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Lathyrane, Premyrsinane, and Related Diterpenes from *Euphorbia boetica*: Effect on in Vitro Neural Progenitor Cell Proliferation

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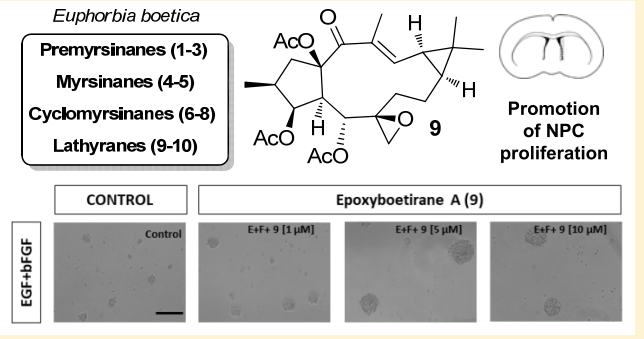
Supporting Information

ABSTRACT: Lathyrane-type diterpenes previously have been proven to promote proliferation of neural precursor cells (NPCs) by targeting and activating one or more protein kinase C (PKC) isozymes. Aiming to find new drug candidates with a lathyrane skeleton to modulate adult neurogenesis through PKC activation, a phytochemical study of a methanol extract of the aerial parts of *Euphorbia boetica* was carried out. Seven new diterpenes, representing the premyrsinane (**1**−**3**), myrsinane (**4**, **5**), and cyclomyrsinane types (**6**, **7**), along with three known diterpenes, belonging to the cyclomyrsinane (**8**) and lathyrane types (**9**, **10**), were isolated. The chemical structures and relative configurations of the new compounds were determined by extensive NMR spectroscopic studies and comparison with known compounds. The absolute configurations for compounds **2**, **3**, **6**, and **7** were proposed, based on a comparison of the experimental ECD spectra of compounds **2** and **7** with those of known related compounds. The activity of lathyrane compounds **9** and **10** as promoters of NPC proliferation was evaluated using a neurosphere assay. Both compounds increased the size of neurospheres in a dose-dependent manner when proliferation was stimulated by the epidermal growth factor and the basic fibroblast growth factor.



Plants of the genus *Euphorbia* (Euphorbiaceae) are well known for the extraordinary chemical diversity of their diterpenoids and the wide variety of pharmacological properties they possess.[1−3] In particular, diterpenes with tigliane, ingenane, and lathyrane skeletons, frequently found in plants of this genus, are known protein kinase C (PKC) activators. They bind to their regulatory domain, mimicking the effect of their physiological activator diacylglycerol.[4] PKC modulation has been linked to a number of relevant physiological events of interest.

Highly lipophilic phorbol esters (tigliane-type diterpenes), despite their ability to activate PKC, are deemed unsuitable for therapeutic applications as a consequence of their strong tumor-promoting activities.[5] In contrast, 12-deoxyphorbols, like prostratin or 12-deoxyphorbol-13-phenylacetate, which are less lipophilic, activate PKC without being tumor promoters.[6,7] Prostratin and related tigliane esters are potentially useful in the treatment of HIV-1, in combination with antiretroviral therapies,[8,9] since they reactivate replication of latent viral reservoirs in CD4$^+$ T-cells,[10] by activating NF-κB through a PKC-dependent pathway.[11,12] Some lathyrane-type diterpenes also mimic prostratin in their ability to reactivate HIV-1 latency[13] through a PKC-dependent pathway.[14] On the other hand, it has been proven that 12-deoxyphorbols, like prostratin or 12-deoxyphorbol-13-phenylacetate, and lathyranes, such as 3,12-di-*O*-acetyl-8-*O*-tigloylingol, promote proliferation of neural precursor cells (NPCs) by targeting and activating one or more PKC isozymes.[15,16] Given the limited ability of the brain to generate neurons in damaged brain areas, compounds promoting NPC proliferation may be suitable for the development of therapies aimed to replace dead neurons in patients with disorders that occur with neuronal death.

Previous studies have shown that lathyrane-type diterpenes with very similar structures differ significantly in their ability to activate PKCs and promote NPC proliferation.[16] Therefore, it

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is important to shed some light on the structural features that contribute to the activity of this type of compound, as well as to their mechanism of action in the interaction with the molecular target.

In the framework of ongoing phytochemical research on *Euphorbia* species, aimed at isolating new bioactive compounds with biological properties related to their ability to modulate PKC isozymes, attention was focused on *Euphorbia boetica* Boiss., an herb endemic to Europe, which is distributed widely in the southern part of the Iberian Peninsula. It is known to produce a large amount of latex, which causes irritation of the human skin. In previous studies, Ferreira et al. described the isolation from this plant of the triterpenes boeticol and euphol,[17] some steroids,[18] a premyrsinane-type diterpene,[18] a lathyrol derivative named euphoboetirane A,[19] and 6,17-epoxylathyrane-type diterpenes, known as epoxyboetiranes A, B, K, L, and M.[20]

In order to find new potential PKC activators with a lathyrane skeleton and study their ability to promote NPC proliferation, the chemical constituents of *E. boetica* were re-examined. This paper reports on the isolation and structural elucidation of seven new diterpenes from this plant, belonging to the premyrsinane (**1**−**3**), myrsinane (**4**, **5**), and cyclomyrsinane types (**6**, **7**), together with three known diterpenes, belonging to the cyclomyrsinane (**8**) and lathyrane types (**9**, **10**). Their chemical structures were established on the basis of the interpretation of their spectroscopic data, including HRESIMS and 1D and 2D NMR. In addition, the biological activity of lathyranes **9** and **10** as facilitators of NPC proliferation is described.

RESULTS AND DISCUSSION

E. boetica was re-collected at "El Pinar del Hierro" in Chiclana de la Frontera, Cádiz (Spain). The aerial parts of the fresh plant were used to obtain a methanol extract, which was partitioned with hexane and dichloromethane, as indicated in the Experimental Section. Both fractions were fractionated by silica gel column chromatography and further purified by HPLC, to afford seven new (**1**−**7**) and three known (**8**−**10**) diterpenoids. The known compounds were identified by comparison of their spectroscopic data with those reported in the literature as a pentacyclic diterpenoid ester of cyclomyrsinane type, isolated from *E. teheranica* (**8**),[21] epoxyboetirane A (**9**) isolated previously from *E. boetica*,[20] and 3,5,15-tri-*O*-acetyllathyrol (**10**), also known as euphoboetirane A, and isolated from *E. boetica*,[19] *E. lathyris*,[22] and *E. insularis*.[23]

Compounds **1**−**3** showed spectroscopic data structurally closely related to diterpenes with a premyrsinane skeleton. Premyrsinanes are a group of tetracyclic or alternatively pentacyclic diterpenes as a consequence of the formation of a tetrahydrofuran ring by intramolecular cyclization involving carbons C-12, C-6, C-13, and C-17.[1]

Compound **1** was isolated as an amorphous solid with a molecular formula of $C_{26}H_{36}O_8$, as deduced from the HRESIMS analysis (*m*/*z* 499.2322 [M + Na]$^+$, calcd for $C_{26}H_{36}O_8Na$, 499.2308). Its spectroscopic data (Table 1) were in accord with a pentacyclic structure similar to that proposed for karajinone A (**11**), previously isolated from *E. decipiens*.[24] The main difference between the ^{1}H NMR spectrum of **1** and that of the previously described premyrsinane **11** was the presence of two signals at δ_H 0.96 and 1.54 ppm in **1** (Table 1), which correlated to a methylene group at δ_C 30.1 ppm in the gHSQC experiment. This methylene group was assigned to C-7 because of its correlation with the proton signal H-17b at δ_H 3.56 ppm in the gHMBC spectrum. These data, together with the absence of aromatic signals, suggested that the benzoyl substituent at C-7 in karajinone A (**11**) was replaced by a hydrogen atom in **1**. In addition, the ^{1}H NMR spectrum of **1** showed the presence of an extra methyl group at δ_H 1.06 ppm (s). This singlet was connected to a primary carbon at δ_C 28.0 ppm (C-18) and to four other carbons at δ_C 25.6 (C-9), 18.4 (C-10), 19.7 (C-11), and 15.9 ppm (C-19) in the gHMBC experiment. These correlations showed that the additional methyl group is attached to C-10 and replaced the acetoxymethyl group of karajinone A (**11**).

The relative configuration of **1** was elucidated on the basis of those described for previously reported natural premyrsinane diterpenes[24−26] and correlations observed in the NOESY

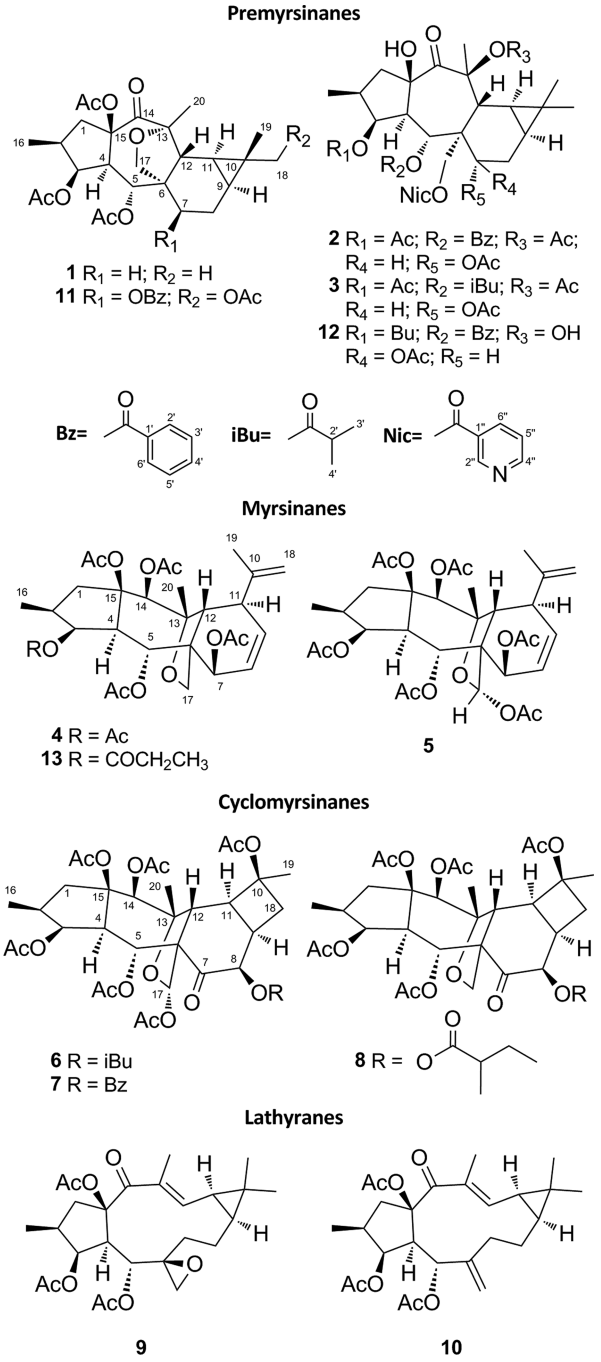

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Table 1. NMR Spectroscopic Data of Premyrsinanes 1−3 in $CDCl_3$ (J in Hz)

