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Biogenetical Related Highly Oxygenated Macrocyclic Diterpenes from Sea Spurge *Euphorbia paralias*

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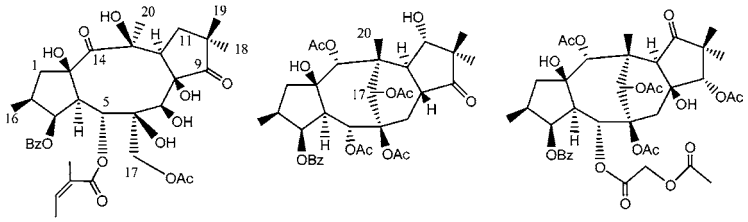
ABSTRACT



pre-segetanin (1) **segetanin A (2)** **segetanin B (3)**

A new tricyclic diterpenoid, named pre-segetanin (1), and two new diterpenes, named segetanin A and B (2 and 3), the latter based on the rare segetane skeleton, have been identified from the whole plant of sea spurge along with four known segetane diterpenes (4–7). Among them, pre-segetanin (1) has an unprecedented carbon skeleton, whose isolation provides a first insight into the biosynthesis of diterpenoids with a segetane skeleton. The stereostructure elucidation of the isolated metabolites was determined by extensive spectroscopic analysis, including 1D and 2D NMR (COSY, TOCSY, HSQC, HMBC, and ROESY) and HRFABMS experiments.

Macrocyclic diterpenes are highly oxygenated diterpenes, which were isolated as the major components of *Euphorbia* plants. Their analysis has attracted increasing attention because of their unique backbone with unusual conformation parameters and their biological properties structure. Investigations on *Euphorbiae* led to the discovery of several new classes of macrocyclic diterpene metabolites based on jatrophane, lathyrane, terracinolide, ingenane, pepluane, paraliane, and segetane skeletons,[1] many of them showing interesting pharmacological properties. In particular, over the past 6 years more than 50 new diterpene compounds were discovered, many of them exhibiting an extremely powerful multidrug resistance (MDR) reversing effect, through inhibition of the drug efflux activity of P-glycoprotein (Pgp),[2−6] and others acting as potential leads to reduce diseases with inflammations.[7,8] The unusual ring assembly of these classes of diterpenes as well as their high oxygenation pattern have brought also a great interest and challenges for developing total synthesis.[9−11] The segetane diterpenoids are the main constituents of *Euphorbia segetalis*,[12] a species that gave the

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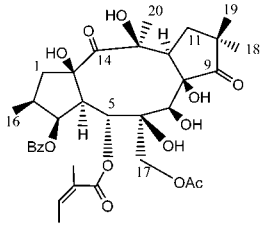
name to the entire skeletal class, and are also isolated from *E. portlandica*[13] and *E. paralias* collected in Turkey,[14] Spain,[15] and Egypt.[16]

Segetane compounds are a unique family of diterpenoids because they are characterized by a modified jatrophane skeleton containing a bicyclo [4.3.1] undecane ring system with up to nine chiral centers.

As part of our ongoing research on the chemistry of *Euphorbia* species[2−8,17] aimed at the isolation of structurally interesting and bioactive diterpenoids, we have analyzed Mediterranean samples of the sea spurge, *Euphorbia paralias.* This, first named *Tithimalo paralio* by Dioscorides,[18] is one of the oldest known species of the large genus *Euphorbia*, and it is also mentioned by Theophrastus in his Enquiry into Plants. According to Pliny, the plant was used to treat illnesses with inflammation, as a purgative, and as a remedy against cancer.[19,20] Examination of its EtOAc extract afforded the new pre-segetanin (**1**) and segetanin A and B (**2** and **3**), along with four known segetane diterpenes (**4**−**7**).[12,15]



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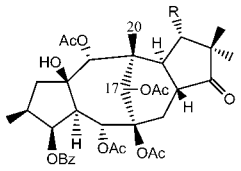
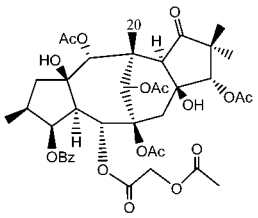
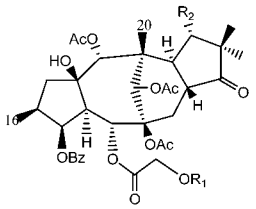


2 R = H

4 R = OAc



3



5 R_1 = Ac, R_2 = OAc

6 R_1 = Ac, R_2 = H

7 R_1 = R_2 = H

Segetanin A and B, as well as the known diterpenes **4**−**7**, are based on the rare segetane skeleton. The remaining compound, pre-segetanin (**1**), is based on an unprecedented backbone, whose isolation showed to be interesting in that it provided a first insight into the biosynthesis of diterpenoids with a segetane skeleton. Here, we describe the structure elucidation and biological evaluation of the isolated diterpenes and propose a biosynthetic route for their formation.

