

Tigliane-Type Diterpenoid Glycosides from *Euphorbia fischeriana*

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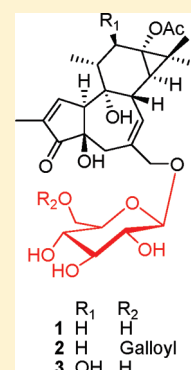
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ABSTRACT: Investigation of whole plants of *Euphorbia fischeriana* afforded three new tigliane-diterpenoid glycosides, fischerosides A–C (1–3), together with 11 known diterpenoids. Fischerosides A–C (1–3) are the first tigliane-type diterpenoid glucosides. Their structures were determined by a combination of 1D and 2D NMR, MS, and acid hydrolysis. Inhibitory activity against HIV-1 was assessed for compounds 1–5. The new compound 3 showed an EC₅₀ value of 0.02 μM and a therapeutic index (TI) of 17.50, while prostratin (4) and 12-deoxyphorbol-13,20-diacetate (5) showed significantly greater anti-HIV-1 activity.



Euphorbia fischeriana Steud (Euphorbiaceae), with the Chinese name “lang-du”, is a perennial herbaceous plant with a milky juice and is distributed mainly in northeastern China. It has been used as a remedy for the treatment of edema, ascites, and cancer in Traditional Chinese Medicine for a long time.¹ Qin's group and others have reported a variety of structurally interesting diterpenoids from the roots of *E. fischeriana*.^{2–8} Among these diterpenoids, jolkinolides A (11) and B (13) showed cytotoxic activities toward sarcoma 180, Ehrlich ascites, and HeLa cells, while 17-hydroxyjolkinolide B (14) was claimed to be a promising anticancer drug candidate as a potent STAT3 signaling inhibitor.^{9–11} Perhaps the most interesting compound from *E. fischeriana* and other species of Euphorbiaceae is prostratin (4, 12-deoxyphorbol-13-acetate), a non-tumor-promoting 12-deoxytigliane diterpenoid that exhibits potent *in vitro* activity in inducing HIV expression in latently infected cell lines and primary cells.^{12–14} Although the mechanism of action has not yet been completely elucidated, prostratin has been advanced into preclinical trials. The challenge of its semi- or total synthesis has drawn great interest in recent years.^{15,16}

These results encouraged us to investigate the whole plants of *E. fischeriana* for new analogues of prostratin^{8,12–14} and led to the isolation of three new tigliane-type diterpenoid glycosides, fischerosides A–C (1–3). Eleven known diterpenoids were also isolated: prostratin (4),⁸ 12-deoxyphorbol-13,20-diacetate (5),¹⁷ 12-deoxyphorbol-13-hexadecanoate (6),⁸ 12-deoxyphorbolaldehyde-13-hexadecanoate (7),⁸ langduin A (8),³ langduin D (9),⁶ *ent*-13S-hydroxy-16-atrisene-3,14-dione (10),¹⁸ jolkinolide A (11),⁹

17-hydroxyjolkinolide A (12),⁴ jolkinolide B (13),⁹ and 17-hydroxyjolkinolide B (14).⁹ Fischerosides A–C (1–3) are the first tigliane-type diterpenoid glycosides. This paper describes the isolation, structural elucidation, and anti-HIV-1 activities of the new compounds.

Compound 1 was isolated as a white, amorphous powder and had the molecular formula C₂₈H₄₀O₁₁, deduced from its high-resolution electrospray ionization mass spectrometry (HRESIMS) at *m/z* 575.2464 [M + Na]⁺ (calcd 575.2468) and ¹³C NMR data (Table 1), indicating nine degrees of unsaturation. The absorption bands in the IR spectrum at 1705 and 1629 cm⁻¹ and the UV maximum at 210 nm indicated the presence of an α,β-unsaturated carbonyl functionality in 1, as in prostratin (4). The ¹H NMR spectrum of 1 (Table 1) showed four methyl protons at δ_H 1.12 (d, *J* = 6.0 Hz), 1.17 (s), 1.19 (s), and 1.64 (s), two trisubstituted olefinic proton signals at δ_H 6.06 (d, *J* = 5.2 Hz) and 7.67 (br s), one acetate proton signal at δ_H 2.05 (s), and an anomeric proton at δ_H 4.87 (d, *J* = 7.8 Hz). The ¹³C and DEPT NMR spectrum (Table 1) displayed signals of two carbonyls, two double bonds, and five methyl, three methylene (one oxygenated one), four methine, and four quaternary carbons (three oxygenated ones). Glucopyranosyl signals were also identified at δ_C 104.6 (d), 75.2 (d), 78.7 (d), 71.9 (d), 78.6 (d), and 62.7 (t), which was confirmed by the results of acid