	1		2		3	
position	δ_H (500 MHz)	δ_C (125 MHz), type	δ_H (500 MHz)	δ_C (125 MHz), type	δ_H (400 MHz)	δ_C (100 MHz), type
1α	3.47, dd (15.0, 10.0)	42.1, CH_2	3.18, dd (8.0, 13.5)	42.8, CH_2	3.17, dd (8.0, 13.6)	42.8, CH_2
1β	1.39, dd (15.0, 10.0)		1.67, t (13.5)		1.63, dd (13.6)	
2α	2.11, m	36.2, CH	1.90, m	37.2, CH	1.84, m	37.4, CH
3α	5.35, t (4.0)	78.1, CH	5.36, t (3.8)	78.4, CH	5.21, t (3.4)	78.5, CH
4α	2.42 dd (11.0, 4.0)	52.3, CH	2.43, dd (11.5, 3.8)	50.3, CH	2.38, dd (11.4, 3.4)	50.4, CH
5β	5.44, d (11.0)	70.4, CH	6.46, d (11.5)	69.9, CH	6.25, d (11.4)	68.9, CH
6		52.9, C		48.1, C		47.6, C
7a	1.54, m	30.1, CH_2	β: 4.97, d (6.0)	70.3, CH	β: 4.68, d (6.8)	70.6, CH
7b	0.96, m					
8α	1.75, m	17.6, CH_2	2.19, m	22.3, CH_2	2.25, m	22.3, CH_2
8β	0.80−0.87, m		1.93, m		1.90, m	
9α	0.80−0.87, m	25.6, CH	0.79−0.76, m	18.9, CH	0.80−0.75, m	18.9, CH
10		18.4, C		18.4, C		18.3, C
11α	0.63, t (7.5)	19.7, CH	0.79−0.76, m	23.8, CH	0.80−0.75, m	23.8, CH
12β	2.25, d (7.5)	39.3, CH	3.58, d (6.7)	35.2, CH	3.46, d (6.3)	34.9, CH
13		88.2, C		85.6, C		85.7, C
14		201.9, C		204.1, C		204.2, C
15		90.0, C		84.2, C		84.0, C
16β	0.90, d (7.0)	14.3, CH_3	0.87, d (6.5)	13.8, CH_3	0.89, d (6.8)	14.1, CH_3
17a	4.06, d (9.3)	74.7, CH_2	5.04, d (11.5)	63.6, CH_2	4.88, d (12.0)	64.3, CH_2
17b	3.56, d (9.3)		4.55, d (11.5)		4.51, d (12.0)	
18α	1.06, s	28.0, CH_3	1.06, s	29.4, CH_3	1.06, s	29.5, CH_3
19β	0.99, s	15.9, CH_3	0.97, s	14.9, CH_3	0.95, s	14.8, CH_3
20β	1.51, s	20.0, CH_3	1.77, s	24.9, CH_3	1.73, s	24.5, CH_3
OCO-3		171.0,[b] C		170.3,[e] C		170.6,[i] C
OCOMe-3	2.07, s[a]	21.0,[c] CH_3	1.92, s[d]	21.3,[f] CH_3	2.11, s[g]	21.3,[j] CH_3
OCO-5		170.5, C		165.2, C		175.0, C
OCOMe-5	2.02, s	21.1, CH_3				
OCOBz-5						
1′				129.3, C		
2′, 6′			7.70, d (7.9)	129.3, CH		
3′, 5′			7.01, m	128.0, CH		
4′			7.15, t (7.9)	132.8, CH		
OCOiBu-5						
2′					2.15, m	33.9, CH
3′					0.92, d (6.8)[h]	18.5,[k] CH_3
4′					0.88, d (6.8)[h]	18.4,[k] CH_3
OCO-7				170.1, C		169.9, C
OCOMe-7			2.17, s	21.3,[f] CH_3	2.12, s	21.2, CH_3
OCO-13				170.7,[e] C		170.9,[i] C
OCOMe-13			2.12, s[d]	20.9,[f] CH_3	2.01, s[g]	21.3,[i] CH_3
OCO-15		170.0,[b] C				
OCOMe-15	2.09, s[a]	21.6,[c] CH_3				
OH-15			4.46, s		4.47, s	
OCONic-17				164.8, C		164.8, C
1″				125.1, C		125.1, C
2″			8.83, d (2.0)	150.6, CH	9.17, d (1.6)	150.5, CH
4″			8.53, dd (5.0, 2.0)	153.1, CH	8.82, dd (5.0, 1.6)	153.9, CH
5″			6.99, m	123.0, CH	7.43, dd (8.0, 5.0)	123.7, CH
6″			7.60, dt (8.0, 2.0)	136.0, CH	8.19, dt (8.0, 1.6)	136.7, CH

[a−j]Interchangeable signals.

experiment. The premyirsinane skeleton is based on a *trans*-fused 5/7/6-tricyclic ring system and one *cis*-fused three-membered ring. In this, the 13,17-epoxy ring, H-4, H-9, and H-11 are α-oriented, and H_3-16 and H-12 are β-oriented. NOESY correlations observed for H-1α/H-2α, H-2α/H-3α, H-3α/H-4α, H-9α/H-11α, and H-11α/H_3-18α indicated that these protons are directed inward in the premyrsinane skeleton, whereas the correlations of H-1β/H_3-16β, H-5β/H-12β, and H-12β/H_3-19β were used to place these protons on the opposite face of the skeleton (Figure 1). NOESY correlations of H_3-19β with H-12β and of H_3-18α with H-11α, where both methyl groups are substituted on the same carbon (C-10), are consistent with the proposed relative configuration for the *gem*-dimethylcyclopropane moiety (Figure 1). These correlations

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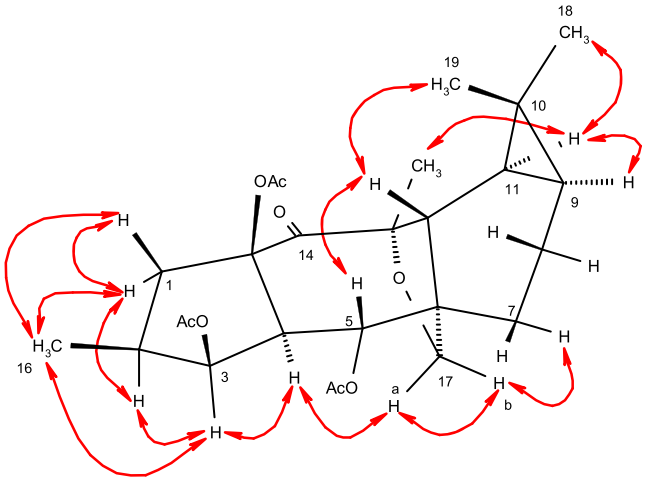

Figure 1. Selected NOESY correlations exhibited by **1**.

allowed **1** to be assigned as (2*S**,3*S**,4*R**,5*R**,6*R**,9*S**,11*S**,12*R**,13*R**,15*R**)-3,5,15-triacetoxy-13,17-epoxypremyrsinol.

Compounds **2** and **3** were isolated as amorphous solids with respective molecular formulas of $C_{39}H_{45}NO_{12}$, according to the ion peak at *m*/*z* 742.2855 $[M + Na]^+$ (calcd for $C_{39}H_{45}NO_{12}Na$, 742.2839), and $C_{36}H_{47}NO_{12}$, according to the ion peak at *m*/*z* 708.3009 $[M + Na]^+$ (calcd for $C_{36}H_{47}NO_{12}Na$, 708.2996). After a perusal of the ^1H and ^{13}C NMR data, part of the unsaturations deduced from their respective elemental formulas could be assigned to up to five carbonyl groups for both compounds, with two aromatic rings for compound **2** and one for compound **3**. A comparison of their spectroscopic data (Table 1) with those of diterpenes from other *Euphorbia* spp. showed that they were very similar to those described for prolifepene B (**12**), previously isolated from *E. prolifera*,[25] suggesting the presence of a common diterpene skeleton, while differences in the substituents and relative configurations were observed.

As mentioned above, analysis of ^{13}C NMR spectra for compounds **2** and **3** revealed the presence of five signals corresponding to the resonances of ester carbonyl carbons. Based on their gHSQC and gHMBC spectra, they corresponded, in both compounds, to the presence of three acetate esters and a nicotinate ester substituted at C-3, C-7, C-13, and C-17, respectively. The long-range couplings of the protons H_2-17 (δ_H 5.04 ppm in **2** and 4.51 and 4.88 ppm in **3**) with the carbonyl carbons O<u>C</u>O-17 (164.8 ppm in **2** and **3**) in the gHMBC spectra demonstrated that a nicotinoyloxy group is located at C-17 in each compound. Furthermore, the presence of a free hydroxy group at C-15 was confirmed by gHMBC correlations between the proton of the hydroxy group (δ_H 4.46 ppm in compound **2**, δ_H 4.47 ppm in compound **3**) and carbons C-4, C-14, and C-15 (δ_C 50.3, 204.1, and 84.2 ppm in compound **2**, δ_C 50.4, 204.2, and 84.0 ppm in compound **3**, respectively).

