Chemical Constituents of the Roots of *Euphorbia micractina*

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Nine minor new tirucallane (**1**−**7**) and euphane (**8** and **9**) triterpenoids including five hydroperoxides, together with 18 known compounds, have been isolated from an ethanolic extract of the roots of *Euphorbia micractina*. Their structures including absolute configurations were elucidated by spectroscopic and chemical analysis. In the in vitro assays, betulin (**10**) showed a selective cytotoxic activity against A2780 ovarian cells with an IC_{50} value of 6.1 μM and inhibitory activity against protein tyrosine phosphatase 1B (PTP1B) with an IC_{50} value of 15.3 μM. Jolkinol B (**11**) showed a potent activity against HIV-1 replication with an IC_{50} value of 12.6 μM. However, compounds **1**−**9** and the other known compounds were inactive in the three assays used.

Species of the genus *Euphorbia* (Euphorbiaceae) are sources of various secondary metabolites with interesting chemical structures and significant bioactivities.[1] Several plants of this genus have long been used for the treatment of various diseases such as edema, ascites, warts, and cancer in the People's Republic of China.[2] *Euphorbia micractina* Boiss. is widely distributed at high altitudes (2700−5000 m) in western mainland China, and its roots are used in Chinese folk medicine for the treatment of tumors and warts.[3] Some structurally interesting diterpenes were reported from this plant.[4−6] As part of a program to access chemical diversity of Chinese traditional medicines and their biological effects, we carried out an investigation of the roots of *E. micractina*. This paper describes the isolation, structure elucidation, and selected in vitro bioassays of nine minor, new triterpenoids including seven tirucallane (**1**−**7**) and two euphane derivatives (**8** and **9**), together with 18 known compounds, from an ethanolic extract of this plant material. Among them, compounds **2**−**4**, **7**, and **8** are uncommon triterpene hydroperoxides.

Results and Discussion

Compound **1**, a white, amorphous solid, $[\alpha]^{20}_D$ −1.3 (*c* 0.08, MeOH), showed IR absorptions for hydroxy (3333 cm^{-1}) and double-bond (1648 cm^{-1}) functional groups. The EIMS of **1** exhibited a molecular ion peak at *m/z* 442 $[M]^{+\bullet}$, and the HREIMS at *m/z* 442.3799 $[M]^{+\bullet}$ indicated that **1** has a molecular formula of $C_{30}H_{50}O_2$ (calcd for $C_{30}H_{50}O_2$, *m/z* 442.3811). The ^1H NMR spectrum of **1** in acetone-d_6 showed signals due to an olefinic methylene at δ 4.88 (br s, H-26a) and 4.74 (br s, H-26b), two oxygen-bearing methines at δ 3.96 (dt, J = 6.0 and 4.2 Hz, H-24) and 3.17 (dt, J = 9.6 and 5.4 Hz, H-3), and two exchangeable hydroxy protons at δ 3.63 (d, J = 4.2 Hz, *H*O-24) and 3.37 (d, J = 5.4 Hz, *H*O-3). In addition, it displayed an olefinic methyl singlet at δ 1.69 (H_3-27), five aliphatic methyl singlets at δ 0.81 (H_3-18), 0.98 (H_3-19), 1.00 (H_3-28), 0.80 (H_3-29), and 0.91 (H_3-30), and an aliphatic methyl doublet at δ 0.94 (J = 6.6 Hz, H_3-21), together with partially overlapped multiplets attributed to aliphatic methylenes and methines between δ 1.10 and 2.20. These data helped classify **1** as a dihydroxylated triterpene containing a terminal double-bond unit.[7,8] This was supported by the ^{13}C NMR and DEPT spectra of **1**, which showed 30 carbon signals consisting of seven methyls, 11 methylenes (one sp^2), five methines (two oxygen-bearing), and seven quaternary carbons (three olefinic) (Table 2).

The structure of **1** was finalized by the analysis of its 2D NMR data. The proton and protonated carbon signals in the NMR spectra of **1** were assigned unambiguously (Tables 1 and 2) on the basis of ^1H−^1H COSY and HMQC spectroscopic analysis. The ^1H−^1H COSY spectrum showed homonuclear coupling correlations from HO-3 through H-3 and H_2-2 to H_2-1, from H-5 through H_2-6 to H_2-7, from H_2-15 through H_2-16, H-17, H-20, H_2-22, and H_2-23 to H-24 (in which H-20 correlated with H_3-21), from H_2-11 to H_2-12, and from H_2-26 to H_3-27. These correlations indicated unambiguously the nature of the partial structural units with vicinal coupling protons in **1** (Supporting Information, Figure S1, units with thick lines). In the HMBC spectrum of **1**, two- and three-bond correlations from H_3-18 to C-12, C-13, C-14, and C-17, from H_3-19 to C-1, C-5, C-9, and C-10, from H_3-21 to C-17, C-20, and C-22, from

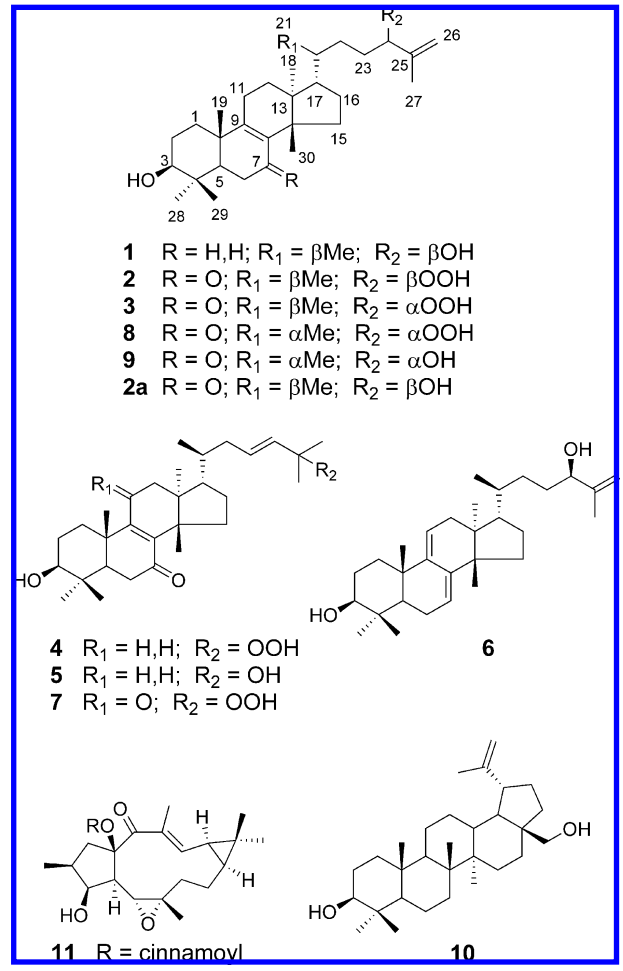

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Table 1. ^{1}H NMR Spectroscopic Data (δ) of Compounds **1**−**9**[a]

