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DITERPENOIDS FROM *EUPHORBIA FISCHERIANA**

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Key Word Index—*Euphorbia fischeriana*; Euphorbiaceae; tigliane diterpenoids; langduin A; 12-deoxyphorbol-13-hexadecanoate; analgesic and sedative.

Abstract—Three tigliane-type diterpenoids, langduin A, 12-deoxyphorbol-13-hexadecanoate and prostratin, were isolated from the roots of *Euphorbia fischeriana*. The first two are new compounds. The third has been isolated for the first time from this plant and showed analgesic and sedative activities in preliminary experiments with mice. The structures have been determined on the basis of spectral methods. Copyright © 1997 Elsevier Science Ltd

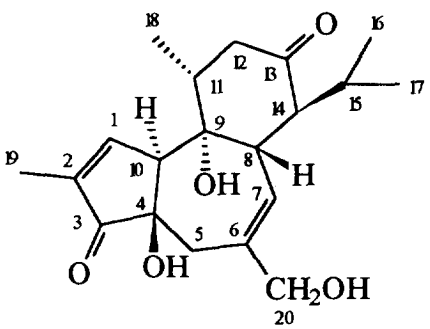
INTRODUCTION

'Lang-Du' is one of the traditional Chinese medicines. It has been used as a pesticide and expectorant, and for the treatment of oedema and indigestion for a long time. According to a nationwide market investigation [1], the original sources of this traditional medicine have been mainly the roots of three plants: *Euphorbia fischeriana*, *E. ebracteolata* (Euphorbiaceae) and *Stellera chamaejasme* (Thymelaeaceae).

Euphorbia fischeriana Steud is a perennial herbaceous plant with milky juice, distributed mainly in north China. Early chemical investigation showed it contains diterpenoids, triterpenoids and steroids. A number of abietane diterpenoids including jolkinolide A and B, and 17-hydroxyjolkinolide A and B have been isolated. Some of these diterpenoids exhibited significant antitumour activities against several tumour lines such as Sarcoma 180 and Ehrlich ascites carcinoma, and hepatocellar carcinoma in mice [2–4]. In the course of our re-examination of the plant roots, several diterpenoids have been isolated from the ether-soluble fraction of the ethanol extract. This paper deals with the isolation and structure elucidation of two new tigliane-type diterpenoids, langduin A (**1**) and 12-deoxyphorbol-13-hexadecanoate (**2**), together with a known compound prostratin (**3**), which has been isolated for the first time from this plant.

RESULTS AND DISCUSSION

Compound **1** gave rise to a $[M]^+$ peak at *m/z* 354.1912 (HRMS), corresponding to the molecular formula $C_{20}H_{28}O_5$. Its IR spectrum indicated the presence of hydroxyl (3400 cm^{-1}), double bond (1640 cm^{-1}, and conjugated carbonyl (1690 cm^{-1}) groups.



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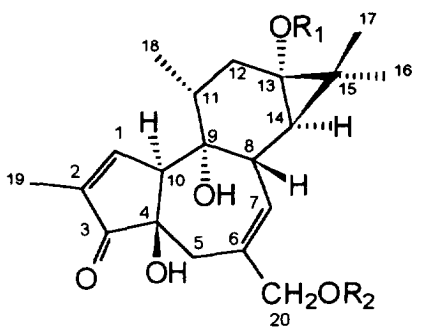

2 R_1=$CO(CH_2)_{14}CH_3$ R_2=H
3 R_1=Ac R_2=H
4 R_1=H R_2=H
5 R_1=Ac R_2=Ac

* Part 1 in the series 'Chemical Studies of Lang-Du, a Traditional Chinese Medicine'.

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The ^{1}H NMR spectrum of **1** contained three methyl doublets (δ 1.04, 1.24 and 1.31), one olefinic methyl singlet (δ 1.73), and signals for two olefinic protons (δ 7.79 *s* and 6.00 *d*, $J = 6.0$) and one oxygenated methylene (δ 4.32 *br s*). D_2O exchange led to the disappearance of two singlet signals (each for one proton) at δ 8.06 and 8.36, indicating the presence of two hydroxyls which was verified by the fragments at *m/z* 330 $[M-H_2O]^+$ and 312 $[M-2H_2O]^+$ in the EI-mass spectrum.

The ^{13}C NMR spectrum of **1** revealed 20 carbons, consisting of four methyls, three methylenes, seven methines and six quaternary carbons. On further study, several characteristic carbon signals were determined, including two carbonyls at δ 208.98 and 213.11, two trisubstituted double bonds at δ 157.88 (CH), 142.66 (C) and 127.21 (CH), 135.11 (C), two oxygenated quaternary carbons at δ 74.65 and 76.55; and one oxygenated methylene at δ 69.15. From the above observations, together with a consideration of its molecular formula, **1** was suggested to have tigliane-type diterpene skeleton. Except as an α,β-unsaturated cyclopentenone, **1** should have two hydroxyls at C-4 and C-9, one double bond between C-6 and C-7 and a hydroxymethylene at C-6 in the proposed skeleton. By comparison of the spectral data of **1** with those of tigliane diterpenes, it was clear that the commonly presented cyclopropane ring was absent and that there was an isopropyl at C-14 and carbonyl at C-13. In the literature there are a few examples of the opening of the cyclopropane ring with formation of an allyl group at C-14 and carbonyl group at C-13. However, **1** was first discovered to have such unique structural character.