On the other hand, while an ester group in both compounds is present at position C-5, their nature was different. For compound **2**, analysis of 1D and 2D NMR spectra showed the presence of a benzoate ester, while in compound **3** these data pointed to the presence of an isobutyrate ester. The long-range coupling between H-5 (δ_H 6.46 ppm in **2** and 6.25 ppm in **3**) and O<u>C</u>O-5 (δ_C 165.2 ppm in **2** and 175.0 ppm in **3**) in the gHMBC spectra placed the ester group at C-5 in both compounds.

The relative configuration of compounds **2** and **3** was elucidated on the basis of comparison with previously reported natural premyrsinane diterpenes, such as prolifepene B (**12**),[25] and the correlations observed in the NOESY experiments. NOESY correlations were observed in both compounds for H-1α/H-2α, H-2α/H-3α, H-3α/H-4α, H-4α/H_3-20α, H-4α/H-17a, H-17a/H-20α, H-17b/H-20α, H17b/H9α, and H17b/H-11α and indicated that these protons are directed inward in the premyrsinane skeleton, whereas the correlations of H-1β/H_3-16β, H-5β/H-7β, H-5β/H-12β, H-12β/15-OH, and H-12β/H_3-19β were used to place these protons on the opposite face of each molecule (Figures 2 and 3). Therefore, H-4, the H_2-17

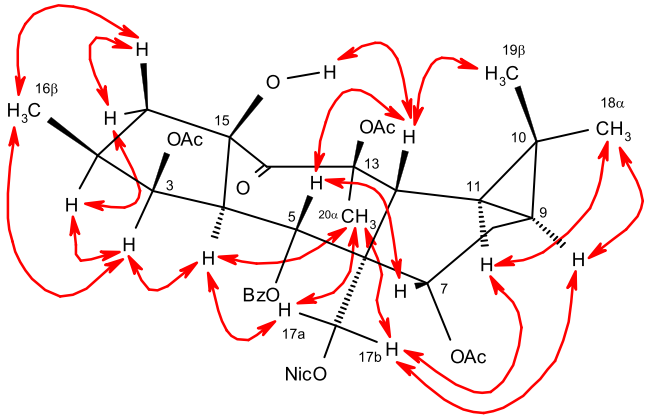

Figure 2. Selected NOESY correlations exhibited by **2**.

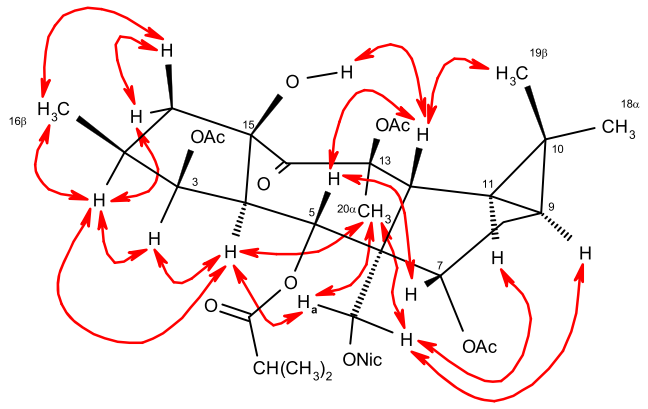

Figure 3. Selected NOESY correlations exhibited by **3**.

methylene group at C-6, H-9, H-11, and H_3-18 are α-oriented, while H-12, OH-15, H_3-16, and H_3-19 are β-oriented. On the other hand, for compounds **2** and **3**, the configuration at C-7 is epimeric to that of prolifepene B (**12**),[25] based on the NOESY correlations for H-7β/H-5β in each compound. Assignment of the relative configuration at C-7 for both compounds based on a comparison of H-7 coupling constants with those of prolifepene (**12**)[25] was not conclusive, as in compounds **2**, **3**, and **12** a nearly bisected conformation of H-7 with H-8a and H-8b could be found. These correlations allowed **2** to be proposed as (2*S**,3*S**,4*R**,5*R**,6*R**,7*S**,9*S**,11*S**,12*R**,13*S**,15*R**)-3,7,13-triacetoxy-5-benzoyloxy-17-nicotinoyloxypremyrsinol and compound **3** as (2*S**,3*S**,4*R**,5*R**,6*R**,7*S**,9*S**,11*S**,12*R**,13*S**,15*R**)-

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3,7,13-triacetoxy-5-isobutiroyloxy-17-nicotinoyloxypremyrsinol.

Compounds **4** and **5** showed characteristic spectroscopic patterns for diterpenes with a myrsinane skeleton, which have a 5/7/6-tricyclic or alternatively a 5/7/6/5-tetracyclic carbon framework if a hemiacetal ring or a 13/17-epoxy is present. The ^{1}H and ^{13}C NMR spectra of both compounds (**4** and **5**) were similar (Table 2). In addition to the signals for the ester moieties, their ^{13}C NMR spectra showed signals for 20 carbons with chemical shifts similar to those of 14-deoxomyrsinols,[27−29] suggesting that these compounds are polyesters of the same parent alcohol.

Table 2. NMR Spectroscopic Data of Myrsinanes 4 and 5 in $CDCl_3$ (J in Hz)

	4		5	
position	δ_H (600 MHz)	δ_C (150 MHz), type	δ_H(500 MHz)	δ_C (125 MHz), type
1α	2.50, dd (15.9, 8.7)	43.4, CH_2	2.48, dd (15.8, 9.3)	43.2, CH_2
1β	2.76, dd (15.9, 11.1)		2.74, dd (15.8, 10.8)	
2α	2.11, m	36.6, CH	2.13, m	36.8, CH
3α	5.22, t (3.6)	77.0, CH	5.15, t (3.8)	76.5, CH
4α	3.04, dd (11.4, 3.6)	51.6, CH	2.95, dd (11.4, 3.8)	51.7, CH
5β	5.86, dd (11.4, 1.8)	69.2,[b] CH	5.96, d (11.4)	69.5, CH
6		54.3, C		56.7, C
7α	4.79, d (6.4)	63.8, CH	5.01, d (6.0)	63.4, CH
8	6.11, ddd (9.6, 6.4, 1.5)	123.4, CH	6.01, ddd (10.0, 6.0, 2.0)	123.1, CH
9	5.82, dd (9.6, 5.4)	133.2, CH	5.75, dd (10.0, 4.5)	133.8, CH
10		147.2, C		147.1, C
11α	3.11, m	41.2, CH	3.23, m	42.8, CH
12β	3.11, m	40.8, CH	3.17, d (5.5)	37.6, CH
13		89.0, C		91.4, C
14α	4.96, s	81.0, CH	4.96, s	79.6, CH
15		89.8, C		89.7, C
16β	0.82, d (7.2)	14.1, CH_3	0.82, d (7.0)	14.2, CH_3
17a	3.97, d (8.6)	69.1,[b] CH_2	6.31, s	97.6, CH
17b	3.49, dd (8.6, 1.8)			
18a	4.82, brs	112.0, CH_2	4.88, brs	112.7, CH_2
18b	4.73, brs		4.85, brs	
19	1.81, s	20.8, CH_3	1.78, s	19.7, CH_3
20β	1.23, s	24.4, CH_3	1.34, s	24.5, CH_3
OCO-3		171.0,[c] C		170.8,[g] C
OCOMe-3	1.99, s[a]	21.0,[d] CH_3	2.01, s[e]	21.0,[h] CH_3
OCO-5		169.3, C		169.0, C
OCOMe-5	1.96, s	20.8, CH_3	2.00, s[f]	21.4,[i] CH_3
OCO-7		170.5, C		170.1, C
OCOMe-7	1.94, s	21.0, CH_3	1.98, s[f]	21.3,[i] CH_3
OCO-14		170.3, C		170.1, C
OCOMe-14	2.02, s	20.9, CH_3	1.98, s[f]	20.8,[i] CH_3
OCO-15		168.4,[c] C		168.2,[g] C
OCOMe-15	2.06, s[a]	22.3,[d] CH_3	2.08, s[e]	22.4,[h] CH_3
OCO-17				169.7, C
OCOMe-17			1.98, s[f]	20.7,[i] CH_3

[a−i]Interchangeable signals.

Compound **4** was isolated as an colorless oil with a molecular formula of $C_{30}H_{40}O_{11}$, according to the ion peak at m/z 599.2484 $[M + Na]^+$ (calcd for $C_{30}H_{40}O_{12}Na$, 599.2468). The ^{1}H and ^{13}C NMR spectra showed signals for five acetoxy groups (Table 2), one more than for 3β-*O*-propionyl-5α,7β,14β,15β-tetra-*O*-acetyl-14-deoxomyrsinol (**13**), isolated previously from *E. prolifera*.[28] The positions of these acetyl groups were deduced from the gHMBC spectrum. Correlations of the protons at δ_H 5.86 (H-5), 4.79 (H-7), and 4.96 ppm (H-14) with the carbonyl carbons at δ_C 169.3 (OCO-5), 170.5 (OCO-7), and 170.3 ppm (OCO-14), respectively, demonstrated the locations of these three acetoxy groups at C-5, C-7, and C-14. However, there were no long-range correlations of protons to the carbonyl carbons of the two remaining acetyl groups. On the basis of the chemical shifts of the proton H-3 (δ_H 5.22 ppm) and the carbon C-3 (δ_C 77.0 ppm) and comparison with the data for compound **13**,[28] one of the two residual acetoxy groups was assigned at C-3, suggesting that the C-3 propoxy moiety in **13** has been replaced by an acetoxy group in compound **4**. Consequently, the remaining acetyl group could only be located at the C-15 quaternary carbon, which was confirmed by comparing the chemical shift of this carbon in **4** (δ_C 89.8 ppm) with that of **13** (δ_C 89.7 ppm).