Received: January 19, 2011

Published: May 02, 2011

Table 1. ^1H and ^{13}C NMR Data for Compounds 1–3

position	1 ^a		2 ^b		3 ^b	
	δ_{H} (J in Hz)	δ_{C}	δ_{H} (J in Hz)	δ_{C}	δ_{H} (J in Hz)	δ_{C}
1	7.67 br s	160.1, CH	7.47 br s	161.3, CH	7.57 br s	161.1, CH
2		133.6, C		134.9, C		134.5, C
3		209.4, C		210.8, C		210.7, C
4		74.2, C		74.7, C		74.8, C
5	3.01 br s (2H)	39.1, CH ₂	2.50 m 2.53 m	39.0, CH ₂	2.52 d (18.1) 2.57 d (18.1)	39.0, CH ₂
6		138.6, C		138.8, C		139.7, C
7	6.06 d (5.2)	132.2, CH	5.63 d (5.1)	133.6, CH	5.68 d (4.8)	132.4, CH
8	3.64 m	39.7, CH	3.07 t (4.9)	40.0, CH	3.27 m	40.2, CH
9		76.5, C		77.9, C		79.9, C
10	3.65 m	57.3, CH	3.19 m	57.1, CH	3.16 m	57.7, CH
11	2.45 m	37.1, CH	1.96 m	37.5, CH	2.01 m	46.1, CH
12	1.91 dd (11.0, 14.5) 2.32 dd (6.6, 14.4)	33.1, CH ₂	1.52 dd (10.9, 14.7) 2.07 dd (7.0, 14.7)	33.0, CH ₂	3.91 d (9.9)	77.6, CH
13		64.6, C		64.6, C		69.5, C
14	1.06 d (5.7)	32.5, CH	0.80 d (5.8)	32.9, CH	1.07 m	36.6, CH
15		23.5, C		24.2, C		27.2, C
16	1.17 s	23.3, CH ₃	1.11 s	23.3, CH ₃	1.21 s	17.5, CH ₃
17	1.19 s	15.9, CH ₃	1.02 s	15.9, CH ₃	1.22 s	24.1, CH ₃
18	1.12 d (6.0)	19.4, CH ₃	0.86 d (6.5)	19.1, CH ₃	1.05 d (6.6)	15.5, CH ₃
19	1.64 s	10.5, CH ₃	1.60 d (1.5)	10.2, CH ₃	1.74 d (1.5)	10.2, CH ₃
20	4.22 m 4.59 d (11.5)	75.3, CH ₂	4.12 d (11.8) 4.16 d (11.8)	76.6, CH ₂	4.07 d (11.7) 4.22 d (11.7)	76.0, CH ₂
Ac		173.0, C		175.0, C		176.0, C
	2.05 s	21.2, CH ₃	2.04 s	21.1, CH ₃	2.10 s	21.1, CH ₃
1'	4.87 d (7.8)	104.6, CH	4.28 d (7.8)	103.5, CH	4.26 d (7.8)	103.9, CH
2'	3.99 m	75.2, CH	3.22 m	75.1, CH	3.15 dd (7.8, 9.0)	75.2, CH
3'	3.89 m	78.7, CH	3.37 m	77.9, CH	3.31 m	78.1, CH
4'	4.18 m	71.6, CH	3.41 m	71.5, CH	3.24 m	71.7, CH
5'	4.20 m	78.6, CH	3.47 m	75.5, CH	3.22 m	78.1, CH
6'	4.35 dd (5.3, 12.0) 4.53 dd (2.0, 12.0)	62.7, CH ₂	4.34 dd (5.3, 11.9) 4.55 dd (1.7, 11.9)	65.1, CH ₂	3.61 dd (5.1, 11.9) 3.83 d (12.4)	62.8, CH ₂
1''				168.2, C		
2''				121.4, C		
3'', 7''			7.06 s	110.1, CH		
4'', 6''				146.5, C		
5''				139.8, C		