position	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	9
1α	1.22 m	1.47 m	1.48 m	1.48 m	1.48 m	1.50 m	1.14 m	1.48 m	1.48 m
1*β*	1.76 m	1.86 dt (12.5, 3.5)	1.86 dt (12.5, 3.5)	1.86 dt (12.5, 3.5)	1.86 dt (12.5, 3.5)	1.81 dt (13.2, 3.0)	2.44 m	1.87 dt (12.5, 3.5)	1.87 (13.0, 3.5)
2	1.63 m	1.69 m	1.69 m	1.70 m	1.70 m	1.67 m	1.69 m	1.69 m	1.69 m
3α	3.17 dt (9.6, 5.4)	3.24 dt (10.0, 5.0)	3.24 dt (10.0, 5.0)	3.24 dt (10.0, 4.5)	3.24 dt (10.0, 5.0)	3.15 dt (9.0, 4.8)	3.26 dt (11.5, 5.5)	3.24 dt (9.0, 5.0)	3.24 dt (10.0, 5.0)
5α	1.14 dd (12.6, 1.8)	1.71 t (9.0)	1.71 t (9.0)	1.71 t (9.0)	1.71 t (9.0)	1.27 dd (11.4, 4.8)	1.67 t (8.5)	1.70 t (9.0)	1.70 t (9.0)
6α	1.44 m	2.30 d (9.0)	2.31 d (9.0)	2.31 d (9.0)	2.31 d (9.0)	2.22 dt (18.0, 4.8)	2.46 d (8.5)	2.31 d (9.0)	2.30 d (9.0)
6*β*	1.44 m	2.30 d (9.0)	2.31 d (9.0)	2.31 d (9.0)	2.31 d (9.0)	2.12 dd (18.0, 11.4)	2.46 d (8.5)	2.31 d (9.0)	2.30 d (9.0)
7	2.12, 1.93 m					5.37 br s			
11α	2.12 m	2.43 dt (21.0, 9.0)	2.44 dt (21.0, 9.0)	2.44 dt (21.0, 9.0)	2.44 dt (21.0, 9.0)	5.24 br s		2.43 dt (21.0, 9.0)	2.43 dt (21.0, 9.0)
11*β*	1.99 m	2.32 ddd (21.0, 8.0, 1.0)	2.32 ddd (21.0, 8.0, 1.0)	2.32 ddd (21.0, 7.0, 1.0)	2.32 ddd (21.0, 7.0, 1.0)			2.33 ddd (21.0, 6.5, 2.0)	2.32 (21.0, 6.5, 2.0)
12	1.74 m; 1.77 m	1.76−1.82 m	1.76−1.82 m	1.76−1.82 m	1.76−1.82 m	2.18 d (4.8)	2.61 d (18.5) 2.42 d (18.5)	1.78−1.83 m	1.76−1.82 m
15α	1.57 m	1.48 m	1.50 m	1.50 m	1.49 m	1.66 m	1.64−1.72 m	1.50 m	1.50 m
15*β*	1.22 m	2.09 ddd (13.0, 10.0, 2.5)	2.10 ddd (13.0, 10.5, 3.0)	2.10 ddd (12.5, 10.0, 2.5)	2.10 ddd (12.5, 10.0, 2.5)	1.33 m	2.08 m	2.09 ddd (13.0, 10.0, 2.5)	2.09 ddd (13.0, 10.0, 2.5)
16α	1.37 m	1.35 m	1.34 m	1.38 m	1.38 m	1.36 m	1.41 m	1.34 m	1.34 m
16*β*	1.94 m	1.92 m	1.94 m	1.99 m	1.99 m	2.0 m	2.05 m	1.92 m	1.92 m
17	1.52 m	1.48 m	1.47 m	1.48 m	1.48 m	1.60 m	1.64−1.72 m	1.48 m	1.48 m
18	0.81 s	0.76 s	0.76 s	0.77 s	0.77 m	0.65 s	0.97 s	0.76 s	0.76 s
19	0.98 s	1.07 s	1.07 s	1.07 s	1.07 m	0.93 s	1.30 s	1.07 s	1.07 s
20	1.45 m	1.44 m	1.43 m	1.51 m	1.50 m	1.42 m	1.53 m	1.50 m	1.50 m
21	0.94 d (6.6)	0.94 d (6.5)	0.94 d (6.5)	0.94 d (6.0)	0.93 d (6.0)	0.92 d (6.6)	0.91 d (7.0)	0.86 d (6.0)	0.86 d (6.0)
22	1.56 m	1.48 m	1.43 m	2.20 m	2.16 m	1.44 m	2.21 m	1.59 m	1.60 m
	0.97 m	1.00 m	1.12 m	1.78 m	1.78 m	1.15 m	1.78 m	1.20 m	1.17 m
23	1.62 m	1.49 m	1.65 m	5.60 m	5.60 br s	1.60 m	5.60 m	1.65 m	1.62 m
	1.40 m		1.33 m			1.42 m		1.33 m	1.37 m
24	3.96 dt (6.0, 4.2)	4.20 t (7.0)	4.20 t (6.5)	5.60 m	5.60 br s	3.96 br s	5.60 m	4.22 t (6.5)	3.96 dt (6.0,5.0)
26	4.88 s	4.90 s	4.90 s	1.26 s	1.22 s	4.89 br s	1.26 s	4.91 s	4.89 s
	4.74 s					4.73 br s			4.74 s
27	1.69 s	1.68 s	1.69 s	1.26 s	1.23 s	1.68 s	1.27 s	1.69 s	1.69 s
28	1.00 s	0.97 s	0.97 s	0.97 s	0.97 s	0.97 s	1.00 s	0.97 s	0.97 s
29	0.80 s	0.88 s	0.86 s	0.88 s	0.88 s	0.88 s	0.89 s	0.88 s	0.88 s
30	0.91 s	0.95 s	0.95 s	0.95 s	0.95 s	0.87 s	1.04 s	0.96 s	0.96 s
3-O*H*	3.37 d (5.4)	3.57 d (5.0)	3.57 d (5.0)	3.57 d (4.5)	3.57 d (5.0)	3.47 d (4.8)	3.59 d (5.5)	3.57 d (5.0)	3.57 d (5.0)
24-O*H*/24-OO*H*	3.63 d (4.2)	10.41 s	10.41 s			3.62 br s		10.41 s	3.65 d (5.0)
25-O*H*/25-OO*H*				9.94 s	3.37 s		9.94 s		

[a] ^{1}H NMR data (δ) were measured in acetone-d_6 for **1** and **6** at 600 MHz and for **2**−**5** and **7**−**9** at 500 MHz. Proton coupling constants (*J*) in Hz are given in parentheses. The assignments were based on DEPT, ^{1}H−^{1}H COSY, HSQC, and HMBC experiments.

Table 2. ^{13}C NMR Spectroscopic Data (δ) of Compounds **1**−**9**[a]

position	1	2	3	4	5	6	7	8	9
1	36.1	35.3	35.3	35.3	35.2	36.6	34.6	35.3	35.2
2	28.8	28.4	28.4	28.3	28.3	28.5	28.2	28.4	28.3
3	78.5	77.7	77.7	77.7	77.7	78.6	77.6	77.7	77.7
4	39.7	39.5	39.5	39.5	39.5	39.7	39.3	39.5	39.5
5	52.0	49.2	49.2	49.2	49.2	49.3	49.1	49.2	49.2
6	19.7	36.3	36.3	36.3	36.2	24.4	36.2	36.3	36.3
7	28.4	197.6	197.6	197.7	197.6	119.5	199.8	197.6	ND[b]
8	134.2	139.2	139.2	139.1	139.1	142.1	150.1	139.2	ND[b]
9	135.3	165.6	165.6	165.7	165.7	146.2	155.3	165.6	ND[b]
10	38.0	40.0	40.0	40.0	39.8	36.9	38.8	40.0	40.0
11	22.1	24.1	24.1	24.1	24.1	115.9	201.9	24.2	24.1
12	31.6	30.7	30.7	30.6	30.5	39.1	51.8	30.7	30.7
13	44.8	45.4	45.3	45.3	45.3	44.7	45.9	45.4	45.4
14	50.7	48.4	48.5	48.4	48.4	50.3	48.4	48.5	48.4
15	30.5	32.3	32.4	32.4	32.4	31.9	32.6	32.3	32.3
16	28.6	29.2	29.2	29.2	29.2	28.6	28.3	29.3	29.3
17	51.0	49.5	49.5	49.3	49.2	51.8	49.6	48.9	49.2
18	15.8	15.9	15.9	15.9	15.9	16.6	18.5	16.1	16.1
19	20.6	18.8	18.8	18.8	18.7	21.2	18.1	18.8	18.7
20	37.2	36.9	37.1	37.4	37.5	36.8	37.1	36.6	36.8
21	19.2	19.0	19.1	19.1	19.1	18.9	18.7	19.3	19.4
22	32.9	32.9	32.9	40.1	40.0	32.72	39.7	31.9	32.7
23	32.6	28.0	28.4	128.7	124.6	32.71	128.3	28.2	32.1
24	76.4	90.1	89.9	137.2	141.6	76.0	137.4	89.8	76.3
25	149.3	145.6	146.0	81.5	70.1	149.6	81.5	146.0	ND[b]
26	110.5	113.8	113.4	25.0[b]	29.3	110.1	25.0	113.5	110.3
27	17.5	16.9	17.1	25.1[b]	29.3	17.9	25.0	17.1	17.7
28	28.6	27.8	27.8	27.8	27.8	28.3	27.9	27.8	27.8
29	16.2	15.6	15.6	15.6	15.6	15.9	15.6	15.6	15.6
30	24.7	24.6	24.7	24.6	24.6	23.6	24.2	24.7	24.7