The relative configuration of compound **4** was established by analysis of the ROESY spectrum and that described for the previously reported myrsinane diterpenes.[1,2] Myrsinol diterpenes show a *trans*-fused 5/7/6-tricyclic ring system, α-orientations for the epoxy ring and H-4, and β-orientations for H_3-16 and the side chain at C-11.[27−29] ROESY correlations observed for H-1α/H-2α, H-1α/H-14α, H-2α/H-3α, H-3α/H-4α, H-7α/H-17a, H-7α/H-17b, H-1β/H_3-16β, H-5β/H-12β, and H-12β/H_3-20β suggested that H-2, H-3, H-4, H-7, and H-14 are α-oriented (directed inward in the premyrsinane skeleton) and H-5, H-12, H-20, and H_3-16 are β-oriented (opposite face of the skeleton) (Figure 4). On the other hand,

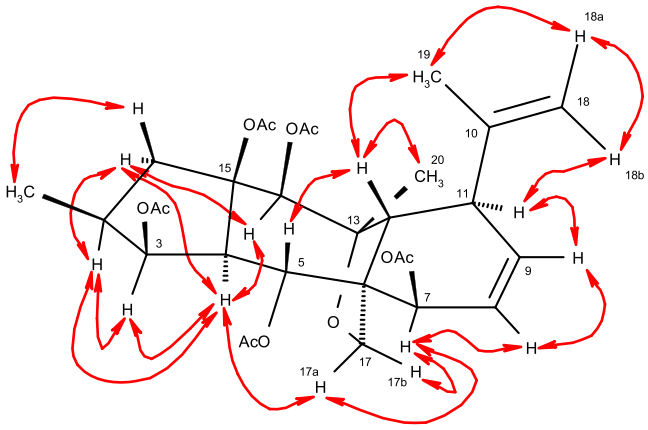

Figure 4. Selected ROESY correlations exhibited by **4**.

ROESY correlations observed for H-12β/H_3-19, H_3-19/H-18a, H-18a/H-18b, H-18b/H-11α, H-11α/H-9, H-9/H-8, and H-8/H-7α confirmed, in turn, that H-11 is α-oriented, as well as H-7. These assignments were consistent with the relative configurations of the reported myrsinane diterpenes and allowed compound **4** to be established as (2*S**,3*S**,4*R**,5*R**,6*R**,7*R**,11*S**,12*R**,13*R**,14*S**,15*R**)-3,5,7,14,15-pentaacetoxy-14-deoxo-13,17-epoxymyrsinol.

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J. Nat. Prod. 2019, 82, 2517−2528

Compound **5** was isolated as an amorphous solid and exhibited a $[M + Na]^+$ ion in its HRESIMS at m/z 657.2524 (calcd for $C_{32}H_{42}O_{13}Na$, 657.2523), providing the molecular formula $C_{32}H_{42}O_{13}$. Its spectroscopic data (Table 2) were in agreement with a tetracyclic structure of a 14-deoxomyrsinol, similar to **4**. The main differences between compounds **5** and **4** were the presence of an additional acetyl group and the lack of the signals for the diastereotopic C-17 methylene protons. Instead, the ^{1}H NMR spectrum of **5** showed the presence of a singlet at δ_H 6.31 ppm, which correlated with a methine at δ_C 97.6 ppm in the gHSQC experiment, pointing to the presence of a hemiacetal ring. The hemiacetal proton was connected to the carbonyl carbon at δ_C 169.7 ppm in the gHMBC experiment, which allowed the additional acetyl group to be located at C-17.

Like **4**, in compound **5** all the hydroxy groups attached to the myrsinane skeleton were esterified with acetyl groups; this feature differentiates both of them from the myrsinanes previously described in the literature. The relative configuration of compound **5** was elucidated by comparison with reported myrsinane diterpenes[27−29] and the correlations observed in the NOESY spectrum (Figure 5). NOESY

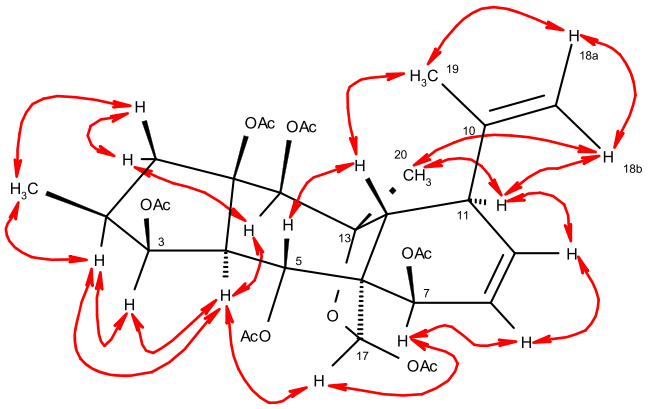

Figure 5. Selected NOESY correlations exhibited by **5**.

correlations observed for H-1α/H-1β, H-2α/H$_3$-16β, H-2α/H-3α, H-2α/H-4α, H-3α/H-4α, H-4α/H-17, H-17/H-7α, H-14α/H-4α, H-14α/H-1α, H-1β/H$_3$-16β, and H-5β/H-12β suggested that H-2, H-3, H-4, H-7, and H-14 are α-oriented and H-5, H-12, and H$_3$-16 are β-oriented (Figure 5). In turn, NOESY correlations observed for H-12β/H$_3$-19, H$_3$-19/H-18a, H-18a/H-18b, H-18b/H-11α, H-18b/H$_3$-20β, H-11α/H$_3$-20β, H-11α/H-9, H-9/H-8, and H-8/H-7α confirmed that H-11 is α-oriented, as well as H-7, and that the only orientation compatible with those correlations for the methyl group substituted at C-13 is β (H$_3$-20β). These correlations allowed compound **5** to be assigned structurally as (2*S**,3*S**,4*R**,5*R**,6*R**,7*R**,11*S**,12*R**,13*R**,14*S**,15*R**,17*S**)-3,5,7,14,15,17-hexacetoxy-14-deoxo-13,17-epoxymyrsinol.

Compounds **6** and **7** showed spectroscopic data closely related structurally to compound **8**,[21] which possesses a cyclomyrsinol-type terpene skeleton. Cyclomyrsinanes are diterpenoids with a 5/7/6/4-tretracyclic or alternatively 5/7/6/4/5-pentacyclic carbon framework if a 13/17-epoxy is present. Both compounds (**6** and **7**) were isolated as amorphous solids. Their respective molecular formulas were determined as $C_{36}H_{48}O_{16}$ (m/z 759.2859 $[M + Na]^+$, calcd for $C_{36}H_{48}O_{16}Na$, 759.2840) and $C_{39}H_{46}O_{16}$ (m/z 793.2686 $[M + Na]^+$, calcd for $C_{39}H_{46}O_{16}Na$, 793.2684), from their HRESIMS data. The ^{1}H and ^{13}C NMR spectra of compounds **6** and **7** were similar (Table 3). A detailed analysis revealed the presence of signals corresponding to six acetoxy groups, one

Table 3. NMR Spectroscopic Data of Cyclomyrsinanes 6 (C_6D_6) and 7 ($CDCl_3$) (J in Hz)

	6		7	
position	δ_H (400 MHz)	δ_C (100 MHz), type	δ_H (500 MHz)	δ_C (125 MHz), type
1α	2.42, dd (15.9, 9.9)	43.2, CH_2	2.46, dd (16.0, 9.5)	42.9, CH_2
1β	3.05, dd (15.9, 8.8)		2.71, dd (16.0, 10.5)	
2α	1.54, m	35.1, CH	2.19, m	35.9, CH
3α	5.17, t (4.5)	76.9, CH	5.39, t (4.0)	76.9, CH
4α	2.56, dd (11.2, 4.4)	52.0, CH	2.96, dd (4.0, 11.3)	51.5, CH
5β	6.05, d (11.2)	68.8, CH	6.07, d (11.3)	67.8, CH
6		65.5, C		64.8, C
7		200.2, C		199.5, C
8α	5.48, d (7.0)	71.2, CH	5.19, d (7.5)	73.5, CH
9α	2.08, m	28.9, CH	2.90, m	29.2, CH
10		77.4, C		77.1, C
11α	2.70, m	42.7, CH	2.82, m	42.6, CH
12β	4.17, d (12.3)	39.6, CH	4.10, d (12.5)	38.9, CH
13		91.7, C		92.0, C
14α	5.30, s	81.0, CH	5.12, s	80.5, CH
15		90.9, C		90.1, C
16β	0.67, d (7.0)	14.2, CH_3	0.85, d (7.0)	14.1, CH_3
17	6.96, s	96.7, CH	6.53, s	96.6, CH
18α	2.36, m	34.8, CH_2	2.40, dd (10.0, 13.0)[f]	36.5, CH_2
18β	2.65, m		2.66, m[f]	
19α	1.42, s	24.7, CH_3	1.66, s	24.4, CH_3
20β	1.32, s	23.2, CH_3	1.27, s	22.7, CH_3
OCO-3		169.6, C		170.3, C
OCOMe-3	1.72, s	20.4,[c] CH_3	2.02, s	20.9, CH_3
OCO-5		170.0, C		169.7, C
OCOMe-5	1.81, s	20.6,[c] CH_3	1.98, s	20.8, CH_3
OCO-8		174.8, C		166.0, C
OCOiBu-8				
2′	2.78, sept (7.0)	34.7, CH		
3′	1.36, d (7.0)[a]	20.4,[c] CH_3		
4′	1.10, d (7.0)[a]	18.9,[c] CH_3		
8-OCOBz				
1′				129.9, C
2′, 6′			8.22, d (8.0)	130.1, CH
4′			7.57, t (8.0)	133.4, CH
3′, 5′			7.42, t (8.0)	128.4, CH
OCO-10		167.9,[d] C		168.3,[i] C
OCOMe-10	1.99, s[b]	23.3,[e] CH_3	1.65, s[g]	23.2,[j] CH_3
OCO-14		170.0, C		170.4, C
OCOMe-14	1.90, s	21.1, CH_3	2.06, s	21.3, CH_3
OCO-15		168.5,[d] C		169.1,[i] C
OCOMe-15	1.83, s[b]	21.4,[e] CH_3	2.00, s[g,h]	21.3,[j] CH_3
OCO-17		168.4, C		169.1, C
OCOMe-17	1.61, s	20.7, CH_3	2.00, s[h]	21.7,[j] CH_3

[a−j]Interchangeable signals.

