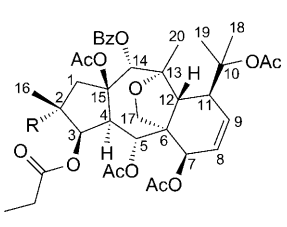
New Cytotoxic Myrsinane-Type Diterpenes from *Euphorbia prolifera*

by **Jie Li**, **Liang Xu***, and **Feng-Peng Wang***

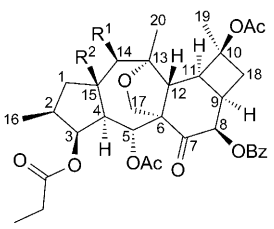
Department of Chemistry of Medicinal Natural Products, West China College of Pharmacy, Sichuan University; No. 17, Duan 3, Renmin Nan Road, Chengdu 610041, P. R. China (phone/fax: +86-28-5501368; e-mail: wfp@scu.edu.cn)

Four new myrsinol diterpenes, proliferins A–D (**1**–**4**, resp.) were isolated from the EtOH extracts of the roots of *Euphorbia prolifera*, along with four known compounds, euphorprolitherin B (**5**), euphorprolitherin D (**6**), SPr5 (**7**), and 14-desoxo-3-*O*-propionyl-5,15-di-*O*-acetyl-7-*O*-nicotinoylmyrsinol-14β-acetate (**8**). Their structures were established on the basis of spectroscopic methods, including HR-ESI-MS, and 1D- and 2D-NMR techniques. The cytotoxicity of compounds **1**, **3**, and **4** against cancer cells was evaluated, with compound **1** being active against A2780 cancer cells.

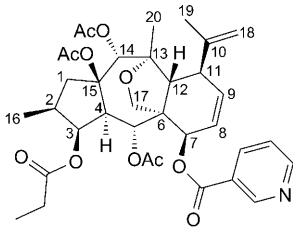
Introduction. – *Euphorbia prolifera* BUCH-HAM. is a perennial herbaceous plant widely distributed in southwest mainland China and used as traditional folk medicine for the treatment of inflammations and tumors [1]. The chemical constituents of the roots have been investigated previously, leading to the identification of five tigliane-type diterpenes [2], seven myrsinane-type diterpenes [3–6], and one ergostanol compound [4]. However, to the best of our knowledge, there has been no report of their bioactivity so far. For the search of antitumor constituents, further phytochemical research on this plant was carried out by our group. Four new myrsinane-type diterpenes, proliferins A–D (**1**–**4**, resp.) together with four known myrsinane-type diterpenes, *i.e.*, euphorprolitherin B (**5**) [6], euphorprolitherin D (**6**) [6], SPr5 (**7**) [3], and 14-desoxo-3-*O*-propionyl-5,15-di-*O*-acetyl-7-*O*-nicotinoylmyrsinol-14β-acetate (**8**) [7], were isolated from the roots of *Euphorbia prolifera*. Details of the isolation and structure elucidation of compounds **1**–**4** are presented herein. The new compounds **1**, **3**, and **4** were evaluated for cytotoxicity against HCT-8, Bel-7402, BGC-823, A549, and A2780 cancer cells.