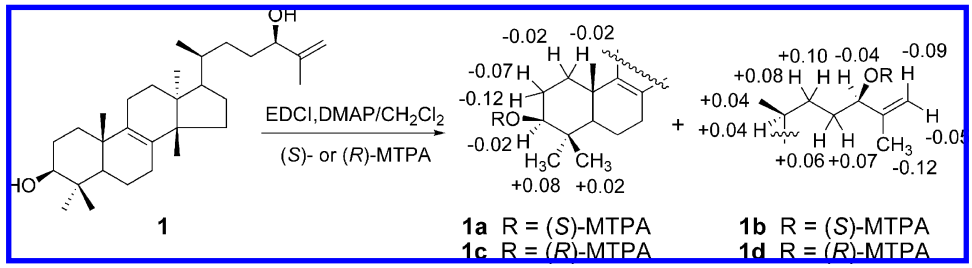
[a] ^{13}C NMR data (δ) were measured in acetone-d_6 for **1**−**4** and **6**−**9** at 150 MHz. and for **5** at 125 MHz. The assignments were based on DEPT, ^{1}H−^{1}H COSY, HSQC, and HMBC experiments. [b] ND means the signal was not detected.

H_3-27 to C-24, C-25, and C-26, from both H_3-28 and H_3-29 to C-3, C-4, and C-5, and from H_3-30 to C-8, C-13, C-14, and C-15 (Supporting Information, Figure S1, arrows), together with the chemical shifts of these protons and carbons, suggested that **1** is a stereoisomer of either tirucalla-8,25-diene-3,24-diol or eupha-8,25-diene-3,24-diol.[8] In a NOE difference experiment of **1**, irradiation of H-3 enhanced H-5 and H_3-28, suggesting that the hydroxy group at C-3 has an equatorial β-orientation. This was in good agreement with the coupling constants between H-3 with H-2a (5.4 Hz) and H-2b (9.6 Hz), indicating that H-3 has an axial α-orientation. Irradiation of H_3-19 enhanced H_3-29 and H_3-30, while irradiation of H_3-30 enhanced H-12a, H-17, and H_3-19, indicating that these protons are oriented on the same side of the ring system. In addition, H-20 and H_3-21 were enhanced by irradiation of H_3-18, while H_3-18 and H-12b were enhanced by irradiation of H_3-21. These NOE enhancements, in combination with the chemical shift of H_3-21,[8] suggested that **1** is a C-24 epimeric isomer of tirucalla-8,25-diene-3β,24-diol rather than of eupha-8,25-diene-3β,24-diol. The absolute configuration at C-24 of **1** was determined by the modified Mosher's method.[9] Compound **1** was treated with (*R*)-(+)- and (*S*)-(−)-α-methoxy-α-(trifuromethyl)phenylacetic acid (MTPA) to yield the corresponding two pairs of (*S*)- and (*R*)-MTPA monoesters, **1a/1c** and **1b/1d** (Scheme 1). The chemical shifts of the two pairs of diastereomers **1a/1c** and **1b/1d** were carefully assigned by analysis of their ^{1}H−^{1}H COSY and NOE difference data. From the MTPA determination rule,[9] the positive and negative $\Delta\delta$ ($\delta_{S\text{-MTPA ester}} - \delta_{R\text{-MTPA ester}}$) values observed for protons were located on the right and on the left side of the MTPA plane, respectively. As shown in Scheme 1, for the pair of diastereomers **1a/1c** the $\Delta\delta$ values for H_2-1 and H_2-2 are negative, whereas those for H_3-28 and H_3-29 are positive. This verified that **1** possesses a 3*S* configuration, which is the same as that of the majority of natural tirucalla-3β-ol derivatives. For the pair of diastereomers **1b/1d** the $\Delta\delta$ values for H_2-26 and H_3-27 are negative, whereas those for H_3-21, H-20, H_2-22, and H_2-23 are positive. This indicated that **1** has a 24*R* configuration.[10,11] Therefore, the structure of **1** was determined as (−)-(24*R*)-tirucalla-8,25-diene-3β,24-diol.

Compound **2**, a white, amorphous solid, $[\alpha]^{20}_D$ −1.9 (*c* 0.05, MeOH), exhibited quasimolecular ion peaks at *m*/*z* 473 $[M + H]^+$ and 495 $[M + Na]^+$ in the positive ESIMS. The molecular formula of **2** was indicated as $C_{30}H_{48}O_4$ by the HRESIMS at *m*/*z* 495.3468 $[M + Na]^+$ (calcd for $C_{30}H_{48}O_4Na$, 495.3445). The IR spectrum of **2** suggested the presence of hydroxy (3313 cm^{-1}) and α,β-unsaturated carbonyl (1714 and 1633 cm^{-1}) groups. The NMR spectroscopic features of **2** in acetone-d_6 were found to be similar to those of **1**. However, comparison of the NMR data of the tetracyclic ring moieties of **1** and **2** showed that the resonance due to a methylene (H_2-7 and C-7) of **1** was replaced by the resonance of a carbonyl carbon (δ 197.6) in **2**. Meanwhile, the resonances of the protons and carbons around the double bond between C-8 and C-9 of **1** were changed significantly in **2** (Tables 1 and 2). This suggested that **2** is a analogue of tirucalla-8,25-dien-3β-ol-7-one and was confirmed by analysis of the 2D NMR data of **2**, which resulted in an unambiguous assignment of the 1D NMR data (Tables 1 and 2). In particular, HMBC correlations from H_2-6 to C-5, C-7, and C-10, from H_3-19 to C-1, C-5, C-9, and C-10, and from both H_3-28 and H_3-29 to C-3, C-4, and C-5 (Supporting Information, Figure S1, arrows), in combination with their chemical shifts, demonstrated unequivocally the presence of an 8-en-7-one unit and a β-hydroxy group at C-3 in **2**. The 1D and 2D NMR data analysis indicated that the side-chain moiety of **2** is very similar to that of **1**. However, the resonances due to H-24, H_2-26, and HO-24 were changed from δ 3.96 (dt, J = 6.0, 4.2 Hz, H-24), 4.88 (s, H-26a), 4.74 (s, H-26b), and 3.63 (d, J = 4.2 Hz, *H*O-24) in **1** to δ 4.20 (t, J = 7.0 Hz, H-24), 4.90 (2H, s, H_2-26), and 10.41 (s, *H*OO-24) of **2**, respectively. Moreover, the resonances of C-24, C-25, and C-26 of **2** were shifted by $\Delta\delta_C$ +13.7, −3.7, and +3.3 ppm, respectively, as compared with those of **1**. These data, in combination with the molecular formula, were consistent with the presence of a hydroperoxy group at C-24 for **2**.[12] The NOESY spectroscopic data (Supporting Information) and the chemical shift of H_3-21 (δ_H 0.94, d, J = 6.5 Hz)[8] of **2** suggested that the relative configurations of the tetracyclic ring moiety and C-20 in **2** are identical to those of **1**. This inference was supported by the CD spectrum of **2**, which showed a positive Cotton effect at λ 339 nm ($\Delta\varepsilon$ +0.97) for a n → π^* transition and a negative Cotton effect at λ 258 nm ($\Delta\varepsilon$ −1.49) for a $\pi \rightarrow \pi^*$ transition. Molecular modeling using the MM2 program indicated that the cyclohexenone moiety of the ring system possesses a twisted boat conformation for the lowest energy conformational isomer (Supporting Information, Figure S2). On the basis of the octant rule for cyclohexenones,[13] the Cotton effects observed indicated a configuration for the tetracyclic ring moiety of **2**, identical to that of **1**. Reduction of **2** with triphenylphosphine yielded **2a**. After an unambiguous assignment of the ^{1}H NMR data of **2a** (Experimental Section) by the ^{1}H−^{1}H COSY experiment, comparison of the ^{1}H NMR data of the side-chain moiety of **2a** and **1** suggested that the absolute configuration at C-24 of **2** and **1** is identical. Therefore, the structure of **2** was determined as (−)-(24*R*),24-hydroperoxytirucalla-8,25-dien-3β-ol-7-one.