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J. Nat. Prod. 2019, 82, 2517−2528

more than for **8**. Five of these acetoxy groups were assigned at C-3, C-5, C-10, C-14, and C-15 by comparison of the spectroscopic data of both compounds (**6** and **7**) with those of **8**.[21] These assignments were corroborated through correlations observed in their gHMBC spectra. The remaining acetoxy group was attached at C-17 based on the observed correlation between the proton H-17 (δ_H 6.96 ppm in **6** and 6.53 ppm in **7**) and the carbonyl carbon O<u>C</u>O-17 (δ_C 168.4 ppm in **6** and 169.1 ppm in **7**) in the gHMBC spectra. Unlike myrsinanes and premyrsinanes, this is the first report of a hemiacetal ring in the cyclomyrsinanes.

In addition to these acetoxy groups, an ester group was present at position C-8 in both compounds. For compound **6**, the presence of two doublets at δ_H 1.10 and 1.36 ppm, each corresponding to a methyl group, connected to a septet at δ_H 2.78 ppm in the COSY experiment, pointed to the presence of an isobutyrate ester. On the other hand, compound **7** showed typical aromatic signals at δ_H 7.42, 7.57, and 8.22 in its ^{1}H NMR spectrum, suggesting the presence of a benzoyloxy group. The long-range coupling between H-8 (δ_H 5.48 ppm in **6** and 5.19 ppm in **7**) and O<u>C</u>O-8 (δ_C 174.8 ppm in **6** and 166.0 ppm in **7**) in the gHMBC spectra placed the ester groups at C-8 in both compounds.

The relative configurations of compounds **6** and **7** were elucidated on the basis of comparison with reported values for cyclomyrsinane diterpenes[21,28,29] and the correlations observed in the NOESY experiments. NOESY correlations observed for H-2α/H-3α, H-2α/H-4α, H-3α/H-4α, H-4α/H-17, H-4α/H-14α, H-14α/H-1α, H-8α/H-9α, H-9α/H-18α, H-9α/H-11α, H-11α/H$_3$-19α, and H-18α/H$_3$-19α in compound **6** revealed they have the same orientation as in compound **8**[21] and suggested that H-2, H-3, H-4, H-8, H-9, H-11, H-14, and H$_3$-19 are α. On the other hand, NOESY correlations observed for H-1α/H$_3$-16β, H-2α/H$_3$-16β, H-5β/H-12β, and H-14α/H$_3$-20β confirmed, in turn, that H-5 H-12, H$_3$-16, and H$_3$-20 are β-oriented (Figure 6). These correlations allowed compound **6**

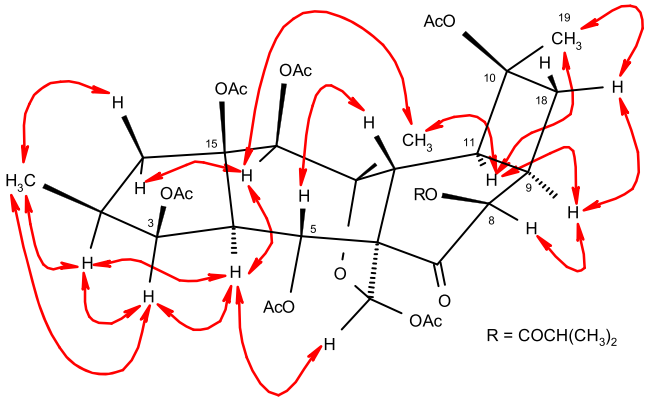

Figure 6. Selected NOESY correlations exhibited by **6**.

to be determined as (2*S**,3*S**,4*R**,5*R**,6*R**,8*R**,9*R**,10*S**,11*S**,12*R**,13*R**,14*S**,15*R**,17*S**)-3,5,10,14,15,17-hexaacetoxy-8-isobutyryloxy-13,17-epoxycyclomyrsinol.

The NOESY correlations observed for H-2α/H-3α, H-2α/H-4α, H-3α/H-4α, H-4α/H-17, H-4α/H-14α, and H-14α/H-1α in compound **7** revealed they also have the same relative configuration as in compound **8**[21] and suggested that H-2, H-3, H-4, and H-14 are α-oriented. The NOESY correlations observed for H-1α/H$_3$-16β, H-2α/H$_3$-16β, H-5β/H-12β, H-14α/H$_3$-20β, H-2′,6′/H-5β, and H-2′,6′/OCOC<u>H</u>$_3$-10 confirmed that H-5, H-12, H$_3$-16, H$_3$-20, and OCOC<u>H</u>$_3$-10 are β-oriented. These observations, together with NOESY correlations between H-8α/H-9α and H-11α/H$_3$-20β, in turn, implied that H-8, H-9, H-11, and H$_3$-19 are α-oriented (Figure 7). Accordingly, compound **7** was assigned as (2*S**,3*S**,4*R**,5*R**,6*R**,8*R**,9*R**,10*S**,11*S**,12*R**,13*R**,14*S**,15*R**,17*S**)-3,5,10,14,15,17-hexaacetoxy-8-benzoyloxy-13,17-epoxycyclomyrsinol.

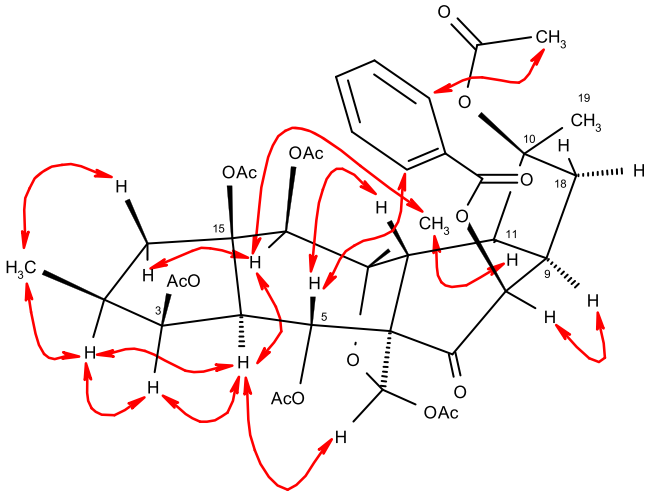

Figure 7. Selected NOESY correlations exhibited by **7**.

Electronic circular dichroism (ECD) spectra for compounds **2** and **7** were measured (see Figures S41 and S42, respectively, in the Supporting Information) and compared to those of prolifepene B and A, respectively.[25] Both series of ECD spectra show close resemblances, noting that the wavelength and sign of the observed Cotton effects presented similar patterns. Therefore, on this basis, the absolute configuration for compounds **2** and **3** can be assigned as 2*S*,3*S*,4*R*,5*R*,6*R*,7*S*,9*S*,11*S*,12*R*,13*S*,15*R*. On the other hand, the absolute configuration for compounds **6** and **7** can be proposed as 2*S*,3*S*,4*R*,5*R*,6*R*,8*R*,9*R*,10*S*,11*S*,12*R*,13*R*,14*S*,15*R*,17*S*.

As mentioned previously, lathyrane-type diterpenes are new drug candidates to modulate adult neurogenesis through PKC activation.[16] Consequently, the effects of lathyrane compounds **9** and **10** were evaluated on NPC proliferation.

To this end, neurosphere assays were used, in which neural progenitor cells isolated from the subventricular zone of postnatal mice were cultured in the presence of growth factors. Under these conditions, cells grow as aggregates named neurospheres, initiated by a neurosphere-forming cell (stem cell or undifferentiated progenitor) and expanded by its progeny (undifferentiated progenitors, glial progenitors, and neuronal progenitors). The size and number of these neurospheres therefore are an indirect measure of NPC proliferation. In particular, the neurosphere size has been well described as an indicator of proliferation of neurosphere-forming cells (mostly undifferentiated progenitors), while the neurosphere number indicates self-renewal and neurosphere-initiating capacity of NPCs together with their viability. Experiments were performed in cultures stimulated with either the epidermal growth factor (EGF), the basic fibroblast growth factor (bFGF), or a combination of both. Increasing concentrations of **9** and **10** (1, 5, and 10 μM) were used to test proliferation. After 72 h, an increase was observed in the

