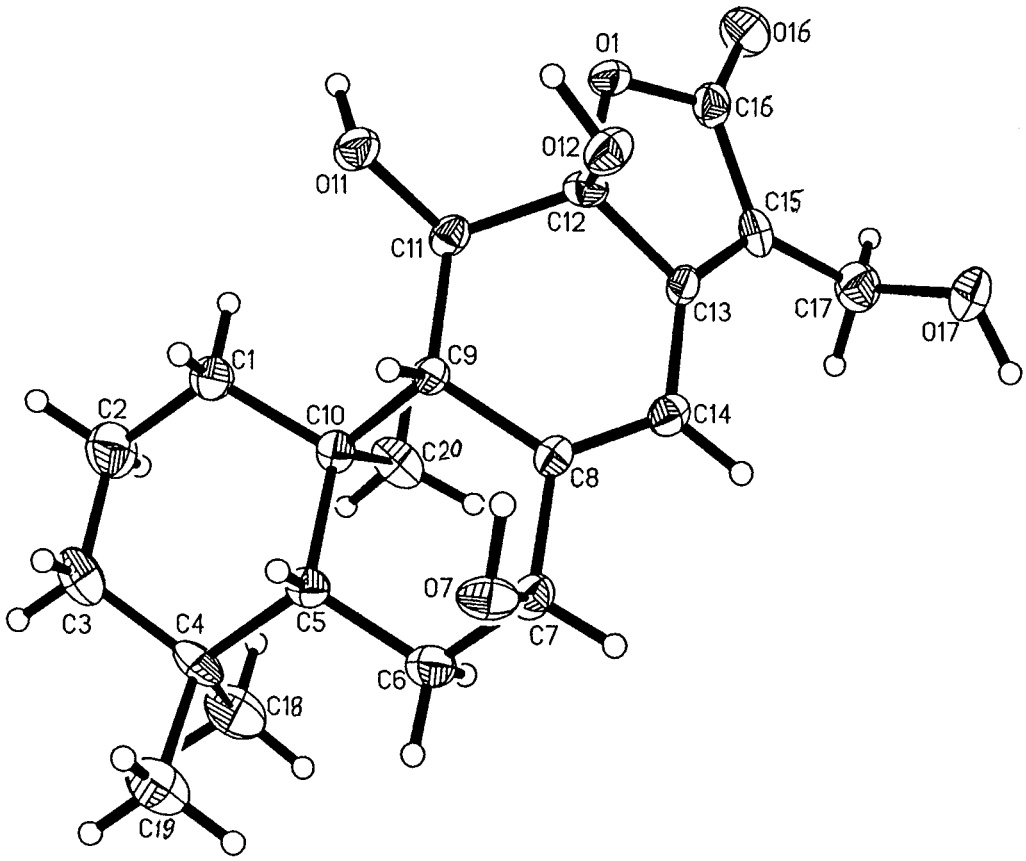


Fig. 3. ORTEP diagram of compound **1**

17-Acetoxyjolkinolide A (**2**) was obtained as a yellowish oil. The molecular formula was deduced as $C_{22}H_{28}O_5$ by FAB-MS (*m*/*z* 395, $[M+Na]^+$), EIMS (*m*/*z* 372, $[M]^+$) and NMR data (Table 1). Its IR spectrum revealed the presence of an α,β-unsaturated γ-lactone (1771 cm^{-1}) and ester carbonyl group (1738 cm^{-1}), with no absorption signals corresponding to OH group. In the ^1H NMR spectrum a signal at δ_H 2.11 (3H, *s*) indicated the presence of an acetoxyl group, which was further confirmed by the characteristic MS fragments at *m*/*z* 329 $[M\text{-}CH_3CO]^+$ and 312 $[M\text{-}HOAc]^+$. The ^{13}C NMR data of **2** were similar to those of 17-hydroxyjolkinolide A (**4**), with the exception of two additional signals for an acetyl group (δ_C 20.7 *q*, 168.5 *s*). Upon acidic hydrolysis, **2** was converted to a product identical with **4** on TLC. Thus, **2** was deduced as 17-acetoxyjolkinolide A.