Compound **3**, a white, amorphous solid, gave spectroscopic data almost identical to those of **2**. Comparison of the ^{1}H−^{1}H COSY spectra of **2** and **3** (Supporting Information) indicated clearly that H-24 of **3** was coupled with two protons having a large difference in their chemical shifts [δ 1.65 (m, H-23a) and 1.33 (m, H-23b)], whereas H-24 of **2** was coupled with two overlapped protons [δ 1.49 (m, H_2-23)]. In addition, resonances for C-23, C-25, and C-26 in the ^{13}C NMR spectrum of **3** were shifted, respectively, by $\Delta\delta_C$ +0.4, +0.4, and −0.4 ppm (Table 2), when compared with those of **2**. These data suggested that **3** is a C-24 epimer of **2**, which was supported by the NOESY and CD spectroscopic data of **3** (Supporting Information). Thus, the structure of **3** was determined as (−)-(24*S*),24-hydroperoxytirucalla-8,25- dien-3β-ol-7-one.

Scheme 1. $\Delta\delta$ ($\delta_S - \delta_R$) Values Obtained from the ^{1}H NMR Spectra of the MTPA Esters **1a**−**1d**



The IR and HRESIMS data of **4** (Experimental Section) demonstrated that it is also an isomer of **2**. Comparison of the ^{1}H NMR data between **4** and **2** indicated that the resonances assigned to the oxymethine (H-24), the olefinic methylene (H_2-26), and the olefinic methyl (H_3-27) of the side-chain moiety of **2** were replaced by resonances attributable to two olefinic methines [δ 5.60 (m, H-23 and H-24)] and two aliphatic methyls attached to an oxygen-bearing quaternary carbon [δ 1.26 (s, H_3-26 and H_3-27)] in **4**. This suggested that the terminal double bond between C-25 and C-26 and the hydroperoxy group at C-24 in **2** were moved in **4** to C-23 and C-24 and C-25, respectively. This was supported by the replacement of the carbon resonances attributed to the terminal double bond and hydroperoxy-bearing methine of the side-chain moiety of **2** by resonances assignable to two methines of a double bond [δ 128.7 (C-23) and 137.2 (C-24)][11,12] and a hydroperoxy-bearing quaternary carbon [δ 81.5 (C-25)] in the ^{13}C NMR spectrum of **4**. The deduction was further confirmed by 2D NMR experiments on **4**, in particular, the HMBC spectrum, in which two- and three-bond correlations from H_3-21 to C-17 and C-20, from H_2-22 to C-20, C-23, and C-24, from H-24 to C-22, C-23, and C-25, from H_2-26 to C-24, C-25, and C-27, and from H_3-27 to C-24, C-25, and C-26, together with their chemical shifts, proved the presence of a 25-hydroperoxy-23-ene unit in the side-chain moiety. Although the H-23 and H-24 signals were partially overlapped in the ^{1}H NMR spectrum of **4** in acetone-d_6, the *trans* geometric configuration of the 23-ene unit was indicated unequivocally by a vicinal coupling constant of 16.2 Hz between H-23 and H-24 observed in the ^{1}H NMR spectrum of this compound in pyridine-d_5.[11] The similarity of the CD (Experimental Section) and specific rotation data between **4** ($[\alpha]^{20}_D$ −3.2) and **2** ($[\alpha]^{20}_D$ −1.9) proposed that they have the same stereochemistry.[8] Therefore, the structure of **4** was determined as (−)-(23*E*),25-hydroperoxytirucalla-8,23-dien-3β-ol-7-one.

Compound **5**, a white, amorphous solid, $[\alpha]^{20}_D$ −2.5 (*c* 0.04, MeOH), gave the molecular formula $C_{30}H_{48}O_3$ (calcd for $C_{30}H_{48}O_3Na$, *m*/*z* 479.3496), as indicated from its positive HRESIMS at *m*/*z* 479.3524 $[M + Na]^+$, one oxygen atom less than that of **4**. The differences in the ^{1}H NMR spectroscopic features between **5** and **4** were that an exchangeable singlet ascribed to a hydroxy proton of **5** at δ 3.37 (s, *H*O-25) replaced the signal assigned to the hydroperoxy proton of **4** [δ 9.94 (s, *H*OO-25)], and the overlapped signals due to H_3-26 and H_3-27 of **5** were shielded by $\Delta\delta_H$ −0.04 and −0.03 ppm, respectively, as compared with those of **4**. In addition, comparison of the ^{13}C NMR spectra of **5** and **4** showed that the resonance attributable to C-25 of **5** was shielded significantly by $\Delta\delta_C$ −11.4 ppm and that the resonances assignable to C-26 and C-27 of **5** were deshielded by $\Delta\delta_C$ +4.3 and +4.2 ppm, respectively. This suggested that **5** is (−)-tirucalla-8,23-diene-3β,25-diol-7-one. Although the resonances for H-23 and H-24 of **5** were overlapped at δ 5.60 in actone-d_6 and at δ 5.94 in pyridine-d_5, a 23*E* configuration was assigned for **5** on the basis of the vicinal coupling constant of 15.6 Hz between H-23 and H-24 observed in the ^{1}H NMR spectrum of **5** in C_6D_6.[14] The CD and specific rotation data of **5** (Experimental Section) supported the same relative configuration in the tetracyclic ring system of **5** as that of **2**. Therefore, the structure of **5** was determined as (−)-(23*E*)-tirucalla-8,23-diene-3β,25-diol-7-one.

Compound **6**, a white, amorphous solid, $[\alpha]^{20}_D$ −23.1 (*c* 0.61, MeOH), exhibited a molecular ion peak at *m*/*z* 440 $[M]^{+\bullet}$ in the EIMS. The molecular formula, $C_{30}H_{48}O_2$, was indicated from the HREIMS at *m*/*z* 440.3640 $[M]^{+\bullet}$ (calcd for $C_{30}H_{48}O_2$, 440.3654). The IR and NMR spectroscopic features of **6** were similar to those of **1**. However, the ^{1}H NMR spectrum of **6** in acetone-d_6 showed signals attributable to two additional olefinic methines for the tetracyclic ring moiety at δ 5.37 (br s, H-7) and 5.24 (br s, H-11), while the ^{13}C NMR and DEPT spectra of **6** displayed signals attributable to two trisubstituted double bonds for the tetracyclic ring moiety at δ 146.2, 142.1, 119.5, and 115.9. In addition, the resonances assignable to C-6, C-8, C-9, and C-12 of **6** were deshielded by $\Delta\delta_C$ +4.7, +7.9, +10.9, and +7.5 ppm, respectively, as compared with those of **1**, whereas the resonance due to C-5 of **6** was shielded by $\Delta\delta_C$ −2.7 ppm. These data indicated that **6** is a derivative of **1** possessing double bonds between both C-7 and C-8 and C-9 and C-11, respectively. This was confirmed by the 2D NMR spectroscopic data analysis of **6**. In particular, HMBC correlations from H_3-19 to C-1, C-5, C-9, and C-10, from H-7 to C-5, C-6, C-9, and C-14, and from H-11 to C-8, C-12, and C-13, in combination with chemical shifts of these protons and carbons, proved the location of the double bonds in **6**. Therefore, the structure of **6** was determined as (−)-(24*R*)-tirucalla-7,9(11),25-triene-3β,24-diol.