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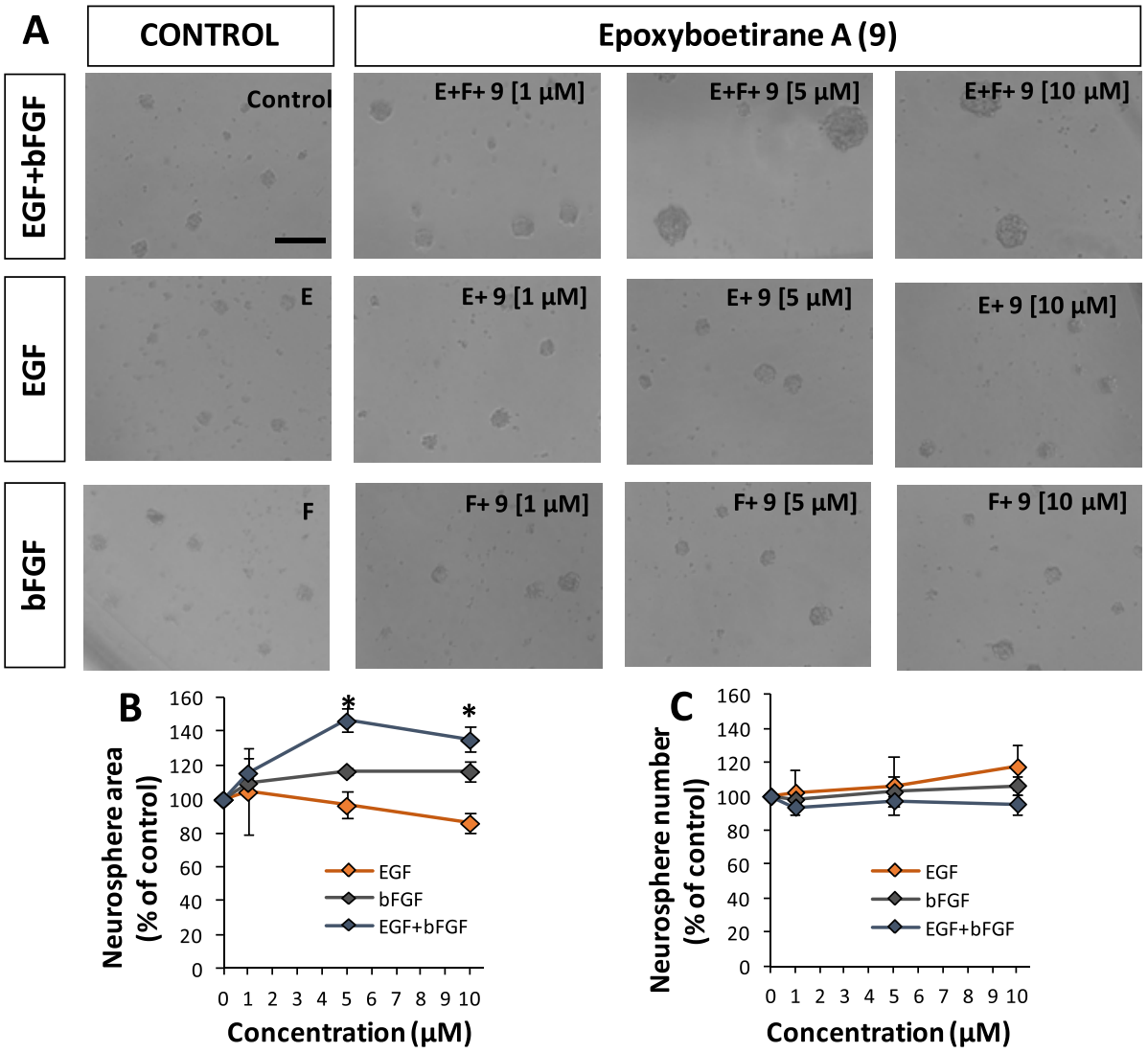

Figure 8. Effects of increasing concentrations of **9** on neural precursor cell (NPC) proliferation in an in vitro assay. Proliferation was evaluated in a neurosphere assay in the presence of EGF (E), bFGF (F), or a combination of both (E+F). (A) Phase-contrast microscopic images of neurospheres cultured for 72 h in the absence or presence of **9** (1, 5, or 10 μM) plus the specific growth factor, or a combination of the two. The scale bar indicates 200 μm. (B) Graph showing the effect of increasing concentrations of **9** on neurosphere area after 72 h in culture. (C) Graph showing the effect of increasing concentrations of **9** on neurosphere number after 72 h in culture (*$p < 0.05$ compared with the control using a Student t test).

size of neurospheres in cultures stimulated with a combination of EGF and bFGF and treated with compound **9** when 5 and 10 μM concentrations were used, while no effect was observed at 1 μM (Figure 8A and B). Interestingly, these effects were only observed in cultures in which proliferation was stimulated by both growth factors, and no statistically significant effect was observed in cultures stimulated with either EGF or bFGF alone. These results indicated that **9** promotes NPC proliferation in a dose-dependent manner only when proliferation is stimulated by both growth factors. Nevertheless, no effect was found in neurosphere number, indicating that the capacity to form new neurospheres (self-renewal and viability) was not affected (Figure 8A and C).

Compound **10** induced a smaller increase in the neurosphere area and only at 5 μM and also in the presence of both growth factors. This compound (**10**) also did not exert any effect on neurosphere number (Figure 9A–C). These results indicated that the modes of action of both compounds were probably similar, although **9** exerted a more potent effect on NPC proliferation compared to **10**.

In contrast, previous reports on the effect of lathyranes on NPC proliferation have described a proliferative effect only in the presence of bFGF or EGF alone, but not with a combination of them both.[16] In particular, the lathyrane ELAC promotes NPC proliferation in a PKCβ-dependent manner in cultures stimulated with bFGF, indicating a role for this lathyrane on activating classical PKC.[16] These results have suggested a role for classical PKC on activating the epidermal growth factor receptor (EGFR) in bFGF-stimulated cultures and the basic fibroblast growth factor receptor (FGFR) in EGF-stimulated cultures. Thus, when both receptors are saturated by EGF and bFGF, no effect can be observed. This is in agreement with recent findings, which demonstrated that classical PKC activation facilitates the release of EGFR ligands such as TGFα.[30]

Regarding the effect of the compounds tested herein, **9** and, to a lesser extent, **10** exerted a synergistic effect on NPC

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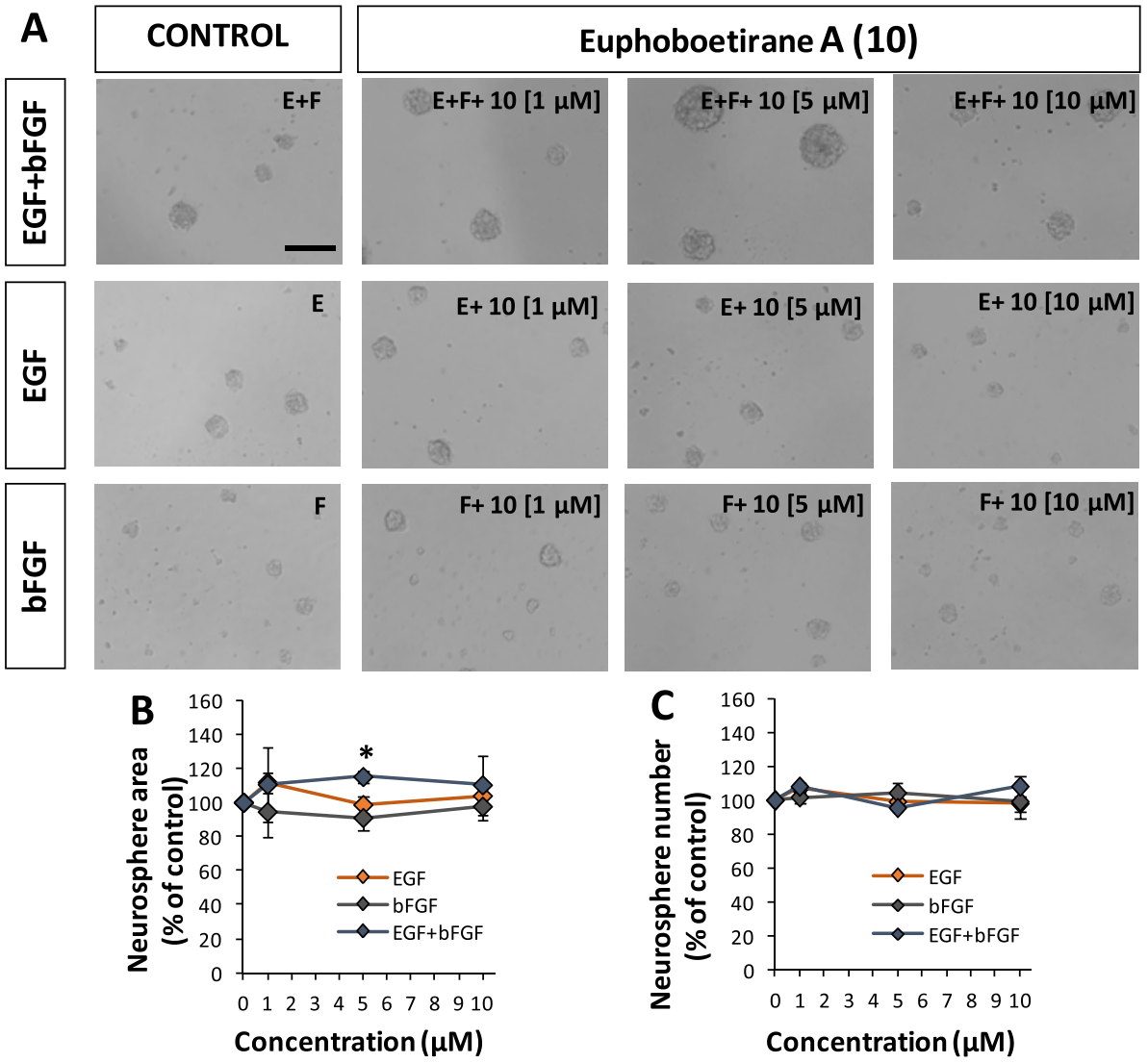

Figure 9. Effects of **10** on neural precursor cell (NPC) proliferation in an in vitro assay. Proliferation was evaluated by a neurosphere assay in the presence of epidermal growth factor (EGF), basic fibroblastic growth (bFGF), or a combination of both. (A) Phase-contrast microscopic images of neurospheres cultured for 72 h in the absence or presence of **10** (1, 5, or 10 μM) plus the specific growth factor or the combination of the two. The scale bar indicates 200 μm. (B) Graph showing the effect of increasing concentrations of **10** on neurosphere area after 72 h in culture. (C) Graph showing the effect of increasing concentrations of **10** on neurosphere number after 72 h in culture (*$p < 0.05$ compared with the control using a Student t test).

proliferation when stimulated with a combination of EGF plus bFGF, suggesting they are acting on receptors different from EGFR or FGFR. Recent reports have shown that novel PKC activation facilitates the release of the ErbB4 receptor ligand neuregulin.[30] Therefore, it is possible that compounds **9** and **10** are activating novel PKC isozymes. This is a reasonable hypothesis since classical PKC isozymes have a C1B regulatory domain with a lower affinity for diacylglycerol (DAG) or its analogues, whereas the novel PKC C1B domain has a higher affinity.[31] According to this, **9** and **10** may be able to activate novel PKCs without activating the classical isozymes, thus exerting an effect through a different signaling pathway.

