Diterpenoids from the Roots of *Euphorbia fischeriana*

Yu-Bo Wang,[†] Rong Huang,[§] Hong-Bing Wang,[†] Hui-Zi Jin,[†] Li-Guang Lou,[†] and Guo-Wei Qin*[,†]

Shanghai Institute of Materia Medica, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai 201203, People's Republic of China, and Laboratory of Analytical Chemistry, Yunnan University, Kunming 650091, People's Republic of China

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From the dried roots of *Euphorbia fischeriana*, seven new diterpenoids, 3α,17-dihydroxy-*ent*-pimara-8(14),15-diene (**1**), 7*β*,11*β*,12*β*-trihydroxy-*ent*-abieta-8(14),13(15)-dien-16,12-olide (**2**), 17-acetoxyjolkinolide B (**3**), 13*β*-hydroxy-*ent*-abiet-8(14)-en-7-one (**4**), 12-deoxyphorbaldehyde-13-acetate (**5**) 12-deoxyphorbaldehyde-13-hexadecacetate (**6**), and 12-deoxyphorbol-13-(9*Z*)-octadecanoate-20-acetate (**7**), and two known compounds, 12-deoxyphorbol-13-decanoate (**8**) and prostratin (**9**), were isolated. The structures of the new compounds were elucidated on the basis of spectroscopic analysis. The structure of compound **1** was confirmed by single-crystal X-ray crystallography. Compounds **3** and **8** exhibited potent cytotoxic activity to Ramos B cells with IC_{50} values of 0.023 and 0.0051 *μ*g/mL, respectively.

Euphorbia fischeriana Steud (Euphorbiaceae) is a perennial herbaceous plant distributed widely in northeast mainland China. The dried plant roots, named "lang-du" in traditional Chinese medicine, are used as a remedy for the treatment of edema, ascites, and cancer.[1] The chemical constituents of the roots have been investigated and a variety of diterpenoids have been reported.[2−7] Two *ent*-abietane diterpenoids, jolkinolides A and B, the major components of the roots, showed cytotoxic activities toward sarcoma 180, Ehrlich ascites, and Hela cells.[8,9] Recently, it has been shown that 12-deoxyphorbol-13-acetate (**9**, prostratin), a known tigliane diterpenoid found in *E. fishceriana* and in other species of Euphorbiaceae, is a protein kinase C activator, potentially useful in the treatment of HIV, as it affects viral reservoirs in latently infected CD4+ T-cells.[10] These results encouraged us to reinvestigate the roots of *E. fischeriana* for additional new bioactive compounds, which has led to the isolation of seven new diterpenoids (**1**−**7**) and two known diterpenoids (**8** and **9**). The compounds isolated were evaluated for cytotoxicity against Ramos B cells.

Compound **1** was obtained as colorless needles and exhibited a molecular ion peak in the HREIMS at *m*/*z* 304.2400, associated with the molecular formula $C_{20}H_{32}O_2$ (calcd 304.2402). The IR spectrum showed absorptions due to hydroxyl (3417 cm^{-1}) and olefinic functionalities (1635 cm^{-1}). The ^1H NMR spectrum exhibited resonances for protons of an ABX system at *δ* 5.06 (dd, $J = 1.8$, 17.3 Hz, H-16a), 5.21 (dd, $J = 1.8$, 10.4 Hz, H-16b), and 5.67 (dd, $J = 10.4$, 17.4 Hz, H-15), an olefinic proton at *δ* 5.35 (1H, d, $J = 1.6$ Hz, H-14), and an oxygenated methine at *δ* 3.26 (1H, dd, $J = 4.0$, 11.6 Hz, H-3*β*), which were very similar to analogous data of 3α-hydroxy-*ent*-pimara-8(14),15-diene.[11] However, **1** exhibited an additional oxygenated methylene signal at *δ* 3.33 (2H, ABq, $J = 10.4$ Hz) instead of a methyl singlet at C-17, indicating that **1** is a derivative of 3α-hydroxy-*ent*-pimara-8(14),15-diene with one more hydroxyl group at C-17. In the HMBC NMR spectrum, the proton at *δ* 3.33 showed long-range correlations with signals at *δ* 29.8 (C-12), 122.9 (C-14), and 143.0 (C-15), supporting the placement of a CH_2OH group at C-17. The proposed relative structure of **1** was confirmed by X-ray crystallography analysis (Figure 1). Therefore, **1** was determined as 3α,17-dihydroxy-*ent*-pimara-8(14),15-diene.