^a Recorded in pyridine-*d*₅, 400 MHz for δ_{H} , 100 MHz for δ_{C} . ^b Recorded in methanol-*d*₄, 500 MHz for δ_{H} , 125 MHz for δ_{C} .

hydrolysis. Comparison of the NMR spectroscopic data of **1** (Table 1) with those obtained for prostratin (**4**) indicated that compound **1** is a glycoside derivate of prostratin, which was supported by the ^1H – ^1H COSY, HMBC (Figure 1), and ROESY spectra. The glucopyranosyl moiety attached to C-20 could be determined by the HMBC correlations from H-1' (δ_{H} 4.87) to C-20 (δ_{C} 75.3). Thus, the structure of **1** was deduced as prostratin 20-*O*- β -D-glucopyranoside, named fischeroside A. This is first example of a tigliane-type diterpenoid glycoside.

Compound **2** gave a molecular formula of C₃₅H₄₄O₁₅ by its HRESIMS at *m/z* 727.2587 [*M* + Na]⁺ (calcd 727.2577) and ^{13}C NMR data (Table 1). The NMR data (Table 1) were similar to those of **1**, with an apparent difference being the presence of a galloyl group [δ_{H} 7.06, (s, H-3'' and H-7''); δ_{C} 168.2 (s, C-1''),

121.4 (s, C-2''), 110.1 (d, C-3'' and C-7''), 146.5 (s, C-4'' and -6''), and 139.8 (s, C-5'')] in **2**, which was confirmed by the mass difference of *m/z* 152 (C₇H₄O₄) between the two compounds. Furthermore, the position of the galloyl group at C-6' of the glucopyranosyl moiety was determined by the HMBC correlations (Figure 1) from H₂-6' [δ_{H} 4.34 (dd, *J* = 5.3, 11.9 Hz) and 4.55 (dd, *J* = 1.7, 11.9 Hz)] to C-1'' (δ_{C} 168.2). On the basis of the above evidence, the structure of **2** was proved to be prostratin 20-*O*-(6'-galloyl)- β -D-glucopyranoside, named fischeroside B.

Compound **3** exhibited a molecular formula of C₂₈H₄₀O₁₂ as derived from the quasimolecular ion peak at *m/z* 591.2413 [*M* + Na]⁺ (calcd 591.2417) in the HRESIMS and ^{13}C NMR data (Table 1), indicating one additional oxygen atom in comparison to compound **1**. The ^1H and ^{13}C NMR data of **3** (Table 1) were

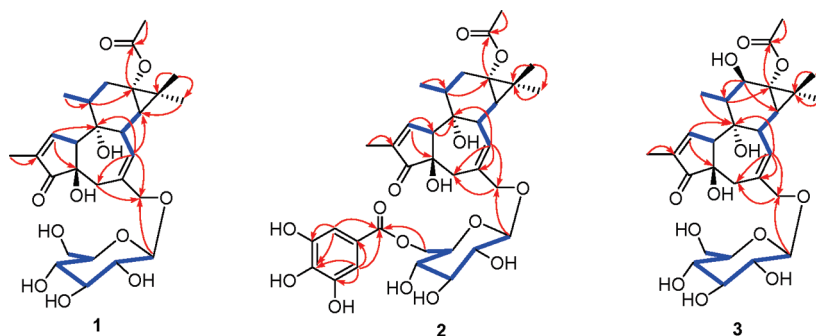


Figure 1. ^1H – ^1H COSY (blue lines) correlations and key HMBC (red arrows) for 1–3.