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were determined with a PerkinElmer 341 polarimeter. IR spectra were recorded on a PerkinElmer Spectrum BX FT-IR spectrophotometer and reported as wavenumber (cm^{-1}). ECD spectra were recorded on a JASCO J-1500 CD spectrometer. ^{1}H and ^{13}C NMR measurements were carried out on Agilent 400, 500, and 600 MHz spectrometers with $SiMe_4$ as the internal reference. Chemical shifts were referenced to $CDCl_3$ (δ_H 7.25, δ_C 77.0) and C_6D_6 (δ_H 7.15, δ_C 128.0). NMR assignments were made using a combination of 1D and 2D techniques and by comparison with those made for previously described compounds, where appropriate. High-resolution mass spectrometry (HRMS) was performed in a Q-TOF mass spectrometer in the positive-ion ESI mode. TLC was performed on Merck Kiesegel 60 F_{254}, 0.25 mm thick. Silica gel 60 (70−230 mesh, Merck) was used for column chromatography. HPLC was performed with a Merck-Hitachi LaChrom apparatus equipped with a UV−vis detector (L 4250) and a differential refractometer detector (RI-7490) and an Elite LaChrom-Hitachi apparatus equipped with a differential refractometer detector (RI-2490). LiChroCART LiChrospher Si 60 (5 μm, 250 mm × 4 mm), LiChroCART LiChrospher Si 60 (10 μm, 250 mm × 10 mm), and LiChroCART ChiraSpher NT, based on silica gel particles coated with the optically active polymer poly(*N*-acryloyl-*S*-phenylalanine ethyl ester), columns (5 μm, 250 mm × 4 mm) were used for isolation experiments.

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Plant Material. The whole plants of *E. boetica* were collected at El Pinar del Hierro in Chiclana de la Frontera, Cádiz, Spain, in June 2011 by Dr. L. Plaza-Aguirre (Consejería del Medio Ambiente of Junta de Andalucía) and J. Luis Rendón (Botanical Garden of San Fernando, Cádiz, Spain).

Extraction and Isolation. The aerial parts of the fresh plant (1.2 kg) were frozen with liquid nitrogen, powdered, and extracted with 2.5 L of MeOH (×3) at room temperature for 24 h. The MeOH extract was evaporated under reduced pressure to yield a crude extract (58.4 g), which was suspended in water (1 L) and then partitioned with hexane (2 L) and dichloromethane (1 L), sequentially. After removing the solvent, the hexane-soluble extract yielded 12.2 g of residue and 0.5 g of the dichloromethane-soluble extract. The residual aqueous fraction was freeze-dried. The hexane and dichloromethane extracts were chromatographed on silica gel in a gradient mixture of *n*-hexane−EtOAc of increasing polarity (0−100%), to afford 32 and 26 fractions, respectively, according to TLC analysis. Final purification of those fractions from the hexane extract that showed the presence of diterpene signals in their ^{1}H NMR spectra was carried out by means of semipreparative and/or analytical and/or chiral analytical HPLC. The hexane fraction yielded (per gram of hexane fraction) compounds **1** (0.5 mg), **2** (3.0 mg), **3** (0.5 mg), **4** (0.5 mg), **5** (2.0 mg), **6** (2.0 mg), **7** (0.5 mg), **8** (1.0 mg), **9** (13.0 mg), and **10** (30.0 mg). Fractions of the dichloromethane extract were analyzed by ^{1}H NMR, detecting the presence of compounds **2**, **3**, and **5**−**9**.

(2S,3S*,4R*,5R*,6R*,9S*,11S*,12R*,13R*,15R*)-3,5,15-Triacetoxy-13,17-epoxypremyrsinol (**1**):* amorphous solid; $[\alpha]_D^{20}$ −31 (*c* 0.13, $CHCl_3$); IR (film) ν_{max} 2926, 1739, 1453, 1371, 1240, 1143, 1024, 967, 753 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 1; gHMBC (selected correlations) C-1 → H_3-16*β*; C-2 → H-1*α*, H-1*β*; C-3 → H-1*α*, H_3-16*β*; C-4 → H-5*β*; C-5 → H-4*α*, H-12*β*, H-17a, H-17b; C-6 → H-12*β*; C-7 → H-17b; C-9 → H_3-18*α*, H_3-19*β*; C-10 → H-12*β*, H_3-18*α*, H_3-19*β*; C-11 → H-12*β*, H_3-18*α*, H_3-19*β*; C-13 → H-17a, H_3-20*β*; C-14 →H-12*β*, H_3-20*β*; C-15 → H-1*β*; C-18*α* →H_3-18*β*; C-19*β* →H_3-18*α*; OCO-5 → H-5*β*, OCOMe-5; HRESIMS *m*/*z* 499.2322 [M + Na]$^+$ (calcd for $C_{26}H_{36}O_8Na$, 499.2308).

*(2S,3S,4R,5R,6R,7S,9S,11S,12R,13S,15R)-3,7,13-Triacetoxy-5-benzoyloxy-17-nicotinoyloxypremyrsinol (**2**):* amorphous solid; $[\alpha]_D^{20}$ −22 (*c* 0.26, $CHCl_3$); ECD (CH_3CN) *λ* (Δ*ε*) 203 (0.33), 214 (0.64), 234 (−0.74), 307 (0.18) nm; IR (film) ν_{max} 3489, 2931, 1720, 1590, 1450, 1370, 1264, 1176, 1111, 1070, 964, 757, 713 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 1; gHMBC (selected correlations) C-1 → H_3-16*β*; C-2 → H-1*α*, H-1*β*, H_3-16*β*; C-3 → H-1*α*, H_3-16*β*; C-4 → H-1*α*, H-5*β*, OH-15; C-5 → H-4*α*, H-7*β*, H-17a, H-17b; C-6 → H-4*α*, H-5*β*, H-7*β*, H-8*β*, H-12*β*, H-17a, H-17b; C-7 → H-5*β*, H-8*β*, H-9*α*, H-17a, H-17b; C-8 → H-7*β*; C-9 → H-7*β*, H-8*β*, H-8*α*; C-10 → H-12*β*, H_3-18*α*, H_3-19*β*; C-11 → H-8*β*, H-12*β*, H_3-18*α*, H_3-19*β*; C-12 → H-7*β*, H-17b, H_3-20*α*; C-13 → H-12*β*, H_3-20*α*; C-14 → H-1*α*, H-1*β*, H-4*α*, 15-OH, H_3-20*α*; C-15 → H-1*α*, H-1*β*, 15-OH; C-16*β* →H-1*β*; C-17 → H-5*β*, H-12*β*; C-18*α* →H_3-19*β*; C-19*β* →H_3-18*α*; C-20*α* → H-12*β*; OCOCH$_3$-3 → OCOCH_3-3; OCOPh-5 →H-5*β*, H_2-2′,6′; OCOCH$_3$-7 → H-7*β*, OCOCH_3-7; OCOCH$_3$-13→ OCOCH_3-13; OCONic-17 → H-17a; HRESIMS *m*/*z* 742.2855 [M + Na]$^+$ (calcd for $C_{39}H_{45}NO_{12}Na$, 742.2839).

*(2S,3S,4R,5R,6R,7S,9S,11S,12R,13S,15R)-3,7,13-Triacetoxy-5-isobutiroyloxy-17-nicotinoyloxypremyrsinol (**3**):* amorphous solid; $[\alpha]_D^{20}$ −16 (*c* 0.20, $CHCl_3$); IR (film) ν_{max} 3486, 2937, 1739, 1590, 1422, 1371, 1249, 1139, 1072, 1022, 966, 754 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 1; gHMBC (selected correlations) C-1 → H_3-16*β*; C-2 → H-1*α*, H-1*β*, H_3-16*β*; C-3 → H-1*α*, H_3-16*β*; C-4 → H-1*α*, H-5*β*, OH-15; C-5 → H-4*α*, H-7*β*, H-17a, H-17b; C-6 → H-4*α*, H-5*β*, H-7*β*, H-8*β*, H-12*β*, H-17a, H-17b; C-7 → H-5*β*, H-8*β*, H-9*α*, H-12*β*, H-17a, H-17b; C-8 → H-7*β*; C-9 → H-7*β*, H-8*α*, H-8*β*, H_3-18*α*, H_3-19*β*; C-10 → H-8*β*, H-12*β*, H_3-18*α*, H_3-19*β*; C-11 → H-8*β*, H-12*β*, H_3-18*α*, H_3-19*β*; C-12 → H-7*β*, H-17b, H_3-20*α*; C-13 → H-12*β*, H_3-20*α*; C-14 → H-1*α*, H-1*β*, H-4*α*,15-OH, H_3-20*α*; C-15 → H-1*α*, H-1*β*, H-15*β*, OH-15; C-16*β* →H-1*β*; C-17 → H-5*β*, H-12*β*; C-18*α* → H-9*α*, H-11*α*, H_3-19*β*; C-19*β* →H_3-18*α*; C-20*α* → H-12*β*; OCOCH$_3$-3 → 3−OCOCH_3-3; OCOCH(CH_3)$_2$-5 → OCOCH-(CH$_3$)$_2$-5; OCOCH(CH$_3$)$_2$-5 → OCOCH(CH_3)$_2$-5; OCOCH-(CH$_3$)$_2$-5→ H-5*β*, OCOCH(CH$_3$)$_2$-5, OCOCH(CH_3)$_2$-5; COCH$_3$-7 → H-7*β*, COCH_3-7; COCH$_3$-13 → COCH_3-13; CONic-17 → H-17a, H-17b; HRESIMS *m*/*z* 708.3009 [M + Na]$^+$ (calcd for $C_{36}H_{47}NO_{12}Na$, 708.2996).