Compound **2** was assigned as $C_{20}H_{28}O_5$ ($[M]^+$, *m*/*z* 348.1943) by HREIMS. The UV and IR spectroscopic data showed absorptions due to hydroxyl groups (3374, 3230 cm^{-1}) and an *α*,*β*,*γ*,*δ*-unsaturated-*γ*-lactone (276 nm, 1718, 1594 cm^{-1}). The ^1H and ^{13}C NMR spectra (Table 1) showed signals for three tertiary methyls (*δ* 0.59, 0.72, 0.81), one olefinic methyl (*δ* 1.65), two oxygenated methines (*δ* 3.65, 4.25), one olefinic proton (*δ* 6.23), and seven quaternary carbons (one oxygenated, one carbonyl, and three olefinic). The above-mentioned data are very similar to those of langduin B [7*β*,11*β*,12*β*,17-tetrahydroxy-*ent*-abieta-8(14),13(15)-dien-16,12-olide], isolated from the same plant,[4] except for the presence of one more methyl group at δ_H 1.65/δ_C 8.4 instead of

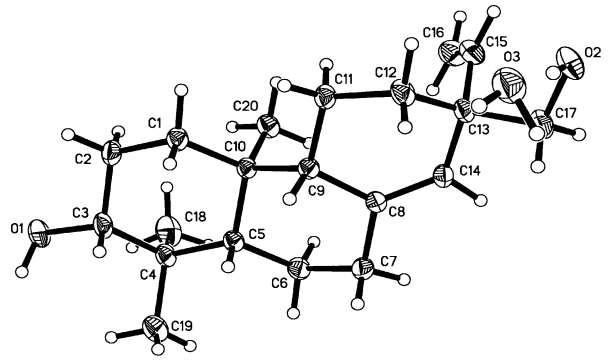
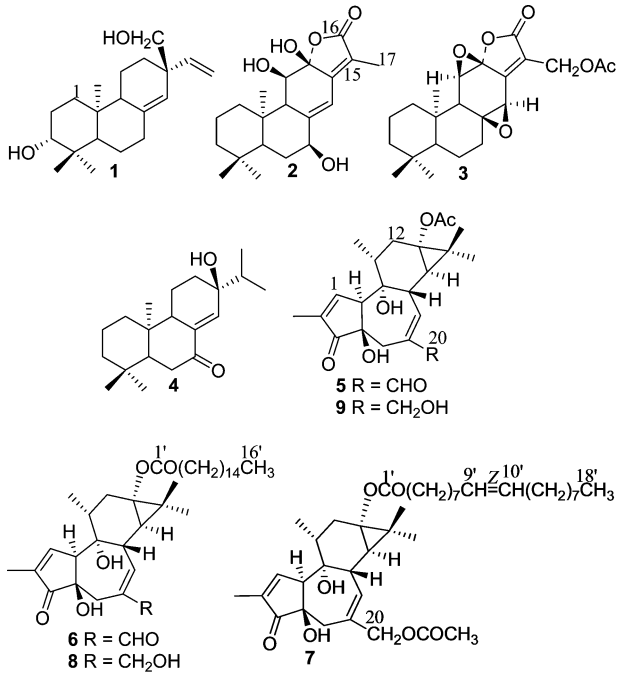


Figure 1. ORTEP structure of compound **1**.



* To whom correspondence should be addressed. Tel: +86-21-50805853. Fax: +86-21-50807088. E-mail: gwqin@mail.shcnc.ac.cn.

[†] Shanghai Institute of Materia Medica.

[§] Yunnan University.

