Bioactive Myrsinol Diterpenoids from the Roots of *Euphorbia prolifera*

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Ⓢ *Supporting Information*

ABSTRACT: Ten myrsinol diterpenes, euphorbiaproliferins A−J (**1**−**10**), along with nine known analogues (**11**−**19**) were isolated from the roots of *Euphorbia prolifera*. Their structures were elucidated by spectroscopic data analysis (IR, ESIMS, HRESIMS, 1D and 2D NMR), and the structure of **1** was confirmed by X-ray crystallography. The diterpenes showed neuroprotective effects against MPP$^+$-induced neuronal cell death in SH-SY5Y cells.

RESULTS AND DISCUSSION
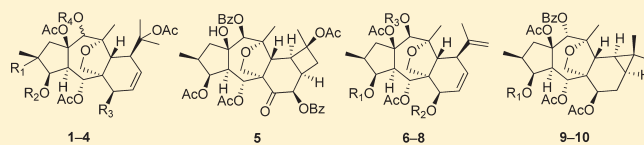
The genus *Euphorbia*, belonging to the family Euphorbiaceae, contains over 2000 species, which are distributed over the tropical and temperate zones of Asia and other parts of the world.[1] Phytochemical investigations on this genus reveal that diterpenoids are the main constituents of the genus with many different parent skeletons including jatrophanes, lathyranes, tiglianes, ingenanes, myrsinols, etc.[2,3] These different diterpenoids possess various biological effects such as antiproliferation, modulability of multidrug resistance, cytotoxic activity, and antimicrobial and anti-inflammatory activity.[2,3] The roots of *Euphorbia prolifera* Buch-Ham are used as a traditional folk medicine for the treatment of inflammation and tumors.[4] Although many bioactive constituents of the genus *Euphorbia* have been reported, phytochemical and pharmacological studies on *E. prolifera* are lacking,[4−9] and there have been no reports on the neuroprotective effects of *E. prolifera* or its constituents. In the course of our ongoing search for bioactive metabolites having neuroprotective effects,[10−12] we investigated the chemical constituents of the roots of *E. prolifera*, whose methanol extract showed neuroprotective effects. Ten new myrsinol-type diterpenes, euphorbiaproliferins A−J (**1**−**10**), and nine known analogues (**11**−**19**) have been isolated from the roots of *E. prolifera* as a result of further chemical investigation for bioactive products. In this paper, we report the isolation and structural elucidation of these myrsinol-type diterpenes and their neuroprotective activities against 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPP$^+$)-induced neuronal cell death in human dopaminergic neuroblastoma SH-SY5Y cells.

■ RESULTS AND DISCUSSION

The ethyl acetate-soluble part of the methanol extract of the roots was fractionated by column chromatography and purified by HPLC to obtain 10 new (**1**−**10**) and nine known (**11**−**19**) compounds. The NMR data of compounds **1**−**19** showed that they are all myrsinol-type diterpenes.

Compound **1** was obtained as white flakes. Its HR-ESIMS provided the molecular formula $C_{39}H_{56}O_{15}$ through the presence of a peak at m/z 787.3510 $[M + Na]^+$ (calcd for $C_{39}H_{56}O_{15}Na$, 787.3517). The ^1H and ^{13}C NMR data (Tables 1 and 2) suggested that compound **1** was a 10,18-dihydromyrsinol-type diterpene with seven acyl groups (four acetyl, two isobutyryl, and one propionyl group) based on the myrsinol diterpenes reported from the genus *Euphorbia*.[2,3,8,9] Further analyses of the HMQC, HMBC, and ^1H−^1H COSY spectra confirmed the presence of the 10,18-dihydromyrsinol-type skeleton and the presence of four acetyl, two isobutyryl, and one propionyl group. The positions of the acyl groups were determined via interpretation of the HMBC data. The correlation of H-3 at δ_H 5.15 with the carbonyl signal at δ_C 172.8 (CO of the propionyl moiety) indicated the presence of the propionyloxy group at C-3. Similarly, the long-range couplings of the protons at δ_H 5.67 (H-5), 4.60 (H-7), and 5.31 (H-14) with the carbonyl carbons at δ_C 168.5, 169.5, and 175.0 demonstrated the presence of the two acetoxy groups and one isobutyryloxy group at C-5, C-7, and C-14, respectively. However, there were no long-range correlations of the protons in the myrsinol-type skeleton to the carbonyl carbons of the remaining two acetyl and isobutyryl groups, and it was therefore assumed that they were attached to quaternary carbons of the myrsinol skeleton. The positions of these remaining acyl groups were further deduced by a NOESY experiment. The NOESY correlations of H-14 (δ_H 5.31) to the methyl protons (δ_H 1.82) of the C-15 acetoxy group, and H$_3$-19 (δ_H 1.29) to the methyl protons (δ_H 1.88) of the C-10 acetoxy group, implied that the two acetoxy groups were located at C-15 and C-10, respectively.[9] Consequently, the isobutyryloxy group could be assigned only to C-2. Thus, the planar structure of **1** was established.
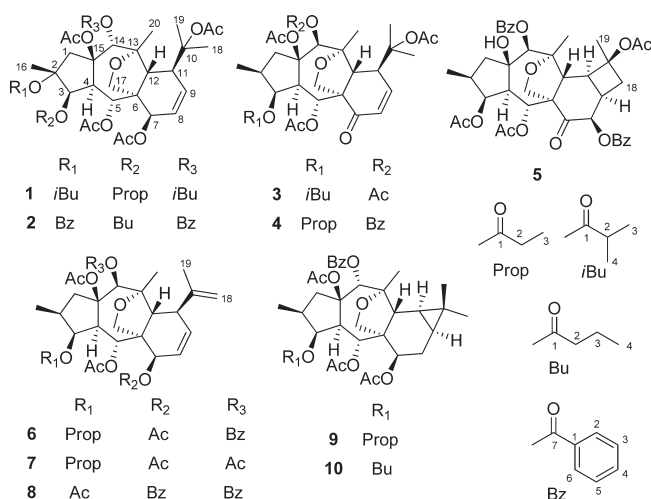
Figure 1. Structures of compounds **1**−**10** from *E. prolifera*.