1 R = Me_2CHCO_2
2 R = BzO
3 R = H
5 R = AcO



4 R^1 = BzO, R^2 = OH
6 R^1 = AcO, R^2 = OH
7 R^1 = AcO, R^2= AcO



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Results and Discussion. – Proliferin A (**1**) was obtained as colorless crystals and exhibited a *quasi*-molecular-ion peak in the HR-ESI-MS at *m/z* 821.3390 (calc. for $C_{42}H_{54}NaO_{15}^{+}$ 821.3355), providing the molecular formula $C_{42}H_{54}O_{15}$. The IR spectrum displayed a prominent absorption band at 1741 cm^{-1}, indicating the presence of CO groups of ester functionalities. The ^{1}H- and ^{13}C-NMR spectra (*Tables 1* and *2*, resp.) showed the presence of seven ester groups, involving four AcO groups (δ(H) 1.98, 2.15, 2.00, 2.11 (4*s*, each 3 H); δ(C) 169.1, 170.6, 170.3, 168.4 (CO); 20.7, 22.4, 20.8, 22.2 (Me)), a (2-methylpropanoyl)oxy group (mpO; δ(H) 0.84, 1.10 (2*d*, $J = 7.2$, each 3 H), 2.25–2.28 (*m*, 1 H); δ(C) 173.5 (*s*), 34.3 (*d*), 18.2 (*q*), 18.8 (*q*)), a BzO group (δ(H) 7.59–8.11 (*m*, 5 H); δ(C) see *Table 2*), and a (propanoyl)oxy group (PO; δ(H) 2.40 (*q*, $J = 7.6$, 2 H), 1.17 (*t*, $J = 7.6$, 3 H); δ(C) 173.3 (*s*), 27.9 (*t*), 8.7 (*q*)). Moreover, the ^{1}H-NMR spectrum showed signals due to four O-bearing CH groups at δ(H) 5.80 (*s*, H–C(14)), 5.94 (*dd*, $J = 10.8, 1.2$, H–C(5)), 5.54 (*d*, $J = 4.0$, H–C(3)), and 4.84 (*d*, $J = 6.4$, H–C(7)); two vicinal olefinic H-atoms at δ(H) 6.19 (*ddd*, $J = 10.0, 6.4, 1.2$, H–C(8)) and 5.91 (*dd*, $J = 10.0, 5.2$, H–C(9)); and a pair of CH_2O H-atoms at δ(H) 4.12 (*d*, $J = 8.8$, H_{α}–C(17)) and 3.51 (*dd*, $J = 8.8, 1.2$, H_{β}–C(17)). The above mentioned data showed similarities to those of euphorprolitherin B (**5**) [6], a myrsinol diterpene isolated from the same plant, except that an AcO group was replaced by a mpO group.

Analyses of the $^{1}H,^{1}H$-COSY, HMQC, and HMBC spectra resulted in the unequivocal assignments of the H- and C-atom resonances (see *Fig. 1*). The relative orientations of seven acyl groups were clarified by the HMBC spectrum and a NOESY experiment. The HMBC cross-peaks between H–C(3) (δ(H) 5.54) and the C=O of PO group (δ(C) 173.3); H–C(5) (δ(H) 5.94) and the C=O of the Ac signal (δ(C) 169.1 (*s*)); H–C(7) (δ(H) 4.84) and the C=O of the AcO group (δ(C) 170.6 (*s*)); and H–C(14) (δ(H) 5.80) and the C=O of the BzO group (δ(C) 165.6 (*s*)) disclosed that the PO, two AcO, and the BzO groups were attached to methine C-atoms C(3), C(5), C(7), and C(14), respectively. The remaining mpO and another two AcO groups were at C(2), C(10), and C(15), respectively, due to the O-bearing quaternary C-atoms with signals at δ(C) 86.4, 85.8, and 90.0. Their relative positions were further established by a NOESY experiment. The NOESY correlations H–C(14)/AcO–C(15) and H–C(19)/AcO–C(10) indicated that two AcO groups were located at C(15) and C(10), respectively (*Fig. 2*). Consequently, the mpO group could only be attached to C(2).

The relative configuration of **1** was assigned to be identical to that of euphorprolitherin B (**5**) due to the high resemblance of chemical shifts and coupling constants of all H-atoms on skeleton C-atoms of these two compounds [6], which was further confirmed by the key NOESY correlations shown in *Fig. 2*.

Thus, on the basis of the above evidence, proliferin A (**1**) was established as 5α,7β,10,15β-O-tetraacetyl-14α-O-benzoyl-14-desoxo-10,18-dihydro-2α-O-isobutyryl-3β-O-propanoylmyrsinol.

Proliferin B (**2**), colorless crystals, has the molecular formula $C_{45}H_{52}O_{15}$, as determined by HR-ESI-MS (*m/z* 855.3152 ($[M+Na]^{+}$); calc. 855.3204). The NMR spectra (*Tables 1* and *2*) indicated the presence of four AcO groups (δ(H) 2.04, 2.00, 2.10, 2.17 (*s*, each 3 H); δ(C) 169.3, 170.4, 170.7, 168.5 (CO); 21.0, 21.3, 22.5, 22.4 (Me)), a PO group (δ(H) 2.40 (*q*, $J = 7.2$, 2 H), 1.19 (*t*, $J = 7.2$, 3 H); δ(C) 173.6 (*s*), 28.1 (*t*), 8.8 (*q*)), and two BzO groups (δ(H) 6.97–7.74 (*m*, 10 H); δ(C), see *Table 2*). The NMR data of **2** exhibited close similarity to those of proliferin A (**1**). Spectroscopic analysis

Table 1. *^{1}H-NMR Data of* **1**–**4** ($CDCl_3$, 400 MHz). δ in ppm, J in Hz.