It has been reported that in all tigliane diterpenoids discovered in nature, H-8 is β, C-9-OH is α and H-10 is α. On comparing the ^{1}H NMR data of **1** with the literature, the orientation of H-8, C-9-OH and H-10 were assigned to be β, α and α, respectively. Previous ^{1}H HMR work showed that $\Delta\delta$ of the two protons in the presence of OH-4α are larger than those with OH-4β [5]. In ^{1}H HMR spectrum of **1**, two unequivalent methylene protons appeared as two doublets at δ 3.05 and 3.18 with $J = 18.6$ and $\Delta\delta$ 0.12 ppm, which agreed with the presence of OH-4β. In addition, the configuration of the group isopropyl at C-14 was deduced by ^{1}H–^{1}H COSY. The proton at δ 2.96 (*dd*, $J = 12.1$, 1.3) showed a cross peak with H-8 at δ 4.32 and was consequently assigned as H-14. The cross peak between H-14 and H-15 was weaker than that between H-8 and H-14, suggesting a smaller J (1.3 Hz) for the interaction between H-14 and H-15 and a larger J (12.1 Hz) for those between H-8 and H-14. These results were confirmed by molecular models, in which the dihedral angle between H-8 and H-14 was found to approach 90° due to the rotational barrier from the large bulk of the isopropyl group, and hence led to the consequently smaller J value. Thus, the configuration of H-14 in **1** was consistent with that in naturally occurring tigliane diterpenoids. In the ^{1}H–^{1}H COSY spectrum, H-11 and H-12 were unambiguously assigned from correlation with the Me-11 signal. The methylene protons at C-12 showed two *dd* signals at δ 2.68 and 2.72 with the same coupling constants of 15.4 Hz and 7.6 Hz, which indicated a boat conformation for ring C (molecular modelling). In addition, a NOE difference spectrum was measured to determine the stereochemistry of **1**. The NOE effects were observed between H-8 and H-11; H-14 and H-18, H-12α and H-14; H-10 and H-18; H-8 and H-5β. Based on the above results, the structure of compound **1** was elucidated as a new tigliane diterpenoid named langduin A.

Compound **2** was assigned the molecular formula $C_{36}H_{58}O_6$ (HRMS). Its IR spectrum showed the presence of hydroxyl (3410 cm^{-1}, and carbonyl (1710 cm^{-1}) groups and double bond (1630 cm^{-1}). The ^{1}H NMR data of **2** (Table 1) was similar to that of 12-deoxyphorbol (**4**) except for additional signals at δ 0.85 (3H, *m*), 1.23 (24H, *s*), 1.58 (2H, *m*) and 2.26 (2H, *t*), indicating that **2** was an ester derivative of 12-deoxyphorbol. The mass spectrum of **2** displayed a discernible $[M]^+$ (*m/z* 586) and three characteristic fragment ion peaks at *m/z* 330, 312 and 294 which suggested that the fragmentation pattern of **2** was similar to that of ester derivatives of 12-deoxyphorbol [6]. The size of the ester moiety [R=$CO(CH_2)_{14}CH_3$] was deduced by subtracting *m/z* 347 from $[M]^+$ and it was confirmed by the presence of the significant peak at *m/z* 330 $[M-ROH]^+$. Two allylic proton signals for H_2-20 at δ 3.94 and 4.01, indicated that the C-20 hydroxy was free. Therefore the ester moiety (hexadecanoyl) had to be at the C-13 position of 12-deoxyphorbol [7].

The stereochemistry of previously published 12-deoxyphorbol derivatives was proved in the same way, except for C-4-OH which can be either α or β. Previous ^{1}H NMR work reported that the $\Delta\delta$ of the two H-5s in the case of a 4α-OH was larger than that of a 4β-OH [5]. In the ^{1}H NMR spectrum of **2**, two unequivalent methylene protons appeared as two doublets at δ 2.43 and 2.53 with $J = 19.0$ Hz and the $\Delta\delta$ was only 0.10 ppm, which agreed with C-4-OH being β. Therefore, **2** was deduced to have a 4β-OH, 8β-H, 9α-OH, 10α-H, 11α-Me, 13α-OR and 14α-H, respectively.

Hydrolysis of **2** with methanolic KOH followed by acetylation (pyridine–Ac_2O) of the resulting alcohol gave 12-deoxyphorbol-13,20-diacetate (**5**) which was proved to be identical in all respects (co-TLC, IR, ^{1}H NMR and MS) with an authentic sample. From all the above evidence, **2** was identified as 12-deoxyphorbol-13-hexadecanoate. The ^{1}H and ^{13}C NMR data of **2** are listed in Table 1.

Compound **3** was elucidated as prostratin (12-deoxyphorbol-13-acetate) by spectroscopic methods (IR, ^{1}H-NMR and MS) [7]. To confirm its structure, **3** was treated with the same procedure as that of **2** to afford the diacetate **5**.