The relative configuration of **1** was elucidated as follows. For the reported natural myrsinol diterpenes, the three rings (5/7/6) forming the myrsinol skeleton are *trans*-fused, H-4 and H$_2$-17 are α-oriented based on biosynthesis considerations, and H$_3$-16, H-12, the side chain at C-11, and the C-15 acyloxy group are β-oriented.[2,3] NOESY correlations observed for H-3/H-4, H-5/H-12, H-7/H$_2$-17, H-12/H-14, and H-12/H$_3$-20, but not for H-4/H-14 and H-3/H-5, suggested that H-3, H-4, and H-7 were α-oriented and H-5, H-12, H-14, and H$_3$-20 were β-oriented. These assignments were consistent with the configuration of reported 10,18-dihydromyrsinol diterpenes.[8,9] In order to confirm the above assignments and the configuration, a single-crystal X-ray crystallographic analysis using anomalous scattering of Cu Kα radiation was carried out. A thermal ellipsoid representation, with the atom numbering indicated, is shown in Figure 2. All of the above evidence confirmed the structure of **1** as 2α,14α-di-*O*-isobutyryl-3β-*O*-propionyl-5α,7β,10,15β-tetra-*O*-acetyl-10,18-dihydromyrsinol, which was named euphorbiaproliferin A.

Compound **2** possessed a molecular formula of C$_{46}$H$_{54}$O$_{15}$ based on the HR-ESIMS (m/z 869.3355 [M + Na]$^+$, calcd for C$_{46}$H$_{54}$O$_{15}$Na, 869.3360). The ^{1}H and ^{13}C NMR spectra suggested that **2** had the same parent skeleton as compound **1** and seven acyl groups (four acetyl, two benzoyl, and one butyryl group), which was supported by the 2D NMR data. The locations of the acyl groups were elucidated by interpretation of the HMBC and NOESY data as in the case of **1**. HMBC correlations of the protons at δ_H 5.51 (H-3), 6.02 (H-5), 4.88 (H-7), and 5.83 (H-14) with the corresponding carbonyl carbons at δ_C 172.9, 169.2, 170.4, and 165.8 indicated that a butyryloxy, two acetoxy, and a benzoyloxy group were attached to C-3, C-5, C-7, and C-14, respectively. The remaining two acetoxy and benzoyloxy groups were located at C-10, C-15, and C-2, respectively, based on the NOESY correlations of H$_3$-19 to the methyl protons of the C-10 acetoxy group and H-14 to the methyl protons of the C-15 acetoxy group.[8,9] The same relative configuration was inferred for euphorbiaproliferin B (**2**) and euphorbiaproliferin A (**1**) on the basis of comparison of the NOESY spectra of **1** and **2**. The structure of euphorbiaproliferin B was elucidated therefore as 2α,14α-di-*O*-benzoyl-3β-*O*-butyryl-5α,7β,10,15β-tetra-*O*-acetyl-10,18-dihydromyrsinol.

Compound **3** was isolated as colorless flakes. It gave the molecular formula C$_{32}$H$_{44}$O$_{12}$ as determined from the HR-ESIMS

and NMR analyses. Apart from the signals for the acyl groups, the main difference of ^{13}C NMR data between **3** and **1** was the presence of a ketocarbonyl carbon (δ_C 197.4) conjugated with the double bond (δ_C 131.6, 140.7) in **3** instead of the oxygenated carbon at C-7 in **1**. The locations of the acyl groups at C-3, C-5, and C-14 were determined by the HMBC correlations of H-3, H-5, and H-14 to the corresponding carbonyl carbons of the acyl groups. The remaining two acetoxy groups needed to be attached at C-10 and C-15, supported by the NOESY correlations of H$_3$-19 to the methyl protons of the C-10 acetoxy group and H-3 (H-4) to H-2′/6′ of the C-2 benzoyloxy group, respectively. The similar myrsinol-type diterpene skeleton of **3** compared to those of compounds **1** and **2** implied the same *trans*-fusion of the three rings.[2,3,8,9] The NOESY correlations of H-3/H-4, H-4/H-14, H-5/H-12, and H-12/H$_3$-20 suggested that the C-3 isobutyryloxy group and the C-14 acetoxy group were both β-oriented, while the C-5 acetoxy group was α-oriented. Additionally, the C-14 carbon signal (δ_C 81.0) was shifted downfield by about 9 ppm, corresponding to the carbon signal of compounds **1** [δ_C 71.8 (C-14)] and **2** [δ_C 73.2 (C-14)], which also implied the different orientation of the C-14 acetoxy group.[9,13] Therefore, euphorbiaproliferin C was elucidated as 3β-*O*-isobutyryl-5α,10,14β,15β-tetra-*O*-acetyl-7-oxo-10,18-dihydromyrsinol.

The molecular formula for compound **4** was determined as C$_{36}$H$_{44}$O$_{12}$ on the basis of the HR-ESIMS. On comparing the chemical shifts for the parent-skeleton carbons in **4** with those for C-1 to C-20 in compound **3**, the close similarity implied that compounds **3** and **4** possessed the same 10,18-dihydromyrsinol skeleton.[2,3,8,9] Following the same NMR procedures used for **3**, the locations of the acyloxy groups in **4** were determined by the analyses of the HMBC and NOESY spectra, which revealed that the three acetoxy groups were attached at C-5, C-10, and C-15, the propionyloxy group was at C-3, and the benzoyloxy group was at C-14, respectively. NOESY correlations of H-4/H-3, H-4/H-14, H-12/H-5, and H-12/H$_3$-20 allowed the stereochemical features to be assigned, which were identical with those of compound **3**. Thus, compound **4** was elucidated as 3β-*O*-propionyl-5α,10,15β-tri-*O*-acetyl-7-oxo-14β-*O*-benzoyl-10,18-dihydromyrsinol, which was named euphorbiaproliferin D.