(2S,3S*,4R*,5R*,6R*,7R*,11S*,12R*,13R*,14S*,15R*)-3,5,7,14,15-Pentaacetoxy-14-deoxo-13,17-epoxymyrsinol (**4**):* colorless oil; $[\alpha]_D^{20}$ −13.4 (*c* 0.11, $CHCl_3$); IR (film) ν_{max} 2939, 1740, 1370, 1233, 1083, 1040, 967, 753, 579 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 2; gHMBC (selected correlations) C-3 → H-1*α*, H_3-16*β*; C-4 → H-1*α*, H-5*β*; C-5 → H-4*α*, H-7*α*, H-12*β*, H-17a, H-17b; C-6 → H-7*α*, H-8, H-11*α*, H-12*β*, H-17a; C-10 → H-11*α*, H-18b, H_3-19; C-11 → H-8, H-18a, H-18b, H_3-19; C-12 → H-7*α*, H-9, H-14*α*, H-17a, H_3-20; C-13 → H-11*α*, H-12*β*, H-14*α*, H-17a, H_3-20*β*; C-15 → H-1*β*; C-17 → H-5*β*, H-12*β*; C-19 → H-11*α*, H-18a, H18b; OCOCH$_3$-5 → H-5*β*, OCOCH_3-5; OCOCH$_3$-7 → H-7*α*, OCOCH_3-7; OCOCH$_3$-4 → H-14*α*, OCOCH_3-14; HRESIMS *m*/*z* 599.2484 [M + Na]$^+$ (calcd for $C_{30}H_{40}O_{11}Na$, 599.2468).

(2S,3S*,4R*,5R*,6R*,7R*,11S*,12R*,13R*,14S*,15R*,17S*)-3,5,7,14,15,17-Hexaacetoxy-14-deoxo-13,17-epoxymyrsinol (**5**):* amorphous solid; $[\alpha]_D^{20}$ −43 (*c* 0.10, $CHCl_3$); IR (film) ν_{max} 2360, 1742, 1370, 1231, 1012 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 2; gHMBC (selected correlations) C-1 → H-14*α*, H_3-16*β*; C-3 → H-1*α*, H_3-16*β*; C-4 → H-5*β*; C-5 → H-12*β*; C-6 → H-7*α*, H-8, H-12*β*; C-10 → H-12*β*, H-19; C-11 → H-9, H-12*β*, H-18a, H-18b, H_3-19; C-12 → H-7*α*, H-9, H-14*α*, H-17, H_3-20*β*; C-13 → H-14*α*, H-17, H-20*β*; C-15 → H-1*β*; C-17 → H-5*β*, H-12*β*; C-19 → H-18a, H-18b; OCOCH$_3$-5 → H-5*β*, OCOCH_3-5; OCOCH$_3$-7 → H-7*α*; OCOCH$_3$ −14 → H-14*α*; OCOCH$_3$-17 → H-17; HRESIMS *m*/*z* 657.2524 [M + Na]$^+$ (calcd for $C_{32}H_{42}O_{13}Na$, 657.2523).

*(2S,3S,4R,5R,6R,8R,9R,10S,11S,12R,13R,14S,15R,17S)-3,5,10,14,15,17-Hexaacetoxy-8-isobutyryloxy-13,17-epoxycyclomyrsinol (**6**):* amorphous solid; $[\alpha]_D^{20}$ +58 (*c* 0.10, $CHCl_3$); IR (film) ν_{max} 2930, 1742, 1370, 1230, 1105, 1009 cm^{-1}; ^{1}H and ^{13}C NMR data see Table 3; gHMBC (selected correlations) C-1 → H-3*α*, H-14*α*, H_3-16*β*; C-2 → H-1*α*, H-1*β*, H_3-16*β*; C-3 → H-1*α*, H_3-16*β*; C-4 → H-1*β*, H-5*β*; C-5 → H-4*α*, H-12*β*, H-17; C-6 → H-4*α*, H-5*β*, H-8*α*, H-12*β*; C-7 → H-8*α*, H-12*β*; C-10 → H-11*α*, H-12*β*, H-18*β*, H_3-19*α*; C-11 → H-8*α*, H-12*β*, H_3-19*α*; C-12 → H-14*α*, H-17, H_3-20*β*; C-13 → H-14*α*, H-17, H_3-20*β*; C-14 → H-1*α*, H-4*α*, H-12*β*, H_3-20*β*; C-15 → H-1*β*, H-3*α*, H-14*α*; C-16 → H-1*β*; C-17 → H-5*β*, H-12*β*; C-18 →H_3-19*α*; OCO-3 → H-3*α*, OCOMe-3; OCO-5 → H-5*β*, OCOMe-5; OCO-8 → H-8*α*, H-2′, H-3′, H-4′; OCO-14 → H-14*α*, OCOMe-14; OCO-17 → H-17, OCOMe-17; HRESIMS *m*/*z* 759.2859 [M + Na]$^+$ (calcd for $C_{36}H_{48}O_{16}Na$, 759.2840).

*(2S,3S,4R,5R,6R,8R,9R,10S,11S,12R,13R,14S,15R,17S)-3,5,10,14,15,17-Hexaacetoxy-8-benzoyloxy-13,17-epoxycyclomyrsinol (**7**):* amorphous solid; $[\alpha]_D^{20}$ +20 (*c* 0.10, $CHCl_3$); ECD (CH_3CN) *λ* (Δ*ε*) 204 (0.35), 229 (−0.48), 251 (0.24), 313 (0.99) nm; IR (film) ν_{max} 2931, 1740, 1432, 1370, 1230, 1171, 1104, 1025, 965, 753, 718 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 3; gHMBC (selected correlations) C-1 → H-3*α*, H-14*α*, H_3-16*β*; C-2 → H-1*α*, H-1*β*, H_3-16*β*; C-3 → H-1*α*, H-5*β* H_3-16*β*; C-4 → H-5*β*; C-5 → H-4*α*, H-12*β*, H-17; C-6 → H-12*β*; C-7 → H-8*α*; C-9 → H-18*α*; C-10 → H-12*β*, H_3-19*α*; C-11 → H-8*α*, H-12*β*, H_3-19*α*; C-12 → H-14*α*, H-17, H_3-20*β*; C-13 → H-14*α*, H-17, H_3-20*β*; C-14 → H-1*α*, H-4*α*, H-12*β*, H_3-20*β*; C-15 → H-1*β*, H-3*α*; C-16 → H-1*β*; C-17 → H-5*β*, H-12*β*; C-18 →H_3-19*α*; OCO-3 → H-3*α*, OCOMe-3 OCO-5 → H-5*β*, OCOMe-5; OCO-8 → H-2′, H-6′, H-8*α*; OCO-10^i → 10−OCOMe-10^g; OCO-14 → H-14*α*, OCOMe-14; OCO-15^i → OCOMe-15^{g,h}; OCO-17 → H-17, OCOMe-17^h (^{g,h,i} interchangeable signals); HRESIMS *m*/*z* 793.2686 [M + Na]$^+$ (calcd for $C_{39}H_{46}O_{16}Na$, 793.2684).

Vertebrate Animals. For the NPC isolation from the subventricular zone (SVZ) 7-day-old mice were used. Animals were housed under controlled conditions (21−23 °C temperature and a light:dark cycle of 12:12), with water and food (AO4 standard maintenance diet; SAFE, Epinary-sur-Orge, France) ad libitum. Care and handling of animals were performed according to the Guidelines of the European Union Council (2010/63/EU) and Spanish Regulations 65/2012 and RD53/2013 for the use of laboratory

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J. Nat. Prod. 2019, 82, 2517−2528

animals. All protocols that involved animals were approved by the "Dirección General de la Producción Agrícola y Ganadera" of the Andalusian "Conserjería de Agricultura Pesca y Desarrollo Rural", protocol number 30/03/2016/038i.

SVZ NPC Isolation and Culture. NPCs were obtained from the SVZ of 7-day-old postnatal mice following the same procedure described by Rabaneda et al.[32] Six animals were used for each independent culture. Cultures of neurospheres were maintained in defined medium (DM) composed of Dulbencco's modified Eagle's medium/F12 medium (1:1 vol/vol) with 1 mg/L gentamicin (Gibco) and B27 supplement (Invitrogen, Carlsbad, CA, USA). EGF (20 ng/mL, Gibco) and bFGF (10 ng/mL, Peprotech, Frankfurt, Germany) were added on cultures to benefit the culture expansion.

Neurosphere Assays. To test the effects of compounds **9** and **10** on NPC proliferation, neurospheres were disaggregated mechanically to single cells and plated onto anti-adherent 96-well plates at a density of 20 000 cells/mL in DM supplemented with EGF (20 ng/mL) and bFGF (10 ng/mL), or only EGF, or only bFGF. Compounds **9** and **10** were added at the same time of seeding, and cells were cultured for 72 h. Triplicates of each condition were performed, and the experiments were done three independent times. Neurosphere number and size were measured as previously described by Rabaneda et al.[32] using ImageJ software.

Statistical Analysis for in Vitro Experiments. Statistical analysis was performed using one-way ANOVA for more than two samples followed by a Bonferroni post-test. The Student's t test was used when only one treatment group was compared with the control. Differences were considered significant at values of $p < 0.05$.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jnatprod.9b00343.

1D and 2D NMR spectra of compounds **1**−**7**; ^{1}H and ^{13}C NMR spectra for compounds **8**−**10**; ECD spectra for compounds **2** and **7** (PDF)

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Notes

The authors declare no competing financial interest.

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