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Table 1. ^{13}C NMR Data of Compounds **1**−**7**

position	1	2	3	4	5	6	7
1	37.1	39.6	41.3	38.9	160.4	160.5	161.3
2	27.6	18.8	18.5	18.4	133.5	133.5	132.9
3	79.1	41.7	39.0	41.8	208.3	208.4	208.9
4	39.0	32.9	33.5	33.2	72.8	72.8	73.7
5	54.0	46.8	53.5	49.8	34.4	34.6	38.9
6	22.1	29.9	20.9	37.5	142.9	142.9	135.2
7	35.7	71.5	35.7	200.7	158.1	158.2	133.7
8	141.3	153.4	67.4	138.9	41.4	41.5	39.5
9	51.6	54.8	47.8	51.8	77.1	77.1	75.9
10	38.3	40.9	39.3	35.9	55.8	55.8	55.8
11	18.5	69.6	61.9	18.6	36.5	36.5	36.3
12	29.8	102.7	85.3	29.7	31.7	31.8	31.9
13	45.1	152.8	154.5	71.8	63.0	63.0	63.6
14	122.9	114.7	55.3	139.5	32.0	32.1	32.4
15	143.0	121.0	128.3	37.8	22.9	22.7	22.6
16	117.4	173.6	167.4	17.6	23.1	23.2	23.2
17	70.5	8.4	54.9	16.2	15.3	15.3	15.3
18	28.4	32.9	33.5	32.6	18.5	18.6	18.6
19	15.7	21.6	21.9	21.2	10.1	10.1	10.1
20	14.7	14.3	15.1	14.1	193.8	193.8	69.4

the C-17 oxygenated methylene of langduin B. The HMBC correlations of H-7 with C-5, C-9, and C-13, H-11 with C-8 and C-10, H-14 with C-9, C-12, and C-15, and H-17 with C-13 and C-16 and the NOE correlations of H-20/H-11 and H-20/H-7 were helpful in determining the structure of **2**. On the basis of the above evidence, **2** was elucidated as 7β,11β,12β-trihydroxy-*ent*-abieta-8(14),13(15)-dien-16,12-olide.

Compound **3** exhibited a molecular formula of $C_{22}H_{28}O_6$ by HREIMS (*m*/*z* 388.1894 $[M]^+$). It gave IR bands for a carbonyl group (1789 cm^{-1}) and a double bond (1685 cm^{-1}). The ^1H NMR spectrum showed the signals for three tertiary methyls (δ_H 0.80, 0.86, 0.94) and two singlet oxygenated methines (δ_H 4.06, 3.99) and the characteristic proton signals of the C-8, 14 and C-11, C-12 epoxy groups in jolkinolide B. Further scrutiny of the ^1H and ^{13}C NMR data also revealed the similarity of **3** to 17-hydroxyjolkinolide B,[12] except for an additional acetyl group in **3** (δ_H 2.11, 3H, s and δ_C 20.7, 170.4), indicating that this isolate is an acetyl derivative of 17-hydroxyjolkinolide B. Furthermore, 17-hydroxyjolkinolide B, a known diterpenoid from the same plant, was acetylated by Ac_2O/pyridine to give an acetate derivative whose ^1H NMR spectroscopic data were identical to those of **3**. On the basis of all of the available information, compound **3** was identified as 17-acetoxyjolkinolide B.

Compound **4**, an amorphous powder, was assigned a molecular formula of $C_{20}H_{32}O_2$ by HREIMS (*m*/*z* 304.2383 $[M]^+$). An α,β-unsaturated ketone group was suggested by the UV (λ_{max} 246 nm, log ϵ 4.54), IR (ν_{max} 1681, 1608 cm^{-1}), and NMR data (δ_H 6.73, 1H, s; δ_C 139.5, 138.9, and 200.7). The ^{13}C NMR and DEPT spectroscopic data revealed the signals for five methyls, six methylenes, four methines (one olefinic), and five tertiary carbons (one oxygenated, one carbonyl). The ^1H NMR spectrum showed also the presence of an isopropyl group (δ_H 0.86, 3H, d, J = 6.9 Hz, 0.96, 3H, d, J = 6.9 Hz, 1.75, 1H, m). This evidence together with the ^{13}C NMR data indicated that **4** is an abietane diterpenoid possessing a structure very similar to those of 13β-hydroxy-abiet-8(14)-en-19-al-7-one[13] and 13β,18-dihydroxy-abiet-8(14)-en-7-one.[14] Furthermore, the C-7 carbonyl, the C-8, C-14 double bond, and the C-13 hydroxyl and isopropyl groups were confirmed by the following HMBC correlations: H-15 with C-14; H-14 with C-7, C-9, C-12; H-16 with C-13, C-14; H-6 with C-5, C-7, C-10; H-20 with C-1, C-5, C-9; and H-18 with C-3, C-5, C-19. Therefore, on the basis of the biogenetic considerations,[15] the structure of compound **4** was assigned as 13β-hydroxy-*ent*-abiet-8(14)-en-7-one.