Compound **5** was obtained as a white powder. Its molecular formula was determined as C$_{40}$H$_{44}$O$_{13}$ from the HR-ESIMS. From the ^{1}H and ^{13}C NMR spectra, three acetoxy and two benzoyloxy groups were evident. The remaining 20 resonances in the ^{13}C NMR spectrum suggested a cyclomyrsinol-type diterpene skelton for **5**,[7−9,13,14] which was demonstrated by the 2D NMR data. The positions of acyloxy groups were determined by HMBC and NOESY experiments. The HMBC correlations of H-3, H-5, H-8, and H-14 to the corresponding carbonyl carbon of the acyloxy groups revealed that the two acetoxy and two benzoyloxy groups were attached at C-3, C-5, C-8, and C-14, respectively. The remaining acetoxy group at C-10 was determined by the NOESY correlations of H$_3$-19 to the methyl protons of the C-10 acetoxy group. Also, the carbon signal for C-15 (δ_C 81.4) was shifted upfield by about 8 ppm, corresponding to the carbon signals (δ_C ~90, C-15) of compounds **1**−**4**, which implied that the substituent at C-15 was a hydroxy, not an acyloxy group.[8,9] NOESY correlations observed for H-3/H-4, H-4/H-14, H-5/H-12, H-12/H$_3$-20, H-8/H-9, H-8/H-11, H-9/H-11, H$_3$-19/H-11, and H$_3$-19/H-9 suggested that the C-3 acetoxy group, the C-8 and C-14 benzoyloxy groups, and the C-10 acetoxy group were β-oriented and the C-5 acetoxy group was α-oriented. These assignments were consistent with the configuration of reported

Table 1. ^{13}C NMR Data (δ_C) of Compounds 1−10 (CDCl$_3$, 100 MHz)[e]

position		1	2	position		3	4	5	6	7	8	9	10
1		46.0	46.5	1		43.9	43.7	50.5	43.4	43.4	43.5	45.2	45.3
2		85.9	87.5	2		36.1	35.6	35.4	36.6	36.6	36.5	37.7	37.8
3		77.4	78.5	3		75.8	76.0	79.1	76.4	76.4	76.9	76.6	76.7
4		46.9	47.7	4		52.2	52.5	50.6	51.3	51.4	51.8	49.9	50.1
5		67.9	68.6	5		66.8	67.0	69.3	68.9	69.0	69.2	68.5	68.6
6		52.6	53.5	6		61.7	61.7	62.9	53.7	54.2	54.5	52.1	52.2
7		62.1	62.9	7		197.4	197.1	205.4	63.5	63.7	64.6	67.7	68.0
8		125.3	125.8	8		131.6	131.6	72.4	122.8	123.3	123.2	23.1	23.3
9		129.4	129.9	9		140.7	140.4	30.6	133.0	133.2	133.5	20.1	20.3
10		85.1	85.8	10		85.3	84.6	78.4	147.0	147.1	146.8	17.1	17.2
11		44.0	44.7	11		42.5	43.4	41.3	41.8	41.1	41.8	17.3	17.4
12		36.5	37.0	12		41.5	41.8	41.8	40.3	40.8	40.5	36.5	36.7
13		89.3	89.9	13		89.2	89.3	89.3	89.1	88.8	89.3	88.0	88.0
14		71.8	73.2	14		81.0	81.8	83.1	81.4	80.9	81.7	73.2	73.4
15		89.4	90.1	15		90.3	90.5	81.4	89.4	89.7	90.0	88.4	88.6
16		18.2	19.0	16		14.0	14.1	14.5	13.8	13.9	14.1	13.8	13.9
17		69.3	69.8	17		72.2	72.0	67.1	68.9	69.1	69.1	69.2	69.4
18		24.6	25.1	18		23.7	23.9	34.7	112.5	111.9	112.8	15.1	15.3
19		20.7	21.2	19		23.9	24.4	25.3	19.8	20.7	20.6	29.9	29.9
20		23.5	24.2	20		23.5	23.7	22.2	24.3	24.3	24.5	24.9	25.0
2-OR	1	174.8	129.1	3-OR	1	176.6	174.1	169.6	173.9	174.0	170.9	174.0	173.4
	2/6	34.2[a]	129.4		2	34.3	27.6	19.5	27.6	27.7	20.8	27.8	36.5
	3/5	19.2	128.1		3	19.3	8.9		8.7	8.8		8.8	17.4
	4	18.2[b]	132.6		4	18.5							13.8
	7		164.7	5-OAc	1	169.4	169.6	170.2	169.0	169.1	168.9	169.1	169.1
3-OR	1	172.8	172.9		2	22.4	21.2	20.6	20.6[c]	20.8[d]	20.7	21.1	21.2
	2	27.3	36.5	7 or 8-OR	1			130.3	170.2	170.3	130.6	170.2	170.2
	3	8.2	17.9		2/6			130.1	20.8[c]	20.8[d]	129.8	21.5	21.5
	4		13.8		3/5			128.5			128.3		
5-OAc	1	168.5	169.2		4			133.4			133.1		
	2	20.3	20.9		7			164.0			165.7		
7-OAc	1	169.5	170.4	10-OAc	1	168.3	167.7	169.2					
	2	20.3	20.9		2	22.9	22.9	21.9					
10-OAc	1	167.8	168.5	14-OR	1	170.1	129.4	129.9	129.5	170.2	129.6	129.4	129.5
	2	21.8	22.3		2/6	21.0	129.7	129.8	129.6	20.9[d]	129.5	129.9	130.1
14-OR	1	175.0	131.0		3/5		128.3	128.4	128.1		127.9	128.5	128.6
	2/6	33.0[a]	129.7		4		133.1	133.3	132.9		132.7	133.3	133.4
	3/5	19.2	128.2		7		165.8	166.9	165.3		165.6	165.4	165.5
	4	18.1[b]	132.7	15-OAc	1	170.4	170.5		167.7	168.4	167.8	168.7	168.8
	7		165.8		2	21.2	22.2		22.1	22.3	22.4	22.3	22.3
15-OAc	1	169.9	170.7										
	2	21.6	22.5										

[a−d] Assignments bearing the same superscript may be interchanged in each column. [e] Assignments of ^{13}C NMR data are based on DEPT, ^{1}H−^{1}H COSY, HMQC, and HMBC experiments.

cyclomyrsinol diterpenes.[7−9,13] Therefore, compound **5** was identified as 3β,5α,10β-tri-O-acetyl-8β,14β-di-O-benzoylcyclomyrsinol and was named euphorbiaproliferin E.