Compound **7**, a white, amorphous solid, $[\alpha]^{20}_D$ +2.7 (*c* 0.04, MeOH), showed IR absorptions for hydroxy (3310 cm^{-1}) and α,β-unsaturated carbonyl (1713 and 1668 cm^{-1}) groups. Its molecular formula, $C_{30}H_{46}O_5$, was indicated from the HRESIMS at *m*/*z* 509.3272 $[M + Na]^+$ (calcd for $C_{30}H_{46}O_5Na$, 509.3237). The NMR spectroscopic features of **7** were similar to those of **4**. Comparison of NMR data (Tables 1 and 2) between **7** and **4** revealed that they have the same side chain and a difference in the tetracyclic ring moiety. The resonances due to C-11 and H_2-11 of **4** were replaced by a carbonyl signal for **7** [δ_C 201.9]. In addition, resonances attributable to C-7, C-8, and C-12 of **7** were deshielded by $\Delta\delta_C$ +2.1, +11.0, and +21.2 ppm, respectively, whereas resonances assignable to C-9 and C-10 of **7** were shielded, in turn, by $\Delta\delta_C$ −10.4 and −1.2 ppm. This suggested that **7** is an 11-oxo derivative of **4**, which was confirmed by comparison of the NMR data between **7** and 11-oxo-kansenonol.[8] Thus, the structure of **7** was assigned as (+)-(23*E*),25-hydroperoxytirucalla-8,23-dien-3β-ol-7,11-dione.

The spectroscopic data of **8** (Tables 1 and 2 and Experimental Section) were consistent with this compound being an isomer of **3**. After the ^{1}H and ^{13}C NMR data of **8** were assigned unambiguously by 2D NMR data analysis, a careful comparison of the data with analogous values for **3** (Tables 1 and 2) revealed that for **8** the resonances for H-20, H-22a, and H-22b were deshielded by $\Delta\delta_H$ +0.07, +0.16, and +0.08 ppm, respectively, whereas the resonances for H_3-21, and C-17, C-20, and C-22 were shielded, in turn, by $\Delta\delta_H$ −0.08 and $\Delta\delta_C$ −0.6, −0.5, and −1.0 ppm. This suggested that **8** is the C-21 epimer of **3**, which was supported by the NOESY data. For compound **8**, NOE correlations very similar to those of **3** were observed except for the appearance of NOEs between H_3-21 and H-16α and the absence of a NOE between H_3-18 and H_3-21.[8] The chemical shift of H_3-21 (δ_H 0.86, d, J = 6.0 Hz) and the positive optical rotation ($[\alpha]^{20}_D$ +6.2) were consistent

with **8** belonging to the euphane series of triterpenoids.[8] Accordingly, the structure of **8** was determined as (+)-(24*S*),24-hydroperoxyeupha-8,25-dien-3β-ol-7-one.

The IR and the NMR spectroscopic features of **9** resembled those of **8**. The HRESIMS at *m*/*z* 479.3539 $[M + Na]^+$ of **9** indicated that it has a molecular formula of $C_{30}H_{48}O_3$ (calcd for $C_{30}H_{48}O_3Na$, 479.3496), with one oxygen less than **8**. Comparison of the ^1H NMR data between these two compounds (Tables 1 and 2) indicated that the triplet for H-24 of **8** at δ_H 4.22 was replaced by a double triplet of **9** at δ_H 3.96 (J = 6.0 and 5.0 Hz), while the singlet for the hydroperoxy proton of **8** at δ_H 10.41 was replaced by an exchangeable doublet for a hydroxy proton of **9** at δ_H 3.65 (J = 5.0 Hz). In addition, as compared with those of **8**, the ^{13}C NMR resonances for C-22 and C-23 of **9** were deshielded, in turn, by $\Delta\delta_C$ +0.8 and +3.9 ppm. Also, the resonances for C-24 and C-26 of **9** were shielded by $\Delta\delta_C$ −13.5 and −3.2 ppm, respectively. However, the ^{13}C NMR resonances of the quaternary carbons C-7, C-8, C-9, and C-25 were not clearly observed for **9** (Supporting Information) due to a limitation of the sample amount available. The differences in the above spectroscopic data, in combination with the similarity in the CD and specific rotation data between **9** and **8**, led to the proposal of the presence of a hydroxy group at C-24 in **9**, replacing the hydroperoxy group of **8**. Therefore, the structure of **9** was assigned as (+)-(24*S*)-eupha-8,25-diene-3β,24-diol-7-one.

The known compounds isolated in the present investigation were identified by comparison of spectroscopic data with those reported in the literature as betulin (**10**),[15] erythrodiol,[16] uvaol,[17] helioscopinolide E,[18] 3-acetoxyhelioscopinolide B,[19] helioscopinolide A,[20] helioscopinolide B,[21] jolkinol D,[22] jolkinol B (**11**),[22,23] latilagascene F,[23] 20-*O*-acetylingenol-3-*O*-(2′*E*,4′*Z*)-decadienoate,[24] glycerol monopalmitate,[25] (*Z*)-12-octadecenic-α-glycerol monoester,[26,27] *p*-hydroxyphenethyl anisate,[28] scopoletin,[29] *E*-cinnamic acid,[30] 1-triacontanol,[31] and β-sitosterol.[32]

In the in vitro bioassays performed in this study, compound **10** showed a selective cytotoxic activity against the A2780 ovarian cancer cell line with an IC_{50} value of 6.1 μM [the positive control camptothecin (CPT) gave an IC_{50} value of 0.3 μM] and inhibitory activity against protein tyrosine phosphatase 1B (PTP1B), with an IC_{50} value of 15.3 μM. The positive control in this assay was oleanolic acid (IC_{50}, 4.2 μM). Compound **11** showed activity against HIV-1 replication with an IC_{50} value of 12.6 μM [the positive control zidovudine (AZT) gave an IC_{50} value of 0.05 μM]. However, compounds **1**−**9** and the other known compounds were inactive in the three assays.

Experimental Section

General Experimental Procedures. Optical rotations were measured on a PE model 343 polarimeter. UV spectra were measured on a Cary 300 spectrometer. CD spectra were recorded on a JASCO-815 CD spectrometer. IR spectra were recorded on a Nicolet 5700 FT-IR microscope instrument (FT-IR microscope transmission). 1D- and 2D-NMR spectra were obtained at 500 or 600 MHz for ^1H and 125 or 150 MHz for ^{13}C, respectively, on INOVA 500 MHz or SYS 600 MHz spectrometers, in acetone-d_6, $CDCl_3$, pyridine-d_5, or C_6D_6, with solvent peaks used as references. EIMS and HREIMS data were measured with a Micromass Autospec-Ultima ETOF spectrometer. ESIMS data were measured with a Q-Trap LC/MS/MS (Turbo Ionspray Source) spectrometer. HRESIMS data were measured using an AccuToFCS JMS-T100CS spectrometer. Column chromatography (CC) was performed with silica gel (200−300 mesh, Qingdao Marine Chemical Inc., Qingdao, People's Republic of China) and Pharmadex LH-20 (Amersham Biosciences, Inc., Shanghai, People's Republic of China). Preparative TLC separation was performed with high-performance silica gel preparative TLC plates ($HSGF_{254}$, glass precoated, Yantai Jiangyou Silica Gel Development Co., Ltd., Yantai, People's Republic of China). HPLC separation was performed on an instrument consisting of a Waters 600 controller, a Waters 600 pump, and a Waters 2487 dual λ absorbance detector, with a Prevail (250 × 10 mm i.d.) column packed with C_{18} (5 μm). TLC was carried out with glass precoated silica gel GF_{254} plates. Spots were visualized under UV light or by spraying with 7% H_2SO_4 in 95% EtOH followed by heating. Unless otherwise noted, all chemicals were obtained from commercially available sources and were used without further purification.