	1	**2**	**3**	**4**
H_α–C(1)	3.18 (*d*, *J* = 17.2)	3.58 (*d*, *J* = 17.6)	2.50 (*dd*, *J* = 15.2, 7.6)	2.47–2.53 (*m*)
H_β–C(1)	2.43 (*d*, *J* = 17.2)	2.51 (*d*, *J* = 17.6)	1.80 (*dd*, *J* = 15.2, 12.4)	1.53 (*dd*, *J* = 15.2, 9.6)
H–C(2)	–	–	1.98–2.02 (*m*)	2.18–2.22 (*m*)
H–C(3)	5.54 (*d*, *J* = 4.0)	5.51 (*d*, *J* = 3.6)	5.36 (*t*, *J* = 3.2)	5.39 (*t*, *J* = 4.0)
H–C(4)	3.72 (*dd*, *J* = 10.8, 4.0)	3.95 (*dd*, *J* = 10.8, 3.6)	3.48 (*dd*, *J* = 11.2, 3.2)	2.76 (*dd*, *J* = 11.2, 4.0)
H–C(5)	5.94 (*dd*, *J* = 10.8, 1.2)	6.02 (*dd*, *J* = 10.8, 1.2)	5.96 (*dd*, *J* = 11.2, 1.2)	5.79 (*dd*, *J* = 11.2, 1.2)
H–C(7)	4.84 (*d*, *J* = 6.4)	4.87 (*d*, *J* = 6.8)	4.86 (*d*, *J* = 6.4)	–
H–C(8)	6.19 (*ddd*, *J* = 10.0, 6.4, 1.2)	6.19 (*ddd*, *J* = 10.4, 6.8, 1.2)	6.17 (*ddd*, *J* = 10.0, 6.4, 1.2)	5.56 (*d*, *J* = 5.2)
H–C(9)	5.91 (*dd*, *J* = 10.0, 5.2)	5.91 (*dd*, *J* = 10.4, 5.8)	5.90 (*dd*, *J* = 10.0, 5.6)	2.80–2.84 (*m*)
H–C(11)	3.17–3.19 (*m*)	3.16–3.18 (*m*)	3.15–3.18 (*m*)	2.62–2.64 (*m*)
H–C(12)	3.17–3.19 (*m*)	3.21 (*d*, *J* = 3.4)	3.28 (*d*, *J* = 3.2)	5.08 (*d*, *J* = 12.0)
H–C(14)	5.80 (*s*)	5.83 (*s*)	5.93 (*s*)	5.20 (*s*)
Me(16)	1.32 (*s*)	1.46 (*s*)	0.76 (*d*, *J* = 6.4)	0.83 (*d*, *J* = 6.8)
H_α–C(17)	4.12 (*d*, *J* = 8.8)	4.19 (*d*, *J* = 8.8)	4.12 (*d*, *J* = 8.4)	4.25 (*d*, *J* = 9.6)
H_β–C(17)	3.51 (*dd*, *J* = 8.8, 1.2)	3.53 (*dd*, *J* = 8.8, 1.2)	3.50 (*dd*, *J* = 8.4, 1.2)	3.69 (*dd*, *J* = 9.6, 1.2)
Me(18) or CH_2(18)	1.64 (*s*)	1.63 (*s*)	1.63 (*s*)	2.59–2.61 (*m*), 2.48–2.52 (*m*)
Me(19)	1.55 (*s*)	1.56 (*s*)	1.55 (*s*)	1.65 (*s*)
Me(20)	1.23 (*s*)	1.16 (*s*)	1.26 (*s*)	1.30 (*s*)
PO–C(3)				
CH_2C(2′)	2.40 (*q*, *J* = 7.6)	2.40 (*q*, *J* = 7.2)	2.35 (*q*, *J* = 7.2)	1.47 (*dq*, *J* = 16.8, 7.2), 1.12 (*dq*, *J* = 16.8, 7.2)
Me(3′)	1.17 (*t*, *J* = 7.6)	1.19 (*t*, *J* = 7.2)	1.16 (*t*, *J* = 7.2)	0.66 (*t*, *J* = 7.2)
AcO–C(5)	1.98 (*s*)	2.04 (*s*)	1.99 (*s*)	1.88 (*s*)
AcO–C(7)	2.15 (*s*)	2.00 (*s*)	2.13 (*s*)	–
AcO–C(10)	2.00 (*s*)	2.10 (*s*)	1.98 (*s*)	2.05 (*s*)
AcO–C(15)	2.11 (*s*)	2.17 (*s*)	2.12 (*s*)	–
BzO–C(2)				
H–C(2′,6′)		7.74 (*d*, *J* = 7.2)		
H–C(3′,5′)		7.34 (*t*, *J* = 7.2)		
H–C(4′)		7.53 (*t*, *J* = 7.2)		
BzO–C(8)				
H–C(2′,6′)				8.40 (*d*, *J* = 8.4)
H–C(3′,5′)				7.55 (*t*, *J* = 8.4)
H–C(4′)				7.65 (*t*, *J* = 8.4)
BzO–C(14)				
H–C(2′,6′)	8.11 (*d*, *J* = 8.0)	7.64 (*d*, *J* = 7.2)	8.12 (*d*, *J* = 7.6)	8.14 (*d*, *J* = 8.4)
H–C(3′,5′)	7.44 (*t*, *J* = 8.0)	6.97 (*t*, *J* = 7.2)	7.48 (*t*, *J* = 7.6)	7.49 (*t*, *J* = 8.4)
H–C(4′)	7.59 (*t*, *J* = 8.0)	7.33 (*t*, *J* = 7.2)	7.61 (*t*, *J* = 7.6)	7.63 (*t*, *J* = 8.4)
mpO–C(2)				
H–C(2′)	2.25–2.28 (*m*)			
H–C(3′)	0.84 (*d*, *J* = 7.2)			
H–C(4′)	1.10 (*d*, *J* = 7.2)			