The ^{1}H and ^{13}C NMR spectra of compounds **6**−**8** were similar. In addition to the signals for the acyl groups in their ^{13}C NMR spectra, chemical shifts for the 20 skeletal carbons were similar to those of 3,5,7,14,15-penta-O-acyl-14-deoxomyrsinol,[13] which implied these compounds were different polyesters of the same parent alcohol. For compound **6**, three acetoxy, one propionyloxy,

and one benzoyloxy group were evident from its ^{13}C and ^{1}H NMR spectra. By using the same HMBC and NOESY experiments as for compounds **1**−**5**, the positions of the acyloxy groups and the configuration of **6** were determined, where H-3, H-4, H-14, and H$_2$-17 were α-oriented and H-5, H-12, and H$_3$-20 were β-oriented.[13] The only difference between **7** and **6** was that the C-14 benzoyloxy group in **6** was replaced by an acetoxy group in **7**. Compound **8** possessed an acetoxy moiety at C-3 instead of the propionyloxy moiety and a benzoyloxy moiety at C-7 instead

Table 2. ^{1}H NMR Data (δ_H) of Compounds 1−5 (CDCl$_3$, 400 MHz)^a

position		1	2	3	4	5
1α		2.98 d (17.3)	3.60 d (17.5)	2.74 dd (15.9, 10.6)	2.88 dd (16.0, 9.8)	2.58 dd (10.6, 4.7)
β		2.11 d (17.3)	2.51 d (17.5)	2.51 d (15.9, 9.3)	2.60 dd (16.0, 9.6)	1.58 dd (10.6, 5.5)
2				2.15 m	2.23 m	2.25 m
3		5.15 d (10.4)	5.51 d (3.9)	5.38 t (3.8)	5.43 t (3.8)	5.39 t (3.8)
4		3.46 dd (10.4, 3.4)	3.95 dd (11.1, 3.9)	3.00 dd (10.9, 3.8)	3.04 dd (10.8, 3.8)	2.79 dd (11.1, 3.8)
5		5.67 d (10.4)	6.02 d (11.1)	5.89 d (10.9)	5.94 d (10.8)	5.87 d (11.1)
7		4.60 d (6.2)	4.88 d (6.5)			
8		5.91 d (9.9, 6.2)	6.20 dd (9.9, 6.5)	6.19 d (10.3)	6.22 d (10.3)	5.58 d (5.4)
9		5.68 d (9.9, 5.4)	5.91 dd (9.9, 5.4)	6.54 dd (10.3, 6.2)	6.56 dd (10.3, 6.2)	2.83 m
11		2.95 m	3.17 m	3.04 m	2.71 m	2.62 m
12		2.84 s	3.22 s	3.52 s	3.67 s	5.10 d (12.1)
14		5.31 s	5.83 s	5.05 s	5.35 s	5.22 s
16		1.13 s	1.46 s	0.81 d (6.8)	0.80 d (6.8)	0.88 d (6.8)
17		3.80 d (8.5)	4.20 d (8.8)	4.25 d (9.1)	4.28 d (9.1)	4.28 d (9.5)
		3.22 d (8.5)	3.54 d (8.8)	3.84 d (9.1)	3.86 d (9.1)	3.72 d (9.5)
18		1.40 s	1.64 s	1.56 s	1.71 s	2.65 m
						2.55 m
19		1.29 s	1.56 s	1.43 s	1.60 s	1.67 s
20		0.89 s	1.16 s	1.20 s	1.23 s	1.33 s
2-OR	2/6	2.28 q (6.8)	7.64 d (7.7)			
	3/5	0.99 d (6.8)	6.97 t (7.7)			
	4	0.99 d (6.8)	7.35 t (7.7)			
3-OR	2	2.13 q (7.6)	2.36 m	2.55 q (6.9)	2.32 q (7.5)	1.19 s
	3	0.92 t (7.6)	1.70 m	1.17 d (6.9)	1.11 t (7.5)	
	4		0.99 t (7.4)	1.17 d (6.9)		
5-OAc	2	1.73 s	2.05 s	2.00 s	2.02 s	1.90 s
7 or 8-OR	2/6	1.71 s	2.00 s			8.43 d (7.2)
	3/5					7.56 t (7.2)
	4					7.65 t (7.2)
10-OAc	2	1.88 s	2.18 s	2.07 s	2.04 s	2.05 s
14-OR	2/6	2.38 q (6.7)	7.74 d (7.6)	2.03 s	7.87 d (7.3)	8.15 d (8.3)
	3/5	0.94 d (6.7)	7.35 t (7.6)		7.41 t (7.3)	7.50 t (8.3)
	4	0.94 d (6.7)	7.53 t (7.6)		7.55 t (7.3)	7.65 t (8.3)
15-OAc	2	1.82 s	2.11 s	1.99 s	1.96 s	

^{a} Assignments of ^{1}H NMR data are based on ^{1}H−^{1}H COSY, HMQC, and HMBC experiments.

of the acetoxy moiety in 6, respectively. Therefore, compounds 6−8 were elucidated as 3β-O-propinoyl-5α,7β,15β-tri-O-acetyl-14β-O-benzoyl-14-deoxomyrsinol (6), 3β-O-propionyl-5α,7β,14β,15β-tetra-O-acetyl-14-deoxomyrsinol (7), and 3β,5α,15β-tri-O-acetyl-7β,14β-di-O-benzoyl-14-deoxomyrsinol (8) and were named euphorbiaproliferins F−H, respectively.