Plant Material. The roots of *Euphorbia micractina* were collected at Zhang County, Gansu Province, People's Republic of China, in September 2002. The plant was identified by Associate Professor Lin Ma (Institute of Materia Medica, Beijing). A voucher specimen (no. 02086) was deposited at the Herbarium of the Department of Medicinal Plants, Institute of Materia Medica.

Extraction and Isolation. The air-dried root of *E. micractina* (11.2 kg) was powdered and extracted with 30 L of 95% EtOH at room temperature for 3 × 48 h. The EtOH extract was evaporated under reduced pressure to yield a dark brown residue (1092 g). The residue was suspended in H_2O (1500 mL) and then partitioned with EtOAc (4 × 1000 mL). After removing the solvent, the EtOAc extract (323.5 g) was subjected to CC over silica gel, eluting with a gradient of increasing acetone concentrations (0−100%) in petroleum ether, to afford 20 fractions (A1−A20) on the basis of TLC analysis. Fraction A9 (6.4 g) was separated by normal-phase silica gel CC, eluting with a gradient of increasing acetone concentrations (5−100%) in petroleum ether, to give 10 subfractions (A9-1−A9-10). Subfraction A9-5 (1.8 g) was further separated by flash column chromatography over reversed-phase silica gel (C-18), eluting with a gradient of increasing EtOH concentrations (0−100%) in H_2O, to afford nine fractions (A9-5-1−A9-5-9). Fraction A9-5-4 (35 mg) was chromatographed over Sephadex LH-20, eluting with petroleum ether−$CHCl_3$−MeOH (5:5:1), to give three further fractions. These were separately purified by reversed-phase semipreparative HPLC, using MeOH−H_2O (70:30) or MeCN−H_2O (70:30) as mobile phase, to yield **2** (1.6 mg), **3** (0.9 mg), **4** (2.4 mg), **5** (0.8 mg), **7** (0.7 mg), **8** (1.4 mg), **9** (0.7 mg), and **10** (22.7 mg). Fraction A9-5-6 (45 mg) was chromatographed over Sephadex LH-20, eluting with petroleum ether−$CHCl_3$−MeOH (5:5:1), and then purified repeatedly by reversed-phase semipreparative HPLC, using MeOH−H_2O (85:15) or MeCN−H_2O (80:20) as mobile phase, to yield **6** (5.6 mg). Fraction A10 (7.6 g) was separated by normal-phase silica gel CC, eluting with a gradient of increasing acetone concentrations (5−100%) in petroleum ether, to give nine subfractions (A10-1−A10-9). Subfraction A10-4 (2.0 g) was further separated by flash column chromatography over reversed-phase silica gel eluting with a gradient of increasing EtOH concentrations (0−100%) in H_2O to afford four fractions (A10-4-1−A10-4-4). Fraction A10-4-2 (800 mg) was chromatographed over Sephadex LH-20, eluting with petroleum ether−$CHCl_3$−MeOH (5:5:1), to give seven fractions (A10-4-2-1−A10-4-2-7). Fraction A10-4-2-4 was purified repeatedly by normal-phase silica gel CC, using $CHCl_3$−MeOH (50:1), to yield **1** (30.6 mg) and **11** (306.3 mg).

(−)-(24*R*)-Tirucalla-8,25-diene-3β,24-diol (1): white, amorphous solid; $[\alpha]^{20}_D$ −1.3 (*c* 0.08, MeOH); UV (MeOH) λ_{max} (log ϵ) 204 (3.41) nm; CD (MeOH) 281 ($\Delta\epsilon$ +0.04), 218 ($\Delta\epsilon$ −0.72), 203 ($\Delta\epsilon$ −0.20); IR ν_{max} 3333, 3071, 2938, 2874, 1648, 1466, 1452, 1373, 1335, 1296, 1249, 1191, 1152, 1095, 1062, 1026, 893, 865, 649 cm^{-1}; ^1H NMR (acetone-d_6, 600 MHz) data, see Table 1; ^{13}C NMR (acetone-d_6, 150 MHz) data, see Table 2; EIMS *m*/*z* 442 $[M]^{+\bullet}$; HREIMS *m*/*z* 442.3799 $[M]^{+\bullet}$ (calcd for $C_{30}H_{50}O_2$, 442.3811).

Preparation of (*S*)- and (*R*)-MTPA Esters of 1. 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI, 75.0 mg), DMAP (53.1 mg), and (*S*)-MTPA (61.3 mg) were added to a solution of **1** (27.6 mg) in dried CH_2Cl_2 (3 mL). The mixture was stirred at room temperature for 24 h. The reaction mixture was evaporated under reduced pressure and then partitioned between $CHCl_3$ and H_2O. After the solvent was removed, the $CHCl_3$ extract was purified by preparative TLC with petroleum ether−EtOAc (6:1), and then HPLC using MeOH−H_2O (99:1), to afford the (*S*)-MTPA esters **1a** (6.5 mg; 16.5%) and **1b** (11.2 mg; 28.4%). By the same procedure, the (*R*)-MTPA esters **1c** (1.4 mg; 8.2%) and **1d** (5.1 mg; 29.8%) were prepared. **1a**: colorless gum; ^1H NMR ($CDCl_3$, 600 MHz) δ 7.54 (2H, m, H-2′ and H-6′ of MTPA moiety), 7.40 (3H, m, H-3′, H-4′, and H-5′ of MTPA moiety), 3.53 (3H, s, MeO of MTPA moiety), 4.93 (1H, br s, H-26a), 4.84 (1H, br s, H-26b), 4.72 (1H, dd, J = 12.0, 4.2 Hz, H-3), 4.02 (1H, dd, J = 6.6 and 4.8 Hz, H-24), 1.81 (1H, m, H-2a), 1.73 (3H, s, H_3-27), 1.64 (1H, m, H-2b), 1.49 (1H, m, H-1a), 1.31 (1H, m, H-1b), 1.24 (1H, m, H-5), 0.96 (3H, s, H_3-19), 0.924 (3H, s, H_3-28), 0.920 (3H, d, J = 5.4 Hz, H_3-21), 0.86 (3H, s, H_3-30), 0.83 (3H, s, H_3-29), 0.76 (3H, s, H_3-18). **1b**: colorless gum; ^1H NMR ($CDCl_3$, 600 MHz) δ 7.52 (2H, m, H-2′ and H-6′ of MTPA moiety), 7.39 (3H, m, H-3′, H-4′, and H-5′ of