Compound **5** exhibited a molecular formula of $C_{22}H_{28}O_6$ by HREIMS (*m*/*z* 388.1873, $[M]^+$). The IR spectrum showed the presence of hydroxyl (3382 cm^{-1}), carbonyl (1708 cm^{-1}), and double-bond (1664 cm^{-1}) absorptions. On the basis of the UV maximum at 240 nm (log ϵ 4.13), **5** was suggested to contain an α,β-unsaturated carbonyl group. The ^{13}C NMR data (Table 1) revealed signals for five methyls (one acetyl), two methylenes, seven methines (one aldehyde, two olefinic), and eight quaternary carbons (two olefinic, two carbonyls, three oxygenated). The ^1H NMR spectrum showed signals for four methyls (δ 0.89, d; 1.08, s; 1.25, s; 1.76, s), two olefinic methines (δ 6.71, q; 7.54, s), one acetate (δ 2.09, s), and one aldehyde (δ 9.41, s). The data obtained for **5** were very similar to that of 12-deoxyphorbol-13-acetate (**9**, prostratin),[16] except that **5** has an aldehyde group instead of a hydroxymethylene at C-20 as in **9**. This was supported by the long-range correlations of H-5/C-20 and H-7/C-20 in the HMBC spectrum. It is rare to find an aldehyde group present at C-20 in the tigliane nucleus. The first example was 12-[2-methylaminobenzoyl]-4-deoxyphorbaldehyde-13-acetate.[17] On the basis of the above spectroscopic information, the structure of **5** was deduced as 12-deoxyphorbaldehyde-13-acetate.

Compound **6**, a colorless oil, exhibited a molecular formula of $C_{36}H_{56}O_6$, as determined from the molecular ion peak at *m*/*z* 584.4077 in the HREIMS. Its ^1H and ^{13}C NMR data (Table 1) showed similarities to those of **5** except for the resonances for the ester residue. The NMR data at δ_H 1.24 (20H, brs) and δ_C 29.1−29.7 indicated that **6** has a long-chain aliphatic acid group instead of an acetate group in **5**. No doublet signals for any additional methyl group were observed in the ^1H NMR spectrum, indicating a linear aliphatic chain in the ester unit of **6**. The chain length of the ester moiety [R = $CO(CH_2)_{14}CH_3$] was deduced by subtracting the molecular weight of 388 of **5** from its $[M]^+$ peak and by comparison with the known compound 12-deoxyphorbol-13-hexadecanoate (**8**).[3] Thus, the structure of **6** was deduced as 12-deoxyphorbaldehyde-13-hexadecanoate.

Compound **7** was isolated as a colorless oil, with the molecular formula of $C_{40}H_{62}O_7$ from the HREIMS (*m*/*z* 654.4500, $[M]^+$) implying 10 degrees of unsaturation. Its EIMS fragment ion peaks at *m*/*z* 594 $[M - 60]^+$, 372 $[M - 282]^+$, 312 $[M - 282 - 60]^+$, and 294 $[M - 282 - 60 - 18]^+$, together with the main ^{13}C NMR data (Table 1), were similar to those of reported compounds, 12-deoxyphorbol-13-angelate-20-acetate[18] and 12-deoxyphorbol-13-phenylacetate-20-acetate,[18] indicating the presence of two ester chains that were elucidated as $[CH_3COO]^+$ and $[C_{17}H_{33}COO]^+$ by MS analysis. When comparing the data with those of prostratin (**9**),[16] one more unsaturated unit remained to be assigned. The NMR data at δ_H 5.31 (m, 2H), 2.25 (t, J = 7.5 Hz, 2H), and 1.22 (m) and δ_C at 130.0, 129.8, and 29.2−29.8 also indicated that **7** contains an aliphatic chain with one double bond with *cis* geometry.[19] Long-range correlations observed in the HMBC spectrum between H-20 and a carbonyl carbon signal at δ_C 173.1 confirmed that the acetyl group is present on C-20, with the aliphatic group thus placed at C-13. Compound **7** was hydrolyzed by 0.9 M HCl in 80% aqueous MeOH at 90 °C for 18 h[20] and gave the resultant fatty acid methyl ester that was analyzed as the methyl ester of 9(*Z*)-octadecenoic acid by GC-MS analysis. On the basis of this information, the structure of **7** was deduced as 12-deoxyphorbol-13-(9*Z*)-octadecenate-20-acetate.