Table 2. *^{13}C-NMR Data of* **1**–**4** ($CDCl_3$, 100 MHz,). δ in ppm.

	1	**2**	**3**	**4**
C(1)	47.5 (*t*)	46.6 (*t*)	45.6 (*t*)	50.6 (*t*)
C(2)	86.4 (*s*)	87.6 (*s*)	38.1 (*d*)	30.8 (*d*)
C(3)	77.6 (*d*)	78.6 (*d*)	76.8 (*d*)	78.9 (*d*)
C(4)	47.7 (*d*)	47.8 (*d*)	50.0 (*d*)	50.6 (*d*)
C(5)	68.5 (*d*)	68.7 (*d*)	68.9 (*d*)	69.3 (*d*)
C(6)	53.4 (*s*)	53.6 (*s*)	53.7 (*s*)	63.0 (*s*)
C(7)	62.8 (*d*)	63.0 (*d*)	63.3 (*d*)	205.4 (*s*)
C(8)	125.8 (*d*)	125.9 (*d*)	125.7 (*d*)	72.4 (*d*)
C(9)	129.8 (*d*)	129.9 (*d*)	129.9 (*d*)	35.5 (*d*)
C(10)	85.8 (*s*)	85.8 (*s*)	85.9 (*s*)	78.4 (*s*)
C(11)	44.6 (*d*)	44.7 (*d*)	44.9 (*d*)	41.4 (*d*)
C(12)	37.0 (*d*)	37.1 (*d*)	37.3 (*d*)	41.9 (*d*)
C(13)	90.0 (*s*)	90.1 (*s*)	90.0 (*s*)	89.3 (*s*)
C(14)	73.5 (*d*)	73.3 (*d*)	73.4 (*d*)	83.1 (*d*)
C(15)	90.0 (*s*)	90.2 (*s*)	89.2 (*s*)	81.3 (*s*)
C(16)	18.9 (*q*)	18.3 (*q*)	14.0 (*q*)	14.5 (*q*)
C(17)	69.8 (*t*)	69.9 (*t*)	70.0 (*t*)	67.2 (*t*)
C(18)	25.1 (*q*)	25.2 (*q*)	25.4 (*q*)	34.7 (*t*)
C(19)	21.2 (*q*)	21.0 (*q*)	21.1 (*q*)	25.4 (*q*)
C(20)	24.1 (*q*)	24.3 (*q*)	24.9 (*q*)	22.3 (*q*)
PO–C(3)				
C=O	173.3 (*s*)	173.6 (*s*)	174.1 (*s*)	173.1 (*s*)
C(2′)	27.9 (*t*)	28.1 (*t*)	28.2 (*t*)	26.3 (*t*)
C(3′)	8.7 (*q*)	8.8 (*q*)	9.1 (*q*)	8.7 (*q*)