Analyses of the ^{13}C and ^{1}H NMR data (Tables 1 and 3) of compounds 9 and 10 revealed that both compounds had a characteristic 6α,17-epoxy-14-deoxopremyrsinol skeleton related to that of the known aleppicatines and euphoppins.[15−17] Apart from the different acyloxy groups in these compounds, the difference between compounds 9 and 10 and the known aleppicatines is that the C-6 acetal moiety in aleppicatines is replaced by methyleneoxy moiety in compounds 9 and 10, as indicated in their DEPT spectra. After defining the skeleton of 6α,17-epoxy-14-deoxo-14-hydroxypremyrsinol for compounds 9 and 10, HMBC and NOESY experiments were performed to define the locations of the acyloxy groups and the configuration. For compound 9, three acetoxy groups at C-5, C-14, and C-15, one

propionyloxy group at C-3, and one benzoyloxy group at C-7 were determined, respectively, by interpretation of the HMBC and NOESY data. The only difference between 10 and 9 was that the C-3 propionyloxy group in 9 was replaced by a butyryloxy group in 10. NOESY correlations observed for H-3/H-4, H-5/H-12, H-7/H$_2$-17, H-9/H-11, H-12/H-14, H-12/H$_3$-20, and H-14/H$_3$-20 in compounds 9 and 10 revealed they had the same configuration and suggested that H-3 and H-7 were α-oriented and H-5 and H-14 were β-oriented.[15] Therefore, the structures of compounds 9 and 10 were elucidated as 3β-O-propinoyl-5α,7β,15β-tri-O-acetyl-14α-O-benzoyl-6α,17-epoxy-14-deoxopremyrsinol (9) and 3β-O-butyryl-5α,7β,15β-tri-O-acetyl-14α-O-benzoyl-6α,17-epoxy-14-deoxopremyrsinol (10) and were named euphorbiaproliferins I and J, respectively.

The nine known myrsinol diterpenes were identified by comparison of experimental and literature spectroscopic data as 14-deoxo-3-O-propionyl-5,15-di-O-acetyl-7-O-benzoylmyrsinol-14β-acetate (11),[13] euphorprolitherin C (12),[8] 14-deoxo-3-O-propionyl-5,15-di-O-acetyl-7-O-benzoylmyrsinol-14β-nicotinoate (13),[13] proliferin
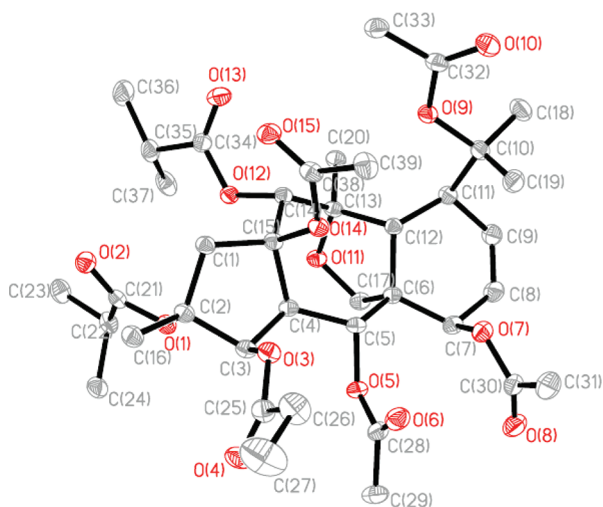
Figure 2. Thermal ellipsoid representation of **1**.

C (**14**),[9] proliferin A (**15**),[9] proliferin B (**16**),[9] euphorprolitherin B (**17**),[8] euphorbia substance SPr 5 (**18**),[4] and premyrsinol-3-propanoate-5-benzoate-7,13,17-triacetate (**19**).[17]

In order to characterize the constituents responsible for the neuroprotective effects of the roots of *E. prolifera*, compounds **1**−**19** were assessed for their neuroprotective activities against MPP$^+$-induced neuronal cell death in SH-SY5Y cells using an established MTT assay with a slight modification.[10,11,18,19] Guanosine was used as a positive control.[20] All the compounds exhibited neuroprotective effects (Table 4). At a concentration of 30 μM, the neuroprotective activities of compounds **2**, **8**−**10**, and **12**−**16** were significantly more effective than that of the positive control. The active compounds (3−30 μM) neither affected the cell viability nor showed any cytotoxcity (MTT assay, data not shown).

Naturally occurring myrsinol diterpenes derived from lathyranes via premyrsinanes are rare, and studies of their biological activities are limited.[2] We successfully isolated 19 myrsinol-type diterpenes including 10 new ones from the roots of *E. prolifera*, and the neuroprotective effects of these myrsinol diterpenes were reported for the first time.

■ EXPERIMENTAL SECTION

General Experimental Procedures. The optical rotations were measured in CH_2Cl_2 using an Autopol IV automatic polarimeter (Autopol Industries Co. Ltd., India). The IR spectra were recorded on a Bio-Rad FTS 6000 Fourier transform infrared (FTIR) spectrometer with KBr disks (DeFelsko Co. Ltd., America). The ESIMS spectra were obtained on an LCQ-Advantage mass spectrometer (Finnigan Co. Ltd., America). HR-ESIMS spectra were recorded by an IonSpec 7.0 T FTICR MS (IonSpec Co. Ltd., America). 1D and 2D NMR data were recorded on a Bruker AV 400 instrument (400 MHz for ^{1}H and 100 MHz for ^{13}C) with TMS as an internal standard. HPLC separations were performed on a CXTH system, equipped with a UV3000 detector at 210 nm (Beijing Chuangxintongheng Instruments Co. Ltd., China) and a YMC-pack ODS-AM (250 × 20 mm) column (YMC Co. Ltd., Japan). X-ray crystallographic analysis was carried out on a Rigaku Saturn 944 CCD diffractometer equipped with a multilayer monochromator and Cu Kα radiation (λ = 1.54187 Å) (Rigaku Co. Ltd., Japan). The structure was solved by direct methods (SHELXL-97), expanded using Fourier techniques, and refined with full-matrix least-squares on F^2

(SHELXL-97). Silica gel was used for column chromatography (200−300 mesh, Qingdao Marine Chemical Group Co. Ltd., China). Chemical reagents for isolation were of analytical grade and purchased from Tianjin Yuanli Co. Ltd., China. Biological reagents were from Sigma Company. Human dopaminergic SH-SY5Y cells were obtained from the American Type Culture Collection.

Plant Material. The roots of *E. prolifera* were collected from Kunming, Yunnan Province, China, in July 2010. The botanical identification was made by one of the authors (Y.G.), and a voucher specimen (No. 20100705) was deposited at the laboratory of the Research Department of Natural Medicine, College of Pharmacy, Nankai University, China.