The two known diterpenoids were identified as 12-deoxyphorbol-13-decanoate (**8**)[3] and prostratin (**9**)[16] by spectral determination.

All isolated compounds were evaluated for cytotoxicity against Ramos B cells. Among these, compounds **3** and **8** showed potent cytotoxic activities, with IC_{50} values of 0.023 and 0.0051 μg/mL, respectively, and were more active than **9** (IC_{50} 0.056 μg/mL).

Experimental Section

General Experimental Procedures. Optical rotations were measured on a Perkin-Elmer model 341 polarimeter. UV spectra were recorded on a Shimadzu UV-2550 UV−visible spectrophotometer. The FT-IR spectra were recorded on a Nicolet Magna FT-IR spectrophotometer. The NMR spectra were recorded on a Bruker AM-400 or an

AV-500 spectrometer with TMS as the internal standard. EI and HREIMS were carried out on a MAT 95 mass spectrometer. GC-MS was performed on a Voyager GC 800 Top spectrometer.

Plant Material. The roots of *E. fischeriana* were purchased from Shanghai Xuhui TCM factory in October 2003 and identified by Prof. Bing-Yang Ding, School of Life and Environmental Sciences, Wenzhou Normal College. A voucher specimen (No. 200310132) is deposited in the herbarium of Shanghai Institute of Materia Medica, Chinese Academy of Sciences.

Extraction and Isolation. The powdered roots of *E. fischeriana* (10 kg) were extracted with 95% EtOH, and the extract was evaporated under reduced pressure to give a residue and then suspended in distilled H_2O and partitioned successively with petroleum ether, $CHCl_3$, and *n*-BuOH. The $CHCl_3$ extract was evaporated to give a residue (883 g). The residue was applied in three portions to a silica gel column, eluting with petroleum ether containing increasing amounts of acetone to give six combined fractions according to TLC detection. The first fraction was subjected to silica gel, eluting with petroleum ether−acetone (30:1), to give **3** (15 mg). The second fraction was chromatographed on silica gel eluting with hexane−acetone (20:1), followed by further separation over LH-20, RP-18, and silica gel columns, to give compounds **6** (43 mg) and **7** (48 mg). Compounds **5** (7 mg), **4** (3 mg), and **8** (2.7 g) was obtained from the third fraction after being repeatedly purified on silica gel eluting with petroleum ether−acetone (15:1). The fourth fraction was further purified on silica gel columns and eluted with petroleum ether−acetone (10:1−5:1). On eluting with petroleum ether−acetone (10:1), compound **1** (18 mg) was obtained after being crystallized in MeOH. The remaining subfractions yielded compound **2** (18 mg) after being purified by LH-20, RP-18, and silica gel columns. Compound **9** (27 mg) was afforded after crystallization in acetone from the fifth fraction.

3α,17-Dihydroxy-*ent*-pimara-8(14),15-diene (1): colorless needles (MeOH); $[\alpha]^{24}$ −38.3 (*c* 0.47, MeOH); IR (KBr) ν_{max} 3417, 2937, 1635, 1035 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 1.16 (1H, m, H-1a), 1.63 (1H, m, H-1b), 1.55 (1H, m, H-2a), 1.19 (1H, m, H-2b), 3.26 (1H, dd, J = 4.0, 11.6 Hz, H-3), 1.06 (1H, dd, J = 2.6, 12.4 Hz, H-5), 1.62 (1H, m, H-6a), 1.41 (1H, m, H-6b), 2.38 (1H, m, H-7a), 2.10 (1H, m, H-7b), 1.69 (1H, m, H-9), 1.53 (1H, m, H-11a), 1.32 (1H, m, H-11b), 1.52 (1H, m, H-12a),1.35 (1H, m, H-12b), 5.35 (1H, d, J = 1.6 Hz, H-14), 5.67 (1H, dd, J = 10.4, 17.4 Hz, H-15), 5.21 (1H, dd, J = 1.8, 10.4 Hz, H-16a), 5.06 (1H, dd, J = 1.8, 17.3 Hz, H-16b), 3.33 (2H, ABq, J = 10.4 Hz, H-17), 1.01 (3H, s, H-18), 0.82 (3H, s, H-19), 0.72 (3H, s, H-20); ^{13}C NMR (100 MHz, $CDCl_3$), see Table 1; EIMS *m/z* 304 $[M]^+$ (5), 273 (100), 135 (74); HREIMS *m/z* 304.2400 $[M]^+$ (calcd for $C_{20}H_{32}O_2$, 304.2402).