*Ac*O–C(5)	169.1 (*s*), 20.7 (*q*)	169.3 (*s*), 21.0 (*q*)	169.2 (*s*), 21.1 (*q*)	170.2 (*s*), 22.6 (*q*)
*Ac*O–C(7)	170.6 (*s*), 22.4 (*q*)	170.4 (*s*), 21.3 (*q*)	170.7 (*s*), 22.7 (*q*)	–
*Ac*O–C(10)	170.3 (*s*), 20.8 (*q*)	170.7 (*s*), 22.5 (*q*)	170.4 (*s*), 21.0 (*q*)	169.3 (*s*), 22.0 (*q*)
*Ac*O–C(15)	168.4 (*s*), 22.2 (*q*)	168.5 (*s*), 22.4 (*q*)	168.6 (*s*), 22.5 (*q*)	–
BzO–C(2)				
C=O		164.8 (*s*)		
C(1′)		129.2 (*s*)		
C(2′,6′)		129.8 (*d*)		
C(3′,5′)		128.2 (*d*)		
C(4′)		132.8 (*d*)		
BzO–C(8)				
C=O				160.1 (*s*)
C(1′)				130.3 (*s*)
C(2′,6′)				130.1 (*d*)
C(3′,5′)				128.5 (*d*)
C(4′)				133.4 (*d*)
BzO–C(14)				
C=O	165.6 (*s*)	165.9 (*s*)	165.5 (*s*)	167.0 (*s*)
C(1′)	130.1 (*s*)	128.4 (*s*)	130.0 (*s*)	130.3 (*s*)
C(2′,6′)	130.0 (*d*)	129.5 (*d*)	130.0 (*d*)	129.9 (*d*)
C(3′,5′)	128.3 (*d*)	128.1 (*d*)	128.6 (*d*)	128.4 (*d*)
C(4′)	133.2 (*d*)	132.6 (*d*)	133.4 (*d*)	133.4 (*d*)
mpO–C(2)				
C(1′)	173.5 (*s*)			
C(2′)	34.3 (*d*)			
C(3′)	18.2 (*q*)			
C(4′)	18.8 (*q*)			

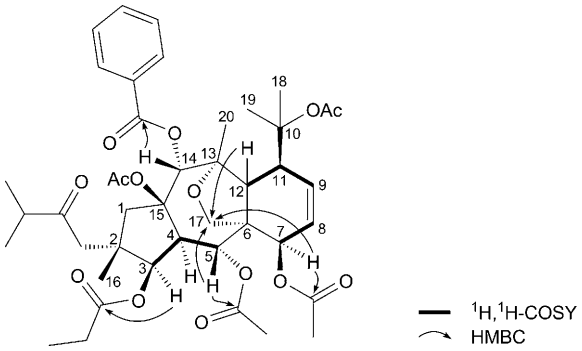

Fig. 1. *Key ^{1}H,^{1}H-COSY and HMBC correlations of proliferin A* (**1**)

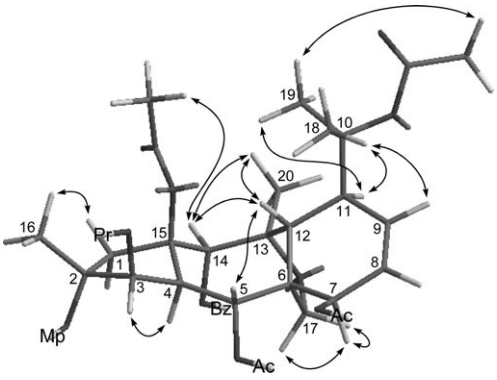

Fig. 2. *Key NOESY correlations of proliferin A* (**1**)