Extraction and Isolation. The air-dried roots of *E. prolifera* (3.8 kg) were powdered and extracted with MeOH (3 × 20 L) under reflux. The solvent was evaporated to obtain a crude extract (900 g). The extract was suspended in H_2O (0.9 L) and partitioned with EtOAc (3 × 0.9 L). The EtOAc-soluble part (150.0 g) was subjected to a silica gel column chromatography, using a gradient of acetone in petroleum ether (1−40%), to give eight fractions (F_1−F_8) based on TLC analyses. Fraction F_4 was separated by MPLC over ODS eluting with a step gradient from 60% to 90% MeOH in H_2O to give three subfractions (F_{4-1}−F_{4-3}). Fraction F_{4-2} was purified by preparative HPLC (YMC-pack ODS-AM, 20 × 250 mm, 79% MeOH in H_2O) to afford compound **1** (t_R = 31 min, 21.9 mg). Further purification of F_{4-3} with the same HPLC system using 83% MeOH in H_2O resulted in the isolation of compound **7** (t_R = 36 min, 13.6 mg). Fraction F_5, using the same procedures for F_4, provided three subfractions, F_{5-1}−F_{5-3}. Compounds **2** (t_R = 34 min, 11.9 mg) and **16** (t_R = 29 min, 13.2 mg) were isolated from F_{5-3} (81% MeOH in H_2O), and compounds **3** (t_R = 25 min, 14.7 mg), **14** (t_R = 37 min, 12.5 mg), **15** (t_R = 42 min, 16.8 mg), **18** (t_R = 28 min, 19.2 mg), and **19** (t_R = 30 min, 13.4 mg) were obtained from F_{5-2} (77% MeOH in H_2O). Compounds **4** (t_R = 27 min, 24.4 mg), **5** (t_R = 49 min, 16.1 mg), **13** (t_R = 56 min, 15.3 mg), and **17** (t_R = 33 min, 19.9 mg) were isolated from F_{6-2} (71% MeOH in H_2O), which was obtained from F_6 by the MPLC. Using the same protocols for the above fractions and subfractions, F_3 yielded three subfractions, F_{3-1}−F_{3-3}. Compounds **6** (t_R = 36 min, 8.9 mg), **9** (t_R = 31 min, 12.7 mg), **10** (t_R = 40 min, 9.9 mg), and **11** (t_R = 25 min,13.4 mg) were isolated from F_{3-2} (84% MeOH in H_2O), and compounds **8** (t_R = 28 min, 12.7 mg) and **12** (t_R = 38 min, 13.4 mg) were obtained from F_{3-3} (86% MeOH in H_2O).

Euphorbiaproliferin A (1): white flakes (MeOH); mp 144−146 °C; $[\alpha]_D^{25}$ −47.0 (*c* 1.0, CH_2Cl_2); IR (KBr) ν_{max} 2983, 2942, 1747, 1468, 1371, 1244, and 1019 cm^{-1}; ^{1}H NMR (400 MHz, CDCl$_3$) and ^{13}C NMR (100 MHz, CDCl$_3$) data, see Tables 1 and 2; ESIMS *m/z* 787 [M + Na]$^+$; HR-ESIMS *m/z* 787.3510 [M + Na]$^+$ (calcd for $C_{39}H_{56}O_{15}$Na, 787.3517).

X-ray Crystal Data of 1 (ref 21)**:** $C_{39}H_{56}O_{15}$, M_r = 764.84, orthorhombic, space group P2(1)2(1)2(1), *a* = 10.2388(5) Å, *b* = 12.9739(8) Å, *c* = 30.5211(16) Å, *V* = 4054.3(4) Å^3, *Z* = 4, D_{calc} =1.253 g/cm^3, crystal dimensions 0.16 × 0.14 × 0.10 mm were used for measurements. The total number of reflections measured was 34 143, of which 7899 were unique and 7519 were observed, $I > 2\sigma(I)$. Final indices: R_1 = 0.0503, wR_2 = 0.1106 for observed reflections, and R_1 = 0.0520, wR_2 = 0.1132 for all reflections.

Euphorbiaproliferin B (2): white powder; $[\alpha]_D^{25}$ −41.3 (*c* 0.3, CH_2Cl_2); IR (KBr) ν_{max} 2959, 2927, 1737, 1452, 1372, 1240, and 1069 cm^{-1}; ^{1}H NMR (400 MHz, CDCl$_3$) and ^{13}C NMR (100 MHz, CDCl$_3$) data, see Tables 1 and 2; ESIMS *m/z* 869 [M + Na]$^+$; HR-ESIMS *m/z* 869.3355 [M + Na]$^+$ (calcd for $C_{46}H_{54}O_{15}$Na, 869.3360).

Euphorbiaproliferin C (3): colorless flakes (MeOH); mp 132−134 °C; $[\alpha]_D^{25}$ −6.8 (*c* 0.3, CH_2Cl_2); IR (KBr) ν_{max} 2960, 2927, 1737, 1457, 1370, 1227, and 1023 cm^{-1};^{1}H NMR (400 MHz, CDCl$_3$) and ^{13}C NMR (100 MHz, CDCl$_3$) data, see Tables 1 and 2; ESIMS *m/z* 643 [M + Na]$^+$; HR-ESIMS *m/z* 643.2724 [M + Na]$^+$ (calcd for $C_{32}H_{44}O_{12}$Na, 643.2730).