MTPA moiety), 3.57 (3H, s, MeO of MTPA moiety), 5.35 (1H, t, J = 7.2 Hz, H-24), 4.97 (1H, br s, H-26a), 4.93 (1H, br s, H-26b), 3.23 (1H, dd, J = 12.0, 4.8 Hz, H-3), 1.76 (1H, m, H-23a), 1.62 (1H, m, H-23b), 1.60 (3H, s, H_3-27), 1.46 (1H, m, H-22a), 1.40 (1H, m, H-20), 1.00 (3H, s, H_3-28), 0.97 (1H, m, H-22b), 0.95 (3H, s, H_3-19), 0.91 (3H, d, J = 6.0 Hz, H_3-21), 0.86 (3H, s, H_3-30), 0.80 (3H, s, H_3-29), 0.75 (3H, s, H_3-18). **1c**: colorless gum; ^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.56 (2H, m, H-2′ and H-6′ of MTPA moiety), 7.40 (3H, m, H-3′, H-4′, and H-5′ of MTPA moiety), 3.57 (3H, s, MeO of MTPA moiety), 4.93 (1H, br s, H-26a), 4.84 (1H, br s, H-26b), 4.74 (1H, dd, J = 11.4, 4.8 Hz, H-3), 4.02 (1H, dd, J = 6.6 and 6.0 Hz, H-24), 1.88 (1H, m, H-2a), 1.76 (1H, m, H-2b), 1.73 (3H, s, H_3-27), 1.51 (1H, m, H-1a), 1.33 (1H, m, H-1b), 1.24 (1H, m, H-5), 0.99 (3H, s, H_3-19), 0.92 (3H, d, J = 6.6 Hz, H_3-21), 0.86 (3H, s, H_3-30), 0.84 (3H, s, H_3-28), 0.81 (3H, s, H_3-29), 0.76 (3H, s, H_3-18). **1d**: colorless gum; ^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.51 (2H, m, H-2′ and H-6′ of MTPA moiety), 7.39 (3H, m, H-3′, H-4′, and H-5′ of MTPA moiety), 3.54 (3H, s, MeO of MTPA moiety), 5.39 (1H, t, J = 7.2 Hz, H-24), 5.06 (1H, br s, H-26a), 4.98 (1H, br s, H-26b), 3.24 (1H, dd, J = 12.0, 4.8 Hz, H-3), 1.72 (3H, s, H_3-27), 1.69 (1H, m, H-23a), 1.56 (1H, m, H-23b), 1.36 (1H, m, H-22a), 1.36 (1H, m, H-20), 1.00 (3H, s, H_3-28), 0.95 (3H, s, H_3-19), 0.89 (1H, m, H-22b), 0.87 (3H, d, J = 6.6 Hz, H_3-21), 0.85 (3H, s, H_3-30), 0.80 (3H, s, H_3-29), 0.73 (3H, s, H_3-18).

(−)-(24*R*),24-Hydroperoxytirucalla-8,25-dien-3*β*-ol-7-one (2): white, amorphous solid; $[\alpha]^{20}_D$ −1.9 (*c* 0.05, MeOH); UV (MeOH) λ_{max} (log ϵ) 202 (3.68), 254 (1.87) nm; CD (MeOH) 339 ($\Delta\epsilon$ +0.97), 258 ($\Delta\epsilon$ −1.49), 237 ($\Delta\epsilon$ −0.61), 221 ($\Delta\epsilon$ −1.17), 207 ($\Delta\epsilon$ −0.53); IR ν_{max} 3313, 2939, 1714, 1633, 1588, 1457, 1403, 1373, 1331, 1267, 1190, 1099, 1033, 980, 916, 863, 803, 689, 607 cm^{-1}; ^{1}H NMR (acetone-d_6, 500 MHz) data, see Table 1; ^{13}C NMR (acetone-d_6, 150 MHz) data, see Table 2; ESIMS *m*/*z* 473 $[M + H]^+$ and 495 $[M + Na]^+$; HRESIMS *m*/*z* 495.3468 $[M + Na]^+$ (calcd for $C_{30}H_{48}O_4Na$, 495.3445).

Reduction of 2. A solution of **2** (0.6 mg) in dried CH_2Cl_2 (1.0 mL) was treated with triphenylphosphine (6.5 mg) at room temperature for 24 h. The reaction mixture was evaporated under reduced pressure and then purified by preparative TLC with $CHCl_3$−EtOAc (10:1) as developing solvent, to afford **2a** (0.5 mg): ^{1}H NMR (acetone-d_6, 600 MHz) δ 4.87 (1H, br s, H-26a), 4.73 (1H, br s, H-26b), 3.95 (1H, dt, J = 5.4, 4.2 Hz, H-24), 3.63 (1H, d, J = 4.2 Hz, O*H*-24), 3.57 (1H, d, J = 4.8 Hz, O*H*-3), 3.24 (1H, dt, J = 8.4, 4.8 Hz, H-3), 2.43 (1H, dt, J = 21.0, 9.0 Hz, H-11a), 2.32 (1H, ddd, J = 21.0, 7.8, 1.0 Hz, H-11b), 2.30 (2H, d, J = 9.0 Hz, H_2-6), 2.09 (1H, ddd, J = 13.0, 10.0, 2.5 Hz, H-15a), 1.93 (1H, m, H-16a), 1.86 (1H, dt, J = 12.6, 3.6 Hz, H-1a), 1.76−1.82 (2H, m, H_2-12), 1.71 (1H, t, J = 9.0 Hz, H-5), 1.69 (2H, m, H_2-2), 1.68 (3H, s, H_3-27), 1.61 (1H, m, H-23a), 1.57 (1H, m, H-22a), 1.48 (1H, m, H-17), 1.48 (1H, m, H-15b), 1.47 (1H, m, H-1b), 1.42 (1H, m, H-20), 1.40 (1H, m, H-23b), 1.34 (1H, m, H-16b), 1.07 (3H, s, H_3-19), 0.98 (1H, m, H-22b), 0.97 (3H, s, H_3-28), 0.95 (3H, s, H_3-30), 0.94 (3H, d, J = 6.6 Hz, H_3-21), 0.87 (3H, s, H_3-29), 0.76 (3H, s, H_3-18).

(−)-(24*S*),24-Hydroperoxytirucalla-8,25-dien-3*β*-ol-7-one (3): white, amorphous solid; $[\alpha]^{20}_D$ −4.7 (*c* 0.08, MeOH); UV (MeOH) λ_{max} (log ϵ) 200 (3.54), 254 (1.91) nm; CD (MeOH) 339 ($\Delta\epsilon$ +0.57), 258 ($\Delta\epsilon$ −0.86), 238 ($\Delta\epsilon$ −0.37), 220 ($\Delta\epsilon$ −0.78); IR ν_{max} 3316, 2936, 2623, 2498, 1711, 1646, 1589, 1452, 1373, 1332, 1268, 1222, 1188, 1097, 1035, 1014, 985, 916, 863, 807, 690, 617, 605 cm^{-1}; ^{1}H NMR (acetone-d_6, 500 MHz) data, see Table 1; ^{13}C NMR (acetone-d_6, 150 MHz) data, see Table 2; ESIMS *m*/*z* 473 $[M + H]^+$ and 967 $[2\ M + Na]^+$; HRESIMS *m*/*z* 495.3463 $[M + Na]^+$ (calcd for $C_{30}H_{48}O_4Na$, 495.3445).

(−)-(23*E*),25-Hydroperoxytirucalla-8,23-dien-3*β*-ol-7-one (4): white, amorphous solid; $[\alpha]^{20}_D$ −3.2 (*c* 0.16, MeOH); UV (MeOH) λ_{max} (log ϵ) 202 (2.99), 254 (1.87) nm; CD (MeOH) 339 ($\Delta\epsilon$ +0.97), 259 ($\Delta\epsilon$ −1.58), 238 ($\Delta\epsilon$ −0.76), 220 ($\Delta\epsilon$ −1.39), 207 ($\Delta\epsilon$ −0.94), 199 ($\Delta\epsilon$ −1.28); IR ν_{max} 3313, 3160, 2939, 2606, 2498, 2360, 2140, 2045, 1938, 1742, 1661, 1647, 1587, 1476, 1445, 1400, 1372, 1331, 1261, 1173, 1096, 1080, 1035, 979, 917, 852, 807, 687, 610 cm^{-1}; ^{1}H NMR (acetone-d_6, 500 MHz) data, see Table 1; ^{13}C NMR (acetone-d_6, 150 MHz) data, see Table 2; ESIMS *m*/*z* 473 $[M + H]^+$ and 495 $[M + Na]^+$; HRESIMS *m*/*z* 495.3478 $[M + Na]^+$ (calcd for $C_{30}H_{48}O_4Na$, 495.3445).