Other diterpenes obtained in the present study were identified as jolkinolide A (**3**) (Uemura & Hirita, 1972; Lal et al., 1990; Liu, Fu, Yang, Zhoo & Fan, 1988), 17-hydroxyjolkinolide A (**4**) (Liu, Yang, Fu, Lou & Lo, 1989; Satti et al., 1986), jolkinolide B (**5**) (Uemura & Hirita, 1972; Liu et al., 1988; Uemura, Katayama & Hirata, 1977), 17-hydroxyjolkinolide B (**6**) (Liu et al., 1988), and *ent*-11β-hydroxyabieta-8(14),13(15)-dien-16,12β-olide (**7**) (Lal et al., 1990), by interpretation of the spectral data (particularly 2D NMR results) and by comparison with published values. The identity of compound **7** was confirmed by X-ray crystallographic analysis, which gave unit cell dimensions similar to those published previously (Lal et al., 1990).

During the course of isolating diterpenes from the plant extract, a number of aromatic compounds were also obtained in pure form. Each of these isolates was characterized by its spectral properties and subsequently identified as octacosyl ferulate (Wandji et al., 1990), scopoletin (Gunasekera, Cordell & Farnsworth, 1980), physcion (Wu et al., 1987), gallic acid (Pouchert & Behnke, 1993), 3,3′-di-*O*-methylellagic acid (Sato, 1987), and 2,4-dihydroxy–6-methoxy-1-acetophenone by comparison with published spectral data and/or reference samples.

Previous studies have reported the cytotoxicity of jolkinolide B against HeLa cells (Uemura et al., 1977), and antitumor effects of jolkinolides A and B in mice bearing S-180, Ehrlich ascites carcinoma and hepatocellar carcinoma (Liu et al., 1988; 1989). In our study, compounds **1** and **3**–**6** were subjected to in vitro cytotoxicity test against tumor cell lines P-388 and SGC7901 at 1 μg/ml concentration. Preliminary results indicated that **6** had strong inhibitory activity in both cell lines while **4** and **5** had relatively weak activity.

3. Experimenal

3.1. General

Mp was uncorr. MS spectra were measured on Finnigan MAT-711 and MAT-441 spectrometers. ^{1}H, ^{13}C and 2D NMR spectra were recorded on Bruker AM-400 MHz and AMX-600 MHz spectrometers using TMS as internal standard. Deuterated solvents (Py-d_5 for **1** and $CDCl_3$ for **2** and **4**–**6**) were used. IR spectra were obtained as KBr pellets on a PE-599B spectrometer. UV data were recorded on a Milton Roy 3000 spectrometer.

3.2. Plant Materials

Dried roots of *E. fischeriana* were purchased from the Shanghai Chinese Medicinal Herbs Corporation and identified by Prof. Zhi-Wei Wang of the Department of Pharmacognosy, Shanghai Medical University. A voucher specimen (No. 93081208) was deposited in the herbarium of the Shanghai Institute of Materia Medica.

3.3. Extraction and isolation

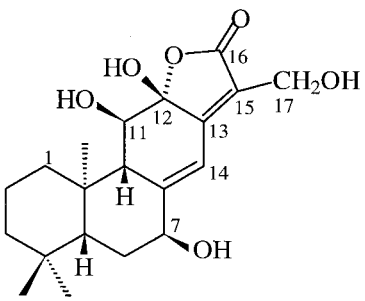
Dried roots (13 kg) were milled and extracted with 95% EtOH. After evaporation, the EtOH extract was suspended in H_2O and extracted with petrol, EtOAc and *n*-BuOH successively to yield fractions of 600, 100 and 300 g, respectively. The petrol extract was subjected to column chromatography on silica gel eluting with petrol and Me_2CO mixtures to afford **2** (21 mg), **4** (620 mg), **3** (1100 mg), **5** (785 mg), **6** (65 mg), **7** (52 mg) and **1** (10 mg). From the EtOAc extract, six aromatic compounds were purified by chromatography using $CHCl_3$–MeOH mixtures as solvent. These compounds were identified as octacosyl ferulate, scopoletin, physcion, gallic acid, 3,3′-di-*O*-methylellagic acid, and 2,4-dihydroxy-6-methoxy-1-acetophenone by interpretation of their spectral data and/or by comparison with authentic samples.