X-ray Crystal Data of 1: $C_{20}H_{33}O_{2.50}$; crystal size (mm) 0.437 × 0.342 × 0.203, colorless prism; space group monoclinic, *C*2; unit cell dimensions a = 24.054(4) Å, b = 6.5688(12) Å, c = 11.748(2) Å; volume 1814.7(6) $Å^3$; Z = 4; formula weight 313.46; density(calcd) 1.147 g/cm^3; absorption coefficient 0.073 mm^{-1}; $F(000)$ = 692. The reflection data were collected on a Bruker Smart Apex CCD diffractometer, using graphite-monochromated Mo Kα radiation, λ = 0.71073 Å. A total of 4706 reflections were collected in the range $1.73° \le \theta \le 25.49°$, of which 3098 unique reflections with $I > 2\sigma(I)$ were utilized for the analysis and for refinement. The final R and R_w were 0.0780 and 0.1892, respectively, with a goodness-of-fit of 0.974. Crystallographic data for the structure reported in this paper have been deposited with the Cambridge Crystallographic Data Centre, deposit No. CCDC 294392. Copies of the data can be obtained, free of charge, on application to the Director, CCDC, 12 Union Road, Cambridge CB21EZ, UK [fax: +44-(0)-1223-336033 or e-mail: deposit@ccdc.cam.ac.uk].

7β,11β,12β-Trihydroxy-*ent*-abieta-8(14),13(15)-dien-16,12-olide (2): white amorphous powder; $[\alpha]^{24}$ −53.4 (*c* 0.61, MeOH); UV (MeOH) λ_{max} 276 (log ϵ 4.51) nm; IR (KBr) ν_{max} 3374, 3230, 1718, 1594, 1402, 1265, 1072 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$+CD_3OD) δ 1.36 (1H, m, H-1a), 1.13 (1H, m, H-1b), 1.35 (1H, m, H-2), 1.34 (1H, m, H-3a), 1.14 (1H, m, H-3b), 1.38 (1H, m, H-5), 1.78 (1H, m, H-6a), 1.49 (1H, m, H-6b), 4.25 (1H, brs, H-7), 2.35 (1H, d, J = 5.7 Hz, H-9), 3.65 (1H, d, J = 6.6 Hz, H-11), 6.23 (1H, d, J = 1.4 Hz, H-14), 1.65 (3H, s, H-17), 0.81 (3H, s, H-18), 0.72 (3H, s, H-19), 0.59 (3H, s, H-20); ^{13}C NMR (125 MHz, $CDCl_3$+CD_3OD), see Table 1; EIMS *m/z* 348 $[M]^+$ (2), 330 (67), 312 (100), 269 (13); HREIMS *m/z* 348.1943 $[M]^+$ (calcd for $C_{20}H_{28}O_5$, 348.1937).

17-Acetoxyjolkinolide B (3): white amorphous powder; $[\alpha]^{24}$ −71.2 (*c* 0.53, MeOH); IR (film) ν_{max} 2948, 2867, 1789, 1685, 1459, 1228 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 1.47 (1H, m, H-1a), 1.23 (1H, m, H-1b), 1.58 (1H, m, H-2), 1.94 (1H, m, H-3a), 1.19 (1H, m, H-3b), 1.11 (1H, dd, J = 2.1, 12.1 Hz, H-5), 1.82 (1H, m, H-6a), 1.49 (1H, m, H-6b), 2.30 (1H, s, H-9), 4.06 (1H, s, H-11), 3.99 (1H, s, H-14), 4.99 (2H, ABq, J = 13.5 Hz, H-17), 0.94 (3H, s, H-18), 0.86 (3H, s, H-19), 0.80 (3H, s, H-20), 2.11 (3H, s, OCOCH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.4 and 20.7 (OAc-17), and other data, see Table 1; EIMS *m/z* 388 $[M]^+$ (5), 299 (61), 149 (80), 69 (100); HREIMS *m/z* 388.1894 $[M]^+$ (calcd for $C_{22}H_{28}O_6$, 388.1885).