revealed that the only structural difference between these two compounds is that the mpO–C(2) group of **1** is replaced by a BzO group in **2**. The relative positions of the acyl groups in **2** were deduced from the HMBC spectrum and a NOESY experiment as in the case of **1**. The cross-peaks in the HMBC spectrum between the H-atoms adjacent to the ester moieties and the corresponding C=O groups permitted locations of the ester groups at the O-bearing C-atoms C(3), C(5), C(7), and C(14), respectively. The remaining two AcO groups and one BzO group were at C(10), C(15), and C(2), respectively, based on the observation of the clear cross-peaks of H–C(14)/AcO and H–C(19)/AcO in the NOESY spectrum. Therefore, the structure of proliferin B (**2**) was identified as 5α,7β,10,15β-O-tetraacetyl-14-desoxo-2α,14α-O-dibenzoyl-10,18-dihydro-3β-O-propanoylmyrsinol.

The HR-ESI-MS of proliferin C (**3**) exhibited a *quasi*-molecular-ion peak ([M + Na]$^+$) at m/z 735.3004 (calc. 735.2987), corresponding to the molecular formula $C_{38}H_{48}O_{13}$ (58 mass units lower than that of euphorprolitherin B (**5**)). The NMR spectra of **3** (*Tables 1* and *2*) were similar to those of 10,18-dihydromyrsinol derivatives such as **1**, **2**, and **5**. Detailed comparison of its NMR spectra with those of **5** indicated that the major difference between these two compounds is the absence of the AcO–C(2) group in proliferin C (**3**), which was supported by the additional signals for the CH(2) group (δ(H) 1.98–2.02 (*m*, 1 H); δ(C) 38.1 (*d*)), and the fact that the H-atom signal of

Me–C(16) was shifted upfield from δ(H) 1.32 to 0.76 and its multiplicity was altered from a *singlet* to a *doublet* ($J = 6.4$).

The structure of **3** was confirmed by 2D-NMR (^{1}H,^{1}H-COSY, HMQC, and HMBC), and its relative configuration was established by comparison with ^{1}H-NMR spectral data of related compounds with myrsinane-type skeletons, which was confirmed by the NOESY spectrum. The cross-peaks H–C(2)/H–C(3), H–C(5)/H_β–C(12), H–C(14)/H_β–C(12), and H–C(7)/CH_2(17) in the NOESY spectrum, combined with the coupling constants between H–C(3) and H–C(2) ($J = 3.2$), and H–C(4) and H–C(5) ($J = 11.2$ Hz), provided the orientation of Me–C(2) as β, PO–C(3) as β, AcO–C(5) as α, AcO–C(7) as β, and BzO–C(14) as α. Therefore, the structure of proliferin C (**3**) was deduced as 5α,7β,10,15β-O-tetraacetyl-14α-O-benzoyl-14-desoxo-10,18-dihydro-3β-O-propanoylmyrsinol.

Proliferin D (**4**) has the molecular formula $C_{41}H_{46}O_{13}$ according to the HR-ESI-MS (*m/z* 769.2836 ([M + Na]$^+$), calc. 769.2831). However, in the ^{13}C-NMR, there are no typical signals of a C(8)=C(9) bond, but instead one isolated CO signal (δ(C) 205.4) was observed. Beside this difference between **4** and typical myrsinol derivatives, the presence of a four-membered ring was detected due to the ^{1}H,^{1}H-COSY interaction between H–C(9) (δ(H) 2.80–2.84 (*m*)) and CH_2(18) (δ(H) 2.59–2.61 (*m*), 2.48–2.52 (*m*)). Further analysis of its ^{1}H- and ^{13}C-NMR spectra revealed that **4** highly resembles euphorprolitherin D (**6**) [6], a cyclomyrsinol derivative isolated from the same plant, with the only difference of an ester substituent at C(14). The HMBC spectrum of **4** showed key correlations for H–C(3) (δ(H) 5.39)/PO (δ(C) 173.1), H–C(5) (δ(H) 5.79)/AcO (δ(C) 170.2), H–C(8) (δ(H) 5.56)/BzO (δ(C) 160.1), and H–C(14) (δ(H) 5.20)/BzO (δ(C) 167.0), leading to the location of the ester groups at C(3), C(5), C(8), and C(14), respectively. The relative configuration was assigned as in euphorprolitherin D (**6**), based on the very similar coupling constants of H_β–C(1)/H–C(2), H–C(2)/H–C(3), H–C(3)/H–C(4), H–C(4)/H–C(5), H–C(5)/H_β–C(17), H–C(8)/H–C(9), and H–C(11)/H–C(12) of these two compounds. The above findings led us to determine the structure of proliferin D (**4**) as 5α,10β-O-diacetoxy-8β,14β-O-dibenzoyl-3β-O-propanoylcyclomyrsinol.