Compound **1**, named pre-segetanin, gave a pseudomolecular ion peak at 661.2858 $[M + H]^+$ in the positive ion HRFABMS, which together with the data obtained by the ^{13}C NMR spectrum suggested a molecular formula of $C_{34}H_{44}O_{13}$. MS data and ^1H (Table 1) and ^{13}C (Table 2) NMR resonances of **1** suggested its polyesterified diterpene nature.

Table 1. ^1H NMR Data ($CDCl_3$) of Compounds **1**−**3**[a]

H	1	2	3
1α	2.54 dd	2.39 dd	2.35 dd
1β	1.79 dd	1.55 dd	1.60 dd[b]
2	2.47 m	2.09 m	2.17 m
3	5.58 dd	5.80 dd	5.74 dd
4	2.99 dd	3.38 dd	3.63 dd
5	5.46 d	5.53 d	5.37 d
7α	3.25 d	1.72 dd	2.49 d
7β		2.22 brd	2.94 d
8		3.68 ddd	
9			6.10 s
11α	2.01 dd	1.82 m[b]	
11β	2.32 dd	2.01 dd[b]	
12	3.08 dd	1.85 m[b]	3.49 s
13			
14		5.22 s	5.38 s
16	1.01 d	0.94 d	0.92 d
17a	3.24 d	6.45 brs	6.51 s
17b	5.24 d		
18	1.10 s	1.08 s	1.01 s
19	1.28 s	1.16 s	1.21 s
20	1.61 s	1.03 s	1.10 s
6-OH	2.04 s		
7- OH	3.49 d		
8-OH	5.14 s		5.02 s
13-OH	4.48 s		
15-OH	3.04 s	2.50 s	2.53 s
5-OAc		1.95 s	
6-OAc		2.06 s	2.09 s
9-OAc			2.11 s
14-OAc		2.19 s	2.23 s
17-OAc	1.80 s	1.94 s	2.04 s
5-OR			4.50 d
			4.43 d
			2.04 s
OAng 3	5.99 brq		
4	1.87 d		
5	1.84 s		
OBz 2,6	8.03 d	7.82 d	7.73 d
3,5	7.50 t	7.47 t	7.41 t
4	7.61 t	7.59 t	7.56 d

[a] J_{H-H} (values in Hz). Compd **1**: 1α−1β = 1β−2 = 13.0; 1α−2 = 8.0; 2−3 = 3−4 = 7−7OH = 3.5; 2−16 = 7.0; 4−5 = 10.5; 11α−11β = 9.0; 11α−12 = 2.0; 11β−12 = 11.0; 17α−17β = 11.5. Compd **2**: 1α−1β = 15.0; 1α−2 = 9.0; 1β−2 = 4−5 = 11.5; 2−3 = 3−4 = 3.5; 2−16 = 7.0; 7α−7β = 7α−8 = 12.5; 7β−8 = 4.0; 8−12 = 16.0; 11α−11β = 11β−12 = 12.0. Compd **3**: 1α−1β = 13.0; 1α−2 = 9.5; 1β−2 = 11.5; 2−3 = 3−4 = 3.5; 2−16 = 7.0; 4−5 = 11−12 = 10.5; 7a−7b = 17. **Ang**: 3−4 = 7.0. **Bz**: 2−3 = 3−4 = 7.4. [b] Overlapped by other signals.