Table 2. Anti-HIV-1 Activities of Compounds 1–5

compound	cytotoxicity, CC_{50} (μM)	anti-HIV-1 activity, EC_{50} (μM)	therapeutic index (TI), $\text{CC}_{50}/\text{EC}_{50}$
1	>0.36	0.17	>2.12
2	>0.28	0.07	>4.00
3	>0.35	0.02	>17.50
4	>0.51	0.00006	>8500
5	0.11	0.0003	>366.67
AZT	4.07	0.000004	1017500

similar to those of **1** except for the absence of a methylene and the presence of a hydroxy group located at C-12 (δ_{C} 77.6) and an oxymethine proton (δ_{H} 3.91, d, $J = 9.9$ Hz) in compound **3**. Therefore, it was supposed that the aglycone of compound **3** was phorbol-13-acetate. This was confirmed by the proton spin system comprising H-11 (δ_{H} 2.01) with H-12 (δ_{H} 3.91) and Me-18 (δ_{H} 1.05) in the ^1H – ^1H COSY spectra and the HMBC correlations (Figure 1) from H-12 (δ_{H} 3.91) to C-11 (δ_{C} 46.1), C-13 (δ_{C} 69.5), C-15 (δ_{C} 27.2), and Me-18 (δ_{C} 15.5). The ROESY correlations of H-12 with H-14 and Me-18 suggested the β -orientation for OH-12. As a result, the structure of **3** was elucidated as phorbol-13-acetate 20-*O*- β -D-glucopyranoside, named fischeroside C.

Compounds **1**–**5** were tested for cytotoxicities against C8166 cells (CC_{50}), and anti-HIV-1 activities were evaluated by the inhibition assay for the cytopathic effects of HIV-1 (EC_{50}), using AZT as a positive control. The results are summarized in Table 2. Compound **3** showed weak activity in preventing the cytopathic effects of HIV-1 in C8166 cells with an EC_{50} of 0.02 μM and a therapeutic index (TI) of 17.50. 12-Deoxyphorbol-13,20-diacetate (**5**) displayed significant anti-HIV-1 activity, with an EC_{50} of 0.003 μM and a TI of 366.67, while prostratin (**4**) showed the strongest anti-HIV-1 activity, with an EC_{50} of 0.00006 μM and a TI of 8500. This assay demonstrated that introducing an *O*-acetyl or glucopyranosyl moiety at C-20 of prostratin (**4**) may dramatically reduce its anti-HIV-1 activity.

EXPERIMENTAL SECTION

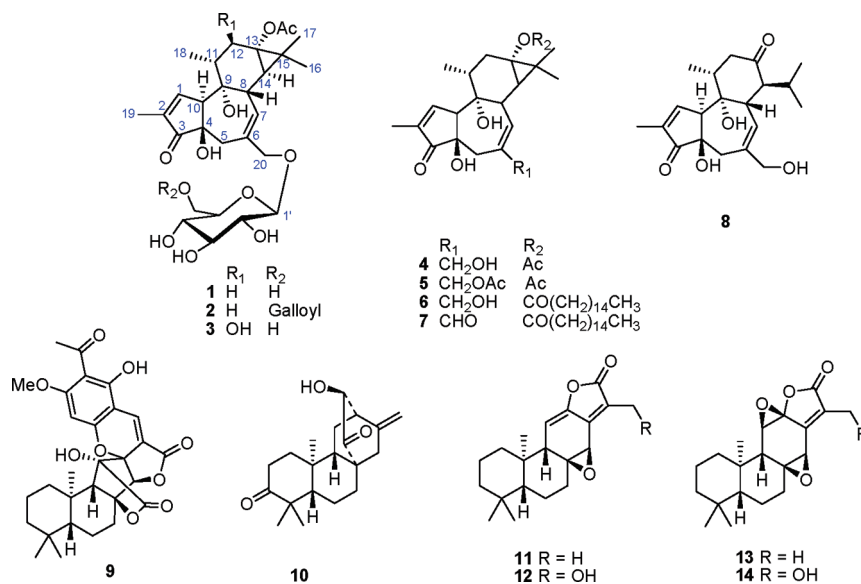
General Experimental Procedures. Optical rotations were recorded on a Horiba SEAP-300 polarimeter. UV spectra were obtained on a Shimadzu UV-2401PC spectrophotometer. IR spectra were measured on a Bio-Rad FTS-135 spectrometer with KBr pellets. NMR spectra were recorded on Bruker AM-400 and DRX-500 instruments.