Proliferins A, B, and D (**1**, **2**, and **4**, resp.) were subjected to evaluation for cytotoxicity against HCT-8, Bel-7402, BGC-823, A549, and A2780 cancer cells. Only compound **1** was found to be cytotoxic against A2780 human ovarian cancer cells (IC_{50} 7.7 μM). The two other compounds were inactive ($IC_{50} > 10$ μM) against all of the above-mentioned cell lines. No correlation between structure and cytotoxicity could be detected.

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Experimental Part

General. Silica gel GF_{254} and *H* (SiO_2; *Qingdao Sea Chemical Factory*, P. R. China) were used for TLC and column chromatography (CC), resp.; spots on TLC were detected with ninhydrin reagent. M.p.: thermal values anal. microscope (uncorrected). Optical rotations: *Perkin-Elmer* 341 polarimeter. IR Spectra: *Nicolet FI-IR 200S* spectrometer. ^{1}H- and ^{13}C-NMR spectra: *Varian Unity-INOVA-400/54* NMR spectrometer in $CDCl_3$ with TMS as the internal standard. ESI-MS and HR-EI-MS: *VG Auto spec 3000* or *Finnigan MAT 90* instrument.

Plant Material. The roots of *Euphorbia prolifera* BUCH-HAM. were collected in Yunnan Province, P. R. China, in October of 2005, and identified by Prof. *Penghua*. A voucher specimen was deposited with the West China College of Pharmacy, Sichuan University.

Extraction and Isolation. The air-dried roots (7.0 kg) were powdered and soaked in 95% EtOH for four times. After concentration *in vacuo*, a crude extract (956 g) was obtained, which was suspended in 800 ml of H_2O, and the suspension was extracted successively with petroleum ether (PE) and AcOEt. The PE-soluble part afforded 456 g of extract, of which 252 g were subjected to SiO_2 CC with PE/acetone gradient system of increasing polarity (9:1, 8:2, 7:3, 6:4, 5:5) to give five fractions. *Fr. 3* was chromatographed over silica-gel column with $CHCl_3$/MeOH 300:1, 200:1, and 99:1 to yield **5** (15 mg), **6** (12 mg), and **7** (18 mg). *Fr. 4* was chromatographed repeatedly over SiO_2 column with MeOH/H_2O (7:3, 8:2, 9:1 to afford **1** (18 mg), **2** (4 mg), and **3** (24 mg). *Fr. 5* was rechromatographed on SiO_2 column with $CHCl_3$/MeOH 200:1, 99:1, 98:2 to give **4** (8 mg) and **8** (16 mg).

Proliferin A (=*(2*R*,3*R*,3a*S*,4*R*,4a*R*,5*R*,8*S*,8a*R*,9*R*,10*R*,10a*R*)-4,5,10a-Tris(acetyloxy)-8-[2-(acetyloxy)propan-2-yl]-2,9-dimethyl-2-[(2-methylpropanoyl)oxy]-3-(propanoyloxy)-2,3,3a,4,5,8,8a,9,10,10a-decahydro-1*H*-9,4a-(epoxymethano)benzo[f]azulen-10-yl Benzoate*; **1**). Colorless crystals (EtOH). M.p. 100–102°. $[\alpha]_D^{20} = -122.7$ ($c = 0.15$, $CHCl_3$). IR (KBr): 3448, 2985, 1741, 1245, 1068. ^{1}H-NMR (400 MHz, $CDCl_3$): *Table 1*. ^{13}C-NMR (100 MHz, $CDCl_3$): *Table 2*. HR-ESI-MS: 821.3390 ($[M + Na]^+$, $C_{42}H_{54}NaO_{15}^+$; calc. 821.3355).