Preparation of 3 from 17-Hydroxyjolkinolide B. 17-Hydroxyjolkinolide B (10 mg) was dissolved in pyridine (1 mL) and Ac_2O (1 mL), and the solution was stirred for 12 h at room temperature. The product was dried under vacuum and purified by a silica gel column (petroleum ether−acetone, 10:1) to furnish a coloreless oil (11.4 mg), identified as **3** by direct comparison with an authentic sample and ^1H NMR analysis.

13β-Hydroxy-*ent*-abiet-8(14)-en-7-one (4): amorphous powder; $[\alpha]^{18}$ −47.0 (*c* 0.14, acetone); UV (MeOH) λ_{max} 246 (log ϵ 4.54) nm; IR (KBr) ν_{max} 3430, 2929, 1681, 1608, 1461, 1253 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 1.83 (1H, m, H-1a), 1.03 (1H, dd, J = 3.6, 5.3 Hz, H-1b), 1.78 (1H, m, H-2a), 1.47 (1H, m, H-2b), 1.47 (1H, m, H-3a), 1.23 (1H, m, H-3b), 1.51 (1H, m, H-5), 2.58 (1H, dd, J = 3.9, 15.0 Hz, H-6a), 2.31 (1H, dd, J = 3.9, 11.0 Hz, H-6b), 1.97 (1H, m, H-9), 1.53 (1H, m, H-11), 1.73 (1H, m, H-12a), 1.47 (1H, m, H-12b), 6.73 (1H, s, H-14), 1.75 (1H, m, H-15), 0.96 (3H, d, J = 6.9, H-16), 0.86 (3H, d, J = 6.9 Hz, H-17), 0.91 (3H, s, H-18), 0.88 (3H, s, H-19), 0.88 (3H, s, H-20); ^{13}C NMR (100 MHz, $CDCl_3$), see Table 1; EIMS *m/z* 304 $[M]^+$ (3), 286 (14), 269 (61), 261 (100); HREIMS *m/z* 304.2383 $[M]^+$ (calcd for $C_{20}H_{32}O_2$, 304.2402).

12-Deoxyphorbaldehyde-13-acetate (5): amorphous powder; $[\alpha]^{18}$ +78.0 (*c* 0.23, acetone); UV (MeOH) λ_{max} 240 (log ϵ 4.13) nm; IR (KBr) ν_{max} 3382, 2925, 1708, 1664, 1265 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 7.54 (1H, s, H-1), 2.60 (2H, ABq, J = 19.5 Hz, H-5), 6.71 (1H, q, J = 2.1 Hz, H-7), 3.38 (1H, t, J = 5.5 Hz, H-8), 3.06 (1H, s, H-10), 2.05 (1H, m, H-11), 2.17 (1H, m, H-12a), 1.61 (1H, m, H-12b), 1.01 (1H, d, J = 5.4 Hz, H-14), 1.08 (3H, s, H-16), 1.25 (3H, s, H-17), 0.89 (3H, d, J = 6.4 Hz, H-18), 1.76 (3H, s, H-19), 9.41 (1H, s, H-20), 2.09 (3H, s, OAc-13); ^{13}C NMR (125 MHz, $CDCl_3$) δ 173.4 and 21.2 (OAc-13), and for other data, see Table 1; EIMS *m/z* 388 $[M]^+$ (1), 370 (1), 328 (46), 310 (100); HREIMS *m/z* 388.1873 $[M]^+$ (calcd for $C_{22}H_{28}O_6$, 388.1886).