(−)-(23*E*)-Tirucalla-8,23-diene-3*β*,25-diol-7-one (5): white, amorphous solid; $[\alpha]^{20}_D$ −2.5 (*c* 0.04, MeOH); UV (MeOH) λ_{max} (log ϵ) 202 (3.13), 254 (2.01) nm; CD (MeOH) 340 ($\Delta\epsilon$ +0.79), 258 ($\Delta\epsilon$ −1.35), 236 ($\Delta\epsilon$ −0.66), 221 ($\Delta\epsilon$ −1.03), 206 ($\Delta\epsilon$ −0.37); IR ν_{max} 3335, 2962, 2932, 1642, 1584, 1454, 1414, 1370, 1333, 1268, 1185, 1148, 1100, 1032, 977, 891, 844, 688, 618 cm^{-1}; ^{1}H NMR (acetone-d_6, 500 MHz) data, see Table 1; ^{13}C NMR (acetone-d_6, 125 MHz) data, see Table 2; ESIMS *m*/*z* 457 $[M + H]^+$, 479 $[M + Na]^+$, and 495 $[M + K]^+$; HRESIMS *m*/*z* 479.3524 $[M + Na]^+$ (calcd for $C_{30}H_{48}O_3Na$, 479.3496).

(−)-(24*R*)-Tirucalla-7,9(11),25-triene-3*β*,24-diol (6): white, amorphous solid; $[\alpha]^{20}_D$ −23.1 (*c* 0.61, MeOH); UV (MeOH) λ_{max} (log ϵ) 205 (3.19), 238 (1.90) nm; CD (MeOH) 236 ($\Delta\epsilon$ −2.12); IR ν_{max} 3391, 3041, 2958, 2926, 2872, 1714, 1655, 1613, 1510, 1459, 1372, 1292, 1243, 1184, 1153, 1110, 1072, 1037, 984, 903, 813 cm^{-1}; ^{1}H NMR (acetone-d_6, 600 MHz) data, see Table 1; ^{13}C NMR (acetone-d_6, 150 MHz) data, see Table 2; EIMS *m*/*z* 440 $[M]^{+\bullet}$; HREIMS *m*/*z* 440.3640 $[M]^{+\bullet}$ (calcd for $C_{30}H_{48}O_2$, 440.3654).

(+)-(23*E*),25-Hydroperoxytirucalla-8,23-dien-3*β*-ol-7,11-dione (7): white, amorphous solid; $[\alpha]^{20}_D$ +2.7 (*c* 0.04, MeOH); UV (MeOH) λ_{max} (log ϵ) 204 (3.33), 219 (1.23), 271 (1.36) nm; CD (MeOH) 274 ($\Delta\epsilon$ +3.03), 233 ($\Delta\epsilon$ −2.96), 214 ($\Delta\epsilon$ −1.43), 202 ($\Delta\epsilon$ −1.91); IR ν_{max} 3310, 3160, 2970, 2930, 1713, 1668, 1458, 1407, 1377, 1230, 1184, 1034, 983, 918, 734, 608 cm^{-1}; ^{1}H NMR (acetone-d_6, 500 MHz) data, see Table 1; ^{13}C NMR (acetone-d_6, 150 MHz) data, see Table 2; ESIMS *m*/*z* 509 $[M + Na]^+$ and 521 $[M + Cl]^-$; HRESIMS *m*/*z* 509.3272 $[M + Na]^+$ (calcd for $C_{30}H_{46}O_5Na$, 509.3237).

(+)-(24*S*),24-Hydroperoxyeupha-8,25-dien-3*β*-ol-7-one (8): white, amorphous solid; $[\alpha]^{20}_D$ +6.2 (*c* 0.08, MeOH); UV (MeOH) λ_{max} (log ϵ) 203 (3.52), 254 (2.11) nm; CD (MeOH) 338 ($\Delta\epsilon$ +0.49), 259 ($\Delta\epsilon$ −0.59), 239 ($\Delta\epsilon$ −0.34), 221 ($\Delta\epsilon$ −0.68), 207 ($\Delta\epsilon$ −0.42), 197 ($\Delta\epsilon$ −0.77); IR ν_{max} 3315, 2932, 1715, 1656, 1586, 1457, 1412, 1373, 1333, 1267, 1184, 1098, 1033, 985, 917, 894, 861, 694, 617 cm^{-1}; ^{1}H NMR (acetone-d_6, 500 MHz) data, see Table 1; ^{13}C NMR (acetone-d_6, 150 MHz) data, see Table 2; ESIMS *m*/*z* 473 $[M + H]^+$ and 495 $[M + Na]^+$; HRESIMS *m*/*z* 495.3475 $[M + Na]^+$ (calcd for $C_{30}H_{48}O_4Na$, 495.3445).

(+)-(24*S*)-Eupha-8,25-diene-3*β*,24-diol-7-one (9): white, amorphous solid; $[\alpha]^{20}_D$ +5.1 (*c* 0.04, MeOH); UV (MeOH) λ_{max} (log ϵ) 203 (3.39), 254 (1.77) nm; CD (MeOH) 337 ($\Delta\epsilon$ +0.50), 255 ($\Delta\epsilon$ −0.58), 239 ($\Delta\epsilon$ −0.42), 220 ($\Delta\epsilon$ −0.78); IR ν_{max} 3320, 2955, 2920, 1713, 1647, 1588, 1457, 1377, 1331, 1303, 1257, 1167, 1076, 1035, 998, 974, 897, 841, 808, 616 cm^{-1}; ^{1}H NMR (acetone-d_6, 500 MHz) data, see Table 1; ^{13}C NMR (acetone-d_6, 150 MHz) data, see Table 2; ESIMS *m*/*z* 457 $[M + H]^+$, 479 $[M + Na]^+$ and 495 $[M + K]^+$; HRESIMS *m*/*z* 479.3539 $[M + Na]^+$ (calcd for $C_{30}H_{48}O_3Na$, 479.3496).

Cells, Culture Conditions, and Cell Proliferation Assay. See ref 33. Camptothecin (CPT) was used as the positive control.

PTP1B Inhibition Assay. See ref 34. Oleanolic acid was used as the positive control.

Anti-HIV Activity Assay. A cell-based VSVG/HIV-1 pseudotyping system was used for evaluating the anti-HIV replication activity of the compounds as described previously.[35] Briefly, vesicular stomatitis virus glycoprotein (VSV-G) plasmid was cotransfected with env-deficient HIV-1 vector, pNL4−3.luc.R$^-$E$^-$,[36] into 293 cells by using a modified $Ca_3(PO_4)_2$ method.[37] Sixteen hours post-transfection, plates were washed by PBS, and fresh DMEM with 10% FBS was added into the plates. Forty-eight hours post-transfection, supernatant, which contained VSVG/HIV-1 virions, was harvested and filtered through a 0.45 μm filter. VSVG/HIV-1 pseudotyped virions were quantified in terms of their p24 concentrations detected by ELISA (ZeptoMetrix, cat. no. 0801111), then diluted to 0.2 ng p24/mL, and used directly or stored at −80 °C.

For the infection assay, 293T cells were plated on 24-well plates at a density of 6×10^4 cells per well, one day prior to infection. Compounds were incubated with target cells for 15 min prior to adding VSVG/HIV-1. The same amount of solvent alone was used as blank control. Forty-eight hours post-infection, cells were lysed in 50 μL of Cell Lysis Reagent (Promega). Luciferase activity of the cell lysate was measured by a FB15 luminometer (Berthold Detection System), according to the manufacture's instructions. In this assay, the positive control was zidovudine (AZT).

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Supporting Information Available: Figure S1, main ^{1}H−^{1}H COSY (thick lines) and HMBC (arrows) correlations of **1**, **2**, and **6** and main HMBC (arrows) correlations of the side chain of **4**. Figure S2, conformation and octant projection diagram of **2**. CD, HRMS, ^{1}H and ^{13}C NMR, ^{1}H−^{1}H COSY, HMQC, HMBC, and NOE spectra of compounds **1**−**4**, **6**, and **8**. CD, HRMS, ^{1}H and ^{13}C NMR of **5**, **7**, and **9**. ^{1}H NMR and ^{1}H−^{1}H COSY of **1a**−**1d** and **2a**. This material is available free of charge via the Internet at http://pubs.acs.org.

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