3.4. Langduin B (*1*)

Colourless crystals, m.p. 220–222°, $[\alpha]^{26}$ −143° (*c* 0.002, EtOH); FABMS *m/z*: 387 $[M+Na]^+$, 365 $[M+H]^+$; EIMS *m/z*: 364 $[M]^+$, 346, 328, 310, 267, 255, 123. UV ($CHCl_3$) λ max (log ε) nm: 276 (5.96); IR (KBr) ν_{max} cm^{-1}: 3400, 1760, 1640, 1460; ^{1}H NMR: δ 1.00, 1.01, 1.11 (each 3H, *s*, Me-18,19,20), 4.86 (1H *br, s*, H-7α), 4.58 (1H, *d, J* = 6.6 Hz, H-11α), 5.04, 5.07 (each 1H, AB *q, J* = 14.7 Hz), 7.31 (1H, *d*, H-14); ^{13}C NMR data, see Table 1.

3.5. 17-Acetoxyjolkinolide A (*2*)

Yellowish oil, $[\alpha]^{20}+70°$ (*c* 0.002, $CHCl_3$); EIMS *m/z*: 372 $[M^+]$, 329, 313, 295, 175, 123. UV ($CHCI_3$) λ max (log ε) nm: 294 (5.99); IR (KBr) ν_{max} cm^{-1}: 1771,



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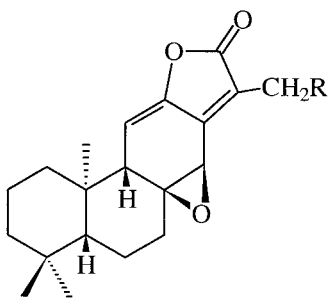
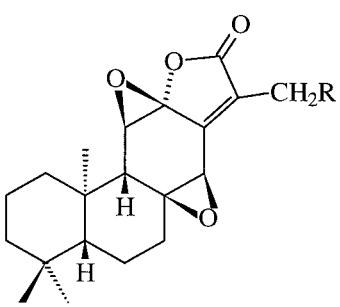
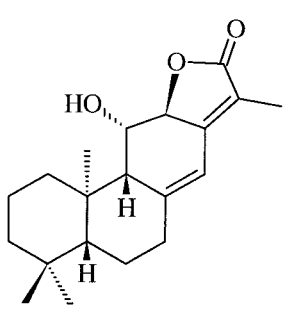


2 R = OAc
3 R = H
4 R = OH



5 R = H
6 R = OH



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1738, 1602; ^{1}H NMR: δ 0.76, 0.87, 0.96 (each 3H, *s*, Me-18,19,20), 2.11 (3H, *s*, OAc), 3.97 (1H, *s*, H-14α), 4.95, 5.03 (each 1H, AB *q*, J = 14.2 Hz), 5.62 (1H, *d*, J = 5 Hz, H-11); ^{13}C NMR data: see Table 1.

3.6. X-ray crystallography of *1*

$C_{20}H_{28}O_6$. Monoclinic, space group $P2_1$, a = 7.642(2) Å, b = 11.174(2) Å, c = 11.301(2) Å, β = 104.50(2)°C, Z = 2, D_{calc} = 1.295 Mg/m^3, MoKα radiation (λ = 0.71073 Å). Diffraction measurements were made at 298 K, using MoKα radiation on a Siemens P4-RA rotating anode diffractometer operating at 50 kV and 200 mA. A total of 2366 reflections were collected to $2\theta_{max} = 50°$ with 1917 unique. Of these 1327 ($F > 4.0\sigma(F)$) were taken as observed and used for refinement. The structure was solved by Direct methods and refined by full-matrix least squares using Siemens SHELXTL program. All hydrogen atoms were located satisfactorily but given idealized geometry $d_{(C—H)}$ = 0.96 Å and allowed to ride with fixed isotropic thermal parameter. A final weighted anisotropic full-matrix refinement gave R = 0.049 and R_w = 0.044 for a weighting scheme $W = [\sigma^2 \mid F \mid + 0.0003\ F^2]^{-1}$ and goodness-of-fit S = 1.29. The largest difference peak was +0.28 eÅ^{-1} and largest difference hole −0.26 eÅ^{-3}. The absolute configuration of the compound was undetermined.

Acknowledgements

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