Chemical shifts (δ) are expressed in ppm with reference to the solvent signals. ESIMS and HRESIMS were carried out on an API QSTAR TOF spectrometer. Semipreparative HPLC was performed on an Agilent 1100 liquid chromatograph with a Zorbax SB-C₁₈ (9.4 mm \times 25 cm) column. Column chromatography was performed on either silica gel (200–300 mesh, Qindao Marine Chemical Inc., Qindao, People's Republic of China), RP-18 gel (LiChroprep, 40–63 μm , Merck, Darmstadt, Germany), or Sephadex LH-20 (Amersham Biosciences, Sweden). Fractions were monitored by TLC, and spots were detected with a UV₂₅₄ lamp and by spraying with 10% H₂SO₄ in EtOH followed by heating at 120 $^{\circ}\text{C}$ for 5 min.

Plant Material. Whole plants of *E. fischeriana* were collected in June 2009 from Panshi, Jilin Province, People's Republic of China, and identified by Dr. En-De Liu of Kunming Institute of Botany. A voucher specimen (No. HY0004) was deposited at the State Key Laboratory of Phytochemistry and Plant Resources in West China.

Extraction and Isolation. The dried and powdered plant material (24 kg) was extracted with 90% EtOH (3 \times 60 L) under reflux for a total of 6 h. After removal of the solvent in vacuo, the residue (4.7 kg) was suspended in H₂O (20 L) and successively extracted with EtOAc (3 \times 20 L). The solvent was removed to give the EtOAc extract (2.25 kg), which was subjected to column chromatography over silica gel eluting with petroleum ether–acetone (from 50:1 to 2:1) to give four fractions, A–D. Fraction D (434 g) was subjected to an MCI gel CHP20P column eluted with a MeOH–H₂O gradient system (7:3 to 9:1) to give two subfractions, D₁ and D₂. Fraction D₁ (67.0 g) was separated on a silica gel column (CHCl₃–MeOH, 80:1 to 10:1) to afford six further fractions, D₁₁–D₁₆. Fraction D₁₁ (1.78 g) was purified by semipreparative HPLC (MeOH–H₂O, 80:20; flow rate 3 mL/min) to give **5** (25 mg). Fraction D₁₂ (8.94 g) was chromatographed on silica gel eluting with petroleum ether–EtOAc (10:1 to 5:1) to afford compound **4** (75 mg). Compound **8** (1.34 g) was obtained from fraction D₁₃ (6.97 g) after being purified on silica gel eluting with CHCl₃–MeOH (30:1 to 20:1). Fraction D₁₄ (8.47 g) was chromatographed on MPLC eluted with petroleum ether–EtOAc (5:1 to 0:1) to afford **1** (59 mg). Fraction D₁₅ (2.73 g) was subjected to a silica gel column eluted with EtOAc, followed by a Sephadex LH-20 column (MeOH), to yield **3** (25 mg). Fraction D₁₆ (1.25 g) was passed through a Sephadex LH-20 column (MeOH) and further purified by semipreparative HPLC (MeOH–H₂O, 45:55; flow rate 3 mL/min) to give **2** (23 mg). Fraction D₂ (230 g) was separated on a silica gel column (CHCl₃–MeOH, 100:1 to 10:1) to afford three combined fractions, D₂₁–D₂₃. Fraction D₂₁ (16.49 g) was passed through a Sephadex LH-20 column (MeOH) followed by silica gel eluting with petroleum ether–EtOAc (100:1 to 40:1) to give **9** (163 mg). Compound **7** (74 mg) was obtained from fraction D₂₂ by a Sephadex LH-20 column (MeOH). Fraction D₂₃ (5.52 g) was subjected to silica gel, eluting with petroleum ether–EtOAc (10:1 to 1:1), to give **6** (65 mg). Fraction C (9.73 g) was subjected to an MCI gel column eluted with a MeOH–H₂O gradient system (70% and 90%) to

Chart 1



give two subfractions, C₁ and C₂. Fraction C₁ (4.5 g) was separated on a silica gel column (CHCl₃–MeOH, 100:1 to 10:1) to afford two combined fractions, C₁₁ and C₁₂. Compound **10** (51 mg) was obtained after being crystallized in MeOH from fraction C₁₁. Fraction C₁₂ was purified on silica gel eluting with CHCl₃–MeOH (150:1 to 30:1) to give **14** (32 mg). Compounds **11** (231 mg), **12** (1.087 g), and **13** (179 mg) were obtained after being crystallized in petroleum ether–CHCl₃ from fractions A, B, and C₂, respectively.