Table 3. ^{1}H NMR Data (δ_H) of Compounds **6−10** (CDCl$_3$, 400 MHz)^a

position		6	7	8	9	10
1α		2.78 dd (15.8, 11.1)	2.76 dd (15.8, 11.1)	2.89 dd (16.0, 10.6)	2.44 dd (14.9, 7.7)	2.48 dd (14.8, 7.7)
β		2.65 dd (15.8, 9.0)	2.51 dd (15.8, 9.0)	2.72 dd (16.0, 9.2)	1.74 dd (14.9, 6.9)	1.77 dd (14.8, 6.2)
2		2.14 m	2.12 m	2.22 m	1.93 m	1.97 m
3		5.27 t (3.5)	5.27 t (3.5)	5.28 t (3.4)	5.26 t (3.2)	5.29 t (3.4)
4		3.15 dd (11.1, 3.5)	3.08 dd (11.0, 3.5)	3.22 dd (11.0, 3.4)	3.46 dd (11.1, 3.2)	3.49 dd (11.0, 3.4)
5		5.92 d (11.1)	5.86 d (11.0)	6.15 d (11.0)	5.78 d (11.1)	5.81 d (11.0)
7		4.81 d (6.4)	4.79 d (6.4)	5.11 d (6.4)	4.65 t (4.4)	4.68 t (4.7)
8		6.06 dd (9.7, 6.4)	6.12 dd (9.4, 6.4)	6.30 dd (9.7, 6.4)	1.70 m	1.72 m
					1.75 m	1.76 m
9		5.76 dd (9.7, 4.8)	5.83 dd (9.4, 4.7)	5.88 dd (9.7, 5.4)	0.79 m	0.83 m
11		3.24 m	3.11 m	3.31 m	0.86 m	0.89 m
12		3.25 s	3.13 s	3.49 d (3.2)	2.72 d (2.9)	2.74 d (2.9)
14		5.18 s	4.97 s	5.31 s	5.87 s	5.90 s
16		0.78 d (6.7)	0.82 d (6.7)	0.85 d (6.7)	0.72 d (6.6)	0.75 d (6.6)
17		4.01 d (8.8)	3.98 d (8.8)	4.16 d (8.7)	4.14 d (8.8)	4.17 d (8.7)
		3.48 d (8.8)	3.50 d (8.8)	3.64 d (8.7)	3.65 d (8.8)	3.67 d (8.7)
18		4.91 s	4.83 s	4.88 s	1.04 s	1.07 s
		4.81 s	4.74 s	4.81 s		
19		1.85 s	1.82 s	1.92 s	1.13 s	1.16 s
20		1.25 s	1.24 s	1.35 s	1.30 s	1.33 s
3-OR	2	2.27 q (7.5)	2.31 q (7.6)	1.86 s	2.33 q (7.6)	2.32 m
	3	1.07 t (7.5)	1.12 t (7.6)		1.13 t (7.6)	1.67 m
	4					0.98 t (7.4)
5-OAc	2	1.94 s	1.96 s	1.99 s	1.96 s	1.99 s
7-OR	2/6	1.95 s	1.95 s	8.06 d (7.8)	1.98 s	2.01 s
	3/5			7.46 t (7.2)		
	4			7.60 t (7.2)		
14-OR	2/6	7.90 d (8.4)	2.02 s	7.99 d (7.8)	8.12 d (7.2)	8.15 d (7.3)
	3/5	7.38 t (8.4)		7.41 t (7.8)	7.46 t (7.2)	7.49 t (7.3)
	4	7.51 t (8.4)		7.55 t (7.8)	7.58 t (7.2)	7.61 t (7.3)
15-OAc	2	2.03 s	2.06 s	2.22 s	2.12 s	2.15 s

^{a} Assignments of ^{1}H NMR data are based on ^{1}H−^{1}H COSY, HMQC, and HMBC experiments.

Euphorbiaproliferin D (4): white powder; $[\alpha]_D^{25}$ −6.5 (c 0.4, CH$_2$Cl$_2$); IR (KBr) ν_{max} 2954, 2926, 1737, 1461, 1371, 1236, and 1024 cm^{-1}; H NMR (400 MHz, CDCl$_3$) and ^{13}C NMR (100 MHz, CDCl$_3$) data, see Tables 1 and 2; ESIMS m/z 691 [M + Na]$^+$; HR-ESIMS m/z 691.2728 [M + Na]$^+$ (calcd for C$_{36}$H$_{44}$O$_{12}$Na, 691.2730).

Euphorbiaproliferin E (5): white powder; $[\alpha]_D^{25}$ +21.2 (c 0.3, CH$_2$Cl$_2$); IR (KBr) ν_{max} 2956, 2927, 1738, 1453, 1374, 1246, and 1072 cm^{-1}; ^{1}H NMR (400 MHz, CDCl$_3$) and ^{13}C NMR (100 MHz, CDCl$_3$) data, see Tables 1 and 2; ESIMS m/z 755 [M + Na]$^+$; HR-ESIMS m/z 755.2677 [M + Na]$^+$ (calcd for C$_{40}$H$_{44}$O$_{13}$Na, 755.2680).

Euphorbiaproliferin F (6): colorless flakes (MeOH); mp 165−167 °C; $[\alpha]_D^{25}$ −49.2 (c 0.2, CH$_2$Cl$_2$); IR (KBr) ν_{max} 2964, 2935, 1740, 1452, 1369, 1243, and 1069 cm^{-1}; ^{1}H NMR (400 MHz, CDCl$_3$) and ^{13}C NMR (100 MHz, CDCl$_3$) data, see Tables 1 and 3; ESIMS m/z 675 [M + Na]$^+$; HR-ESIMS m/z 675.2771 [M + Na]$^+$ (calcd for C$_{36}$H$_{44}$O$_{11}$Na, 675.2781).

Euphorbiaproliferin G (7): colorless flakes (MeOH); mp 72−74 °C; $[\alpha]_D^{25}$ −19.8 (c 0.4, CH$_2$Cl$_2$); IR (KBr) ν_{max} 2959, 2927, 1739, 1461, 1370, 1234, and 1040 cm^{-1}; ^{1}H NMR (400 MHz, CDCl$_3$) and ^{13}C NMR (100 MHz, CDCl$_3$) data, see Tables 1 and 3; ESIMS m/z 613 [M + Na]$^+$; HR-ESIMS m/z 613.2610 [M + Na]$^+$ (calcd for C$_{32}$H$_{41}$O$_{11}$Na, 613.2625).

Euphorbiaproliferin H (8): white powder; $[\alpha]_D^{25}$ −19.0 (c 0.4, CH$_2$Cl$_2$); IR (KBr) ν_{max} 2959, 2929, 1741, 1452, 1371, 1266, and 1026 cm^{-1}; ^{1}H NMR (400 MHz, CDCl$_3$) and ^{13}C NMR (100 MHz, CDCl$_3$) data, see Tables 1 and 3; ESIMS m/z 723 [M + Na]$^+$; HR-ESIMS m/z 723.2776 [M + Na]$^+$ (calcd for C$_{40}$H$_{44}$O$_{11}$Na, 723.2781).