Proliferin B (=*(2*R*,3*R*,3a*S*,4*R*,4a*R*,5*R*,8*S*,8a*R*,9*R*,10*R*,10a*R*)-4,5,10a-Tris(acetyloxy)-8-[2-(acetyloxy)propan-2-yl]-2,3,3a,4,5,8,8a,9,10,10a-decahydro-2,9-dimethyl-3-(propanoyloxy)-1*H*-9,4a-(epoxymethano)benzo[f]azulene-2,10-diyl Dibenzoate*; **2**). Colorless crystals (EtOH). M.p. 156–158°. $[\alpha]_D^{20} = -138.5$ ($c = 0.2$, $CHCl_3$). ^{1}H-NMR (400 MHz, $CDCl_3$): *Table 1*. ^{13}C-NMR (100 MHz, $CDCl_3$): *Table 2*. HR-ESI-MS: 855.3152 ($[M + Na]^+$, $C_{45}H_{52}NaO_{15}^+$; calc. 855.3204).

Proliferin C (=*(2*R*,3*R*,3a*S*,4*S*,4a*S*,5*S*,8*R*,8a*S*,9*S*,10*S*,10a*S*)-4,5,10a-Tris(acetyloxy)-8-[2-(acetyloxy)propan-2-yl]-2,3,3a,4,5,8,8a,9,10,10a-decahydro-2,9-dimethyl-3-(propanoyloxy)-1*H*-9,4a-(epoxymethano)benzo[f]azulen-10-yl Benzoate*; **3**). Colorless crystals (acetone). M.p. 196–198°. $[\alpha]_D^{20} = -110.2$ ($c = 0.45$, $CHCl_3$). IR (KBr): 3449, 2984, 1732, 1637, 1242. ^{1}H-NMR (400 MHz, $CDCl_3$): *Table 1*. ^{13}C-NMR (100 MHz, $CDCl_3$): *Table 2*. HR-ESI-MS: 735.3004 ($[M + Na]^+$, $C_{38}H_{48}NaO_{13}^+$; calc. 735.2987).

Proliferin D (=*(1*R*,2a*S*,3*S*,4a*R*,5*S*,5a*S*,6*R*,7*R*,8a*S*,9*S*,10*S*,10a*S*,10b*R*)-1,5-Bis(acetyloxy)-tetradecahydro-8a-hydroxy-1,7,10-trimethyl-4-oxo-6-(propanoyloxy)-1*H*-10,4a-(epoxymethano)cyclobuta[3,4]benzo[1,2-f]azulene-3,9-diyl Dibenzoate*; **4**). Colorless crystals (EtOH). M.p. 168–170°. $[\alpha]_D^{20} = +73.3$ ($c = 0.75$, $CHCl_3$). IR (KBr): 3448, 2935, 1742, 1266. ^{1}H-NMR (400 MHz, $CDCl_3$): *Table 1*. ^{13}C-NMR (100 MHz, $CDCl_3$): *Table 2*. HR-ESI-MS: 769.2836 ($[M + Na]^+$, $C_{41}H_{46}NaO_{13}^+$; calc. 769.2831).

Cytotoxicity Assay. Cytotoxity against the HCT-8, Bel-7402, BGC-823, A549, and A2780 cells was evaluated by using the MTT (=3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide) method according to the protocols described in [8].

REFERENCES

[1] Kunming Institute of Botany of Academia Sinica, 'Folk Medicinal Plants of Yunnan', Kunming, 1970, Vol. 1, p. 98.
[2] D. Wu, B. Sorg, E. Hecher, *Phytother. Res.* **1994**, *8*, 95.
[3] D. Wu, B. Sorg, E. Hecher, *J. Nat. Prod*. **1995**, *58*, 408.
[4] J. Zhang, C.-J. Yang, D.-G. Wu, *Acta Bot. Yunnan.* **1995**, *17*, 111.
[5] J. Zhang, C.-J. Yang, D.-G. Wu, *Chin. Trad. Herb. Drugs* **1998**, *29*, 73.
[6] W.-J. Zhang, D.-F. Chen, A.-J. Hou, *Chin. J. Chem*. **2004**, *22*, 103.
[7] F. Jeske, J. Jakupovic, W. Berendsohn, *Phytochemistry* **1995**, *40*, 1743.
[8] M. C. Alley, D. A. Scudiero, A. Monks, M. L. Hursey, M. J. Czerwinski, D. L. Fine, B. J. Abbott, J. G. Mayo, R. H. Shoemaker, M. R. Boyd, *Cancer Res.* **1988**, *48*, 581.

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