In our preliminary pharmacological tests, **3** showed significant analgesic and sedative activities. The 92%

Table 1. ^1H NMR and ^{13}C NMR data for compounds **1** (C_5D_5N) and **2** ($CDCl_3$)

	1				**2**			
	^1H NMR			^{13}C NMR	^1H NMR			^{13}C NMR
Position	δ		J(Hz)	δ	δ		J(Hz)	δ
1	7.79	*s*		157.9	7.55	*br s*		161.2
2	—			142.7	—			132.8
3	—			209.0	—			209.4
4	—			74.7	—			73.8
5α	3.18	*d*	18.6	38.3	2.43	*d*	19.0	38.6
β	3.05	*d*	18.6		2.53	*d*	19.0	
6	—			135.1	—			139.9
7	6.00	*d*	6.0	127.2	5.65	*d*	4.1	130.4
8	4.32	*m*		45.8	2.99	*m*		39.1
9	—			76.6	—			76.1
10	3.67	*br s*		58.9	3.23	*bs*		55.7
11	3.30	*m*		37.8	1.96	*m*		36.3
12α	2.72	*dd*	15.4, 7.6	48.0	1.51	*dd*	11.0, 14.2	31.9
β	2.68	*dd*	15.4, 7.6		2.03	*dd*	6.9, 14.2	
13	—			213.1	—			63.3
14	2.96	*dd*	12.1, 1.3	55.8	0.80	*d*	5.2	32.6
15	2.15	*m*		29.0	—			22.7
16	1.04	*d*	7.0	22.1	1.16	*s*		23.2
17	1.24	*d*	6.8	17.7	1.04	*s*		15.3
18	1.31	*d*	6.7	18.8	0.86	*d*	6.2	18.6
19	1.73	*d*	1.1	10.4	1.65	*d*	1.7	10.1
20	4.32	*s*		69.2	3.94	*d*	12.6	68.3
$\underline{C}OCH_2(CH_2)_{12}CH_2CH_3$					4.01	*d*	12.6	176.0
$CO\underline{CH_2}(CH_2)_{12}CH_2CH_3$					2.26	*t*	7.5	34.6
$COCH_2\underline{CH_2}(CH_2)_{12}CH_2CH_3$					1.58	*m*		24.8
$COCH_2\underline{(CH_2)_{12}}CH_2CH_3$					1.23	*bs*		22.7, 29.1 (6), 29.2, 29.3, 29.4, 29.6, 31.9
$COCH_2(CH_2)_{12}CH_2\underline{CH_3}$					0.85	*m*		14.1

and 62% inhibitions were observed in sedative experiments with 20 mg/kg (p.o.) and 1 mg/kg (s.c.) in mice, respectively. 96% and 48% inhibitions were found in analgesic experiments with 20 mg/kg (p.o.) and 1 mg (s.c.) in mice, respectively. Only a few diterpenoids are reported to possess such kind of activities.

EXPERIMENTAL

General. Mps: uncorr; MS: Finnigan-MAT-711 and MAT-441; ^1H, ^{13}C and 2D NMR; Bruker AM-400 spectrometer, with TMS or solvent as int. standard; IR: KBr.

Plant material. Dried roots of *Euphorbia fischeriana* ('Lang-Du' of traditional Chinese medicine) were purchased from the Shanghai Chinese Medicinal Herbs Corporation and identified by Professor Zhi-wei Wang, Department of Pharmacognosy, Shanghai Medical University. A voucher specimen is deposited in the Herbarium of Shanghai Institute of Materia Medica, Chinese Academy of Sciences.

Extraction and isolation. Powdered plant roots were extracted with 95% EtOH. The EtOH extracts, after removal of the solvent, were suspended in H_2O and then extracted with petrol, Et_2O and *n*-BuOH successively to yield fractions of 1000, 400 and 300 g, respectively. The Et_2O extracts were subjected to CC on silica gel, eluting with petrol–Me_2CO (3:1) to afford **1** (30 mg), **2** (150 mg) and **3** (400 mg).

Langduin A (**1**). Needles, mp 200–201°, $[\alpha]_D^{29}+122.2°$. HR-EIMS *m/z*: 348.1931 [M^+], $C_{20}H_{28}O_5$ required: 348.1937. IR ν_{max}^{KBr} cm^{-1}. 3400 (OH), 1690 (C=O), 1640 (C = C); ^1H and ^{13}C NMR: Table 1.

12-Deoxyphorbol-13-hexadecanoate (**2**). Resin, $[\alpha]_D^{12}+43.2°$ (*c* 0.21, EtOH). HRMS *m/z*: 586.4226 [M]$^+$, $C_{36}H_{58}O_6$ required: 586.4233. EIMS *m/z* 586, 568, 550, 429, 361, 330, 312 (100), 294; UV λ_{max}^{EtOH} nm (log ε): 237 (3.91); IR ν_{max}^{KBr} cm^{-1} 3410 (OH), 1710 (C=O), 1630 (C=C); ^1H and ^{13}C NMR: Table 1.

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