12-Deoxyphorbaldehyde-13-hexadecacetate (6): oil; $[\alpha]^{20}$ +52.0 (*c* 0.28, acetone); UV (MeOH) λ_{max} 238 (log ϵ 4.36) nm; IR (KBr) ν_{max} 3378, 2923, 1712, 1629, 1191 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 7.54 (1H, s, H-1), 2.66 (2H, ABq, J = 19.6 Hz, H-5), 6.71 (1H, q, J = 2.1 Hz, H-7), 3.38 (1H, t, J = 5.4 Hz, H-8), 3.06 (1H, s, H-10), 2.04 (1H, m, H-11), 1.62 (1H, m, H-12a), 2.14 (1H, m, H-12b), 0.98 (1H, d, J = 5.3 Hz, H-14), 1.07 (3H, s, H-16), 1.23 (3H, s, H-17), 0.89 (3H, d, J = 6.8 Hz, H-18), 1.76 (3H, t, J = 1.1 Hz, H-19), 9.41 (1H, s, H-20), 2.31 (2H, t, J = 7.4 Hz, H-2′), 1.62 (2H, m, H-3′), 1.24 (20H, brs, H-4′−H-13′), 1.62 (2H, m, H-14), 1.24 (2H, m, H-15), 0.89 (overlapping, H-16′); ^{13}C NMR (125 MHz, $CDCl_3$) δ 176.2 (C-1′), 34.4 (C-2′), 24.8 (C-3′), 29.1−29.7 (C-4′−C-13′), 31.9 (C-14′), 22.8 (C-15′), 14.1 (C-16′), and for other data, see Table 1; EIMS *m/z* 584 $[M]^+$ (16), 566 (60), 328 (35), 310 (100); HREIMS *m/z* 584.4077 $[M]^+$ (calcd for $C_{36}H_{56}O_6$, 584.4077).

12-Deoxyphorbol-13-(9*Z*)-octadecanoate-20-acetate (7): oil; $[\alpha]^{24}$ +22.6 (*c* 0.71, $CHCl_3$); UV (MeOH) λ_{max} 252 (log ϵ 4.07) nm; IR (film) ν_{max} 3399, 2925, 1722, 1261, 756 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 7.56 (1H, s, H-1), 2.41 (2H, ABq, J = 19.0 Hz, H-5), 5.67 (1H, d, J = 4.1 Hz, H-7), 2.97 (1H, t, J = 5.1 Hz, H-8), 3.24 (1H, brd, J = 2.2 Hz, H-10), 1.96 (1H, m, H-11), 1.91 (1H, m, H-12a), 1.55 (1H, m, H-12b), 0.80 (1H, d, J = 5.3 Hz, H-14), 1.16 (3H, s, H-16), 1.03 (3H, s, H-17), 0.85 (3H, d, J = 6.6 Hz, H-18), 1.73 (3H, dd, J = 1.2, 2.8 Hz, H-19), 4.43 (2H, ABq, J = 12.3, 7.3 Hz, H-20), 2.25 (2H, t, J = 7.5 Hz, H-2′), 1.55 (2H, m, H-3′), 1.22 (16H, brs, H-4′-H-7′ and H-12′-H-15′), 1.97 (4H, m, H-8′ and H-11′), 5.31 (2H, m, H-9′ and H-10′), 2.03 (2H, m, H-16′), 1.22 (2H, m, H-17), 0.89 (3H, t, J = 7.5 Hz, H-18′), 2.01 (3H, s, OAc-20); ^{13}C NMR (125 MHz, $CDCl_3$) δ 173.6 (C-1′), 34.3 (C-2′), 24.9 (C-3′), 29.2−29.8 (C-4′−C-8′ and C-12′−C-15′), 31.8 (C-16′), 22.8 (C-17′), 14.1 (C-18′), 173.4 and 21.2 (OAc-

20), and for other data, see Table 1; EIMS *m*/*z* 654 $[M]^+$ (1), 372 (7), 312 (100), 294 (92); HREIMS *m*/*z* 654.4500 $[M]^+$ (calcd for $C_{40}H_{62}O_7$, 654.4496).

Methanol Hydrolysis of 7. Compound **7** (20 mg) was refluxed with 0.9 M HCl in 80% aqueous MeOH (5 mL) at 90 °C for 18 h. The solution was extracted with hexane, and the combined organic layer was dried (Na_2SO_4) and evaporated. The residue was purified by column chromatography (silica gel, hexane−EtOAc, 10:1) to give the part of the fatty acid methyl ester, which was determined as 9*Z*-ocadecanoic acid by GC-MS and database detection.

Biological Testing. Human Burkitt's lymphoma Ramos B cells (purchased from American Type Culture Collection) were maintained at 37 °C (5% CO_2) in tissue culture dishes filled with growth medium (RPMI 1640 medium with 10% heat-inactivated fetal bovine serum, 100 kU/L penicillin, and 200 kU/L streptomycin). Cells (5×10^4 cells/mL) were incubated with different concentrations of compounds for 72 h. Cell proliferation was determined by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay as described previously.[21] The assays were carried out in triplicate in at least three independent experiments. Prostratin (**9**) was used as positive control.

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