Euphorbiaproliferin I (9): colorless needles (MeOH); mp 102−104 °C; $[\alpha]_D^{25}$ −29.7 (c 0.4, CH$_2$Cl$_2$); IR (KBr) ν_{max} 2955, 2927, 1742, 1369, 1241, and 1025 cm^{-1}; ^{1}H NMR (400 MHz, CDCl$_3$) and ^{13}C NMR (100 MHz, CDCl$_3$) data, see Tables 1 and 3; ESIMS m/z 677 [M + Na]$^+$; HR-ESIMS m/z 677.2937 [M + Na]$^+$ (calcd for C$_{36}$H$_{46}$O$_{11}$Na, 677.2938).

Euphorbiaproliferin J (10): white powder; $[\alpha]_D^{25}$ −40.0 (c 0.4, CH$_2$Cl$_2$); IR (KBr) ν_{max} 2958, 2928, 1742, 1452, 1369, 1240, and 1024 cm^{-1}; ^{1}H NMR (400 MHz, CDCl$_3$) and ^{13}C NMR (100 MHz, CDCl$_3$) data, see Tables 1 and 3; ESIMS m/z 691 [M + Na]$^+$; HR-ESIMS m/z 691.3087 [M + Na]$^+$ (calcd for C$_{37}$H$_{48}$O$_{11}$Na, 691.3094).

Bioassay Procedure. Human dopaminergic neuroblastoma SH-SY5Y cells were cultured at 37 °C in DMEM supplemented with 10% (v/v) inactivated fetal bovine serum and 100 U/mL penicillin/streptomycin under a water-saturated atmosphere of 95% air and 5% CO$_2$. The cells were disassociated by incubation with 1 mM ethylene glycol-bis(2-aminoethyl ether)-N,N,N',N'-tetraacetic acid in phosphate-buffered saline for 15 min and seeded in 96-well culture plates (1 × 10^4 cells/well). Cells

Table 4. Neuroprotective Effects of Compounds 1−19 against MPP⁺-Induced SH-SY5Y Cell Death

compd	cell viability (%)[a]		
	30 μM	10 μM	3 μM
1	92.7 ± 4.5^b	83.5 ± 5.0^b	75.1 ± 5.7^b
2	109.3 ± 3.6^b	93.2 ± 3.9^b	74.8 ± 5.2^b
3	81.3 ± 4.4^b	75.4 ± 3.8^b	68.3 ± 5.9^b
4	88.6 ± 4.2^b	71.3 ± 2.5^b	68.7 ± 4.6^b
5	82.1 ± 3.4^b	76.0 ± 4.0^b	67.1 ± 3.2^b
6	93.5 ± 6.2^b	87.1 ± 4.4^b	74.5 ± 5.5^b
7	88.7 ± 2.9^b	78.3 ± 4.3^b	65.6 ± 5.4
8	99.4 ± 3.7^b	89.8 ± 4.6^b	83.6 ± 4.7^b
9	101.3 ± 7.0^b	88.9 ± 4.5^b	84.8 ± 4.0^b
10	104.2 ± 5.7^b	89.2 ± 3.3^b	79.8 ± 5.2^b
11	94.2 ± 3.7^b	86.5 ± 4.7^b	78.1 ± 3.8^b
12	102.6 ± 5.4^b	91.5 ± 4.4^b	75.5 ± 3.2^b
13	96.5 ± 2.7^b	87.4 ± 4.8^b	77.7 ± 3.6^b
14	95.1 ± 5.5^b	84.5 ± 2.9^b	76.7 ± 4.2^b
15	97.8 ± 5.1^b	85.2 ± 6.4^b	74.4 ± 3.4^b
16	112.3 ± 3.6^b	95.4 ± 4.8^b	82.1 ± 3.0^b
17	92.2 ± 5.4^b	81.7 ± 5.1^b	70.3 ± 4.2^b
18	84.1 ± 4.6^b	77.3 ± 6.0^b	69.6 ± 3.4^b
19	78.5 ± 5.7^b	70.3 ± 3.7^b	61.5 ± 4.4
guanosine[c]	87.3 ± 4.2^b	77.3 ± 3.9^b	68.1 ± 3.3^b

[a] Neuroprotective effects of test compounds against MPP⁺-induced SH-SY5Y cell death. Cell viability: 100 (vehicle control); 59.4 ± 3.9 (treated only with MPP⁺, $p < 0.01$, compared with the vehicle control). Data are presented as mean $\pm$ SEM from triplicate samples. [b] $p < 0.05$, compared with the group treated only with MPP⁺. [c] Guanosine was used as a positive control.

were incubated at 37 °C under a 5% CO_2 humidified air incubator for 24 h. Cells were pretreated for 2 h with various concentrations (3, 10, 30 μM) of compounds before incubation in medium containing 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPP⁺). MTT dissolved in phosphate-buffered saline was added at the end of incubation to a final concentration of 0.5 mg/mL. After incubation for 4 h at 37 °C and 5% CO_2, the supernatants were removed and the formed formazan crystals in the viable cells were measured at 490 nm using a microplate reader (BioTek Instruments, Inc., USA). Experiments were carried out in triplicate. Statistical analysis was performed by one-way analysis of variance (ANOVA) followed by *post hoc* multiple comparisons using the Student−Newman−Keuls method. The data are expressed as mean $\pm$ SEM of three assays.

■ ASSOCIATED CONTENT

ⓈSupporting Information. 1D, 2D NMR and MS spectra of compounds **1**−**10** and the cif file of the X-ray data. This material is available free of charge via the Internet at http://pubs.acs.org.

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■ ACKNOWLEDGMENT

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(21) Crystallographic data for compound **1** have been deposited in the Cambridge Crystallographic Data Centre (CCDC 842555). Copies of the data can be obtained, free of charge, on application to the director, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: +44-(0)1223-336033 or e-mail: deposit@ccdc.cam.ac.uk).