Fischeroside A (1): white, amorphous powder; [α]_D¹⁷ +15.2 (c 0.12, MeOH); UV (MeOH) λ_{\max} (log ϵ) 238 (3.35), 210 (3.38) nm; IR (KBr) ν_{\max} 3420, 2983, 1705, 1629, 1378, 1263, 1078, 1044 cm⁻¹; ¹H and ¹³C NMR data, see Table 1; positive ESIMS m/z 575 [M + Na]⁺; positive HREIMS m/z 575.2464 [M + Na]⁺ (calcd for C₂₈H₄₀O₁₁Na, 575.2468).

Fischeroside B (2): white, amorphous powder; [α]_D¹⁷ +16.2 (c 0.17, MeOH); UV (MeOH) λ_{\max} (log ϵ) 272 (3.64), 218 (4.09) nm; IR (KBr) ν_{\max} 3423, 2824, 1700, 1616, 1450, 1378, 1333, 1233, 1077, 1039 cm⁻¹; ¹H and ¹³C NMR data, see Table 1; positive ESIMS m/z 727 [M + Na]⁺; positive HREIMS m/z 727.2587 [M + Na]⁺ (calcd for C₃₅H₄₄O₁₅Na, 727.2577).

Fischeroside C (3): white, amorphous powder; [α]_D¹⁷ +38.0 (c 0.11, MeOH); UV (MeOH) λ_{\max} (log ϵ) 232 (3.41), 210 (3.51) nm; IR (KBr) ν_{\max} 3423, 2824, 1700, 1628, 1378, 1264, 1078, 1043 cm⁻¹; ¹H and ¹³C NMR data, see Table 1; positive ESIMS m/z 591 [M + Na]⁺; positive HREIMS m/z 591.2413 [M + Na]⁺ (calcd for C₂₈H₄₀O₁₂Na, 591.2417).

Acid Hydrolysis of Compounds 1–3. Compound **1** (10 mg) was hydrolyzed with 4 M TFA–dioxane (1:1 v/v, 2 mL) for 4 h at 90 °C. After cooling, the reaction mixture was neutralized with 1 M NaOH and filtered. The filtrate was extracted with CHCl₃. Through TLC comparison with an authentic sample using CHCl₃–MeOH–H₂O (8:2:0.2) as a developing system, D-glucose was detected in the water layer (R_f = 0.11). The aqueous solution was further concentrated to dryness and subjected to silica gel chromatography eluting with CHCl₃–MeOH (9:1) to give D-glucose (2 mg), [α]_D¹⁷ +40.0 (c 0.2, MeOH). By the same method used for **1**, the sugar moiety in compounds **2** and **3** was identified as D-glucose.

Anti-HIV-1 Assay. Cytotoxicity against C8166 cells (CC₅₀) was assessed using the MTT method, and anti-HIV-1 activity was evaluated

by the inhibition assay for the cytopathic effects of HIV-1 (EC₅₀).¹⁹ AZT (zidovudine) was used as the positive control anti-HIV-1 drug.

ASSOCIATED CONTENT

Supporting Information. 1D and 2D NMR (HSQC, COSY, HMBC, ROESY) spectra for fischerosides A–C (**1–3**) are available free of charge via the Internet at <http://pubs.acs.org>.

AUTHOR INFORMATION

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ACKNOWLEDGMENT

This work was supported by the Young Academic and Technical Leader Raising Foundation of Yunnan Province (2008PY066) and funds (P2010-ZZ02) from the State Key Laboratory of Phytochemistry and Plant Resources in West China.

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