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Table 2. ^{13}C NMR Data ($CDCl_3$) of Compounds **1**−**3**[a]

H	1	2	3
1	53.79 t	50.02 t	47.61 t
2	38.93 d	37.29 d	36.61 d
3	77.96 d	81.03 d	80.85 d
4	61.63 d	47.70 d	47.60 d
5	63.90 d	67.50 d	68.98 d
6	87.93 s	83.90 s	82.96 s
7	63.90 d	31.96 t	29.29 t
8	76.17 s	46.60 d	75.45 s
9	212.66 s	219.30 s	79.45 d
10	44.11 s	45.45 s	48.03 s
11	34.53 t	36.29 t	208.23 s
12	48.84 d	42.84 d	50.75 d
13	82.09 s	45.99 s	47.45 s
14	220.00 s	75.91 d	75.50 d
15	83.90 s	82.34 s	69.71 s
16	13.66 q	14.35 q	14.42 q
17	65.22 t	70.57 d	69.57 d
18	26.26 q	24.91 q	18.96 q
19	27.21 q	26.74 q	23.29 q
20	23.62 q	25.82 q	21.82 q

[a] Compd **1**: 17-OAc, 173.88 s, 20.55 q; OAng, 172.71 s, 128.40 s (C-2), 137.86 d (C-3), 15.64 q (C-4), 20.47 q (C-5); OBz, 165.30 s, 129.72 s (C-1), 129.66 d (C-2, C-6), 128.42 d (C-3, C-5), 133.10 d (C-4). Compd **2**: 5-OAc, 169.60 s, 21.09 q; 6-OAc, 169.32 s, 20.71 q, 14-OAc, 170.44 s, 21.13 q; 17-OAc, 169.48 s, 20.66 q; OBz, 165.86 s, 129.20 s (C-1), 129.16 d (C-2,C-6), 128.79 d (C-3, C-5), 133.36 d (C-4). Compd **3**: 6-OAc, 169.91 s, 20.50 q; 9-OAc, 170.49 s, 20.80 q; 14-OAc, 170.14 s, 20.72 q; 17-OAc, 171.20 s, 20.94 q; 5-OR, 167.09 s, 60.19 q, 170.14 s, 20.14 q; OBz, 165.81 s, 129.46 s (C-1), 128.96 d (C-2,C-6), 128.52 d (C-3, C-5), 133.35 d (C-4).

In particular, the presence of one acetate, one angelate, and one benzoate was easily deduced from the typical signals in the ^1H and ^{13}C NMR spectra (Tables 1 and 2). The structural elements recognized among the remaining 20 ^{13}C NMR signals (Table 2) were four methyls (one secondary and three tertiary by ^1H NMR), two keto groups, and eight oxygenated sp^3 carbons (one primary, three secondary, and four tertiary). Three methines, two methylenes, and one quaternary carbon accounted for the remaining signals (Table 2). Data arising from a combined analysis of 2D COSY and HSQC spectra of **1** allowed us to sequence the multiplets of the core diterpene structure into two spin systems (C-1 to C-5 including C-16, C-11 to C-12), to detect two isolated protonated carbons (C-7 and C-17, a methine and a methylene, respectively) and to correlate the directly linked proton and carbon atoms, respectively, providing useful clues to identify the diterpenoid framework. However, to this aim, conclusive information came from the series of $^{2,3}J_{C-H}$ correlations in a 2D HMBC spectrum (Figure 1) that, allowing the connection of the above substructures, pointed to an unprecedented tricyclic skeleton for pre-segetanin. The above 2D NMR analysis of **1** showed also the location of oxygenated carbons at positions 3, 5, 6, 7, 8, 9 (ketone), 13, 14 (ketone), and 15 of this skeleton. Five of these oxygen atoms are present as free hydroxyl groups. Four were located at the unprotonated C-6, C-8, C-13, and C-15 by the observation of HMBC cross-peaks of the exchangeable protons with related carbons (Figure 1). The location of the

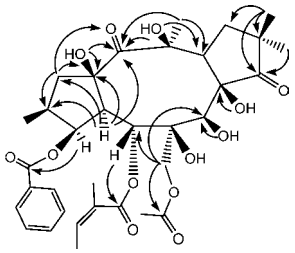

Figure 1. Selected HMBC correlations exhibited by **1**.

last hydroxyl group (exchangeable proton doublet) at C-7 came from an analogous HMBC cross-peak between OH-7 (δ 3.49) and C-7 (δ 63.90) and from the vicinal coupling (J = 3.5 Hz) of OH-7 with H-7 (δ 3.25). Analogously, the 2D HMBC spectrum was used to infer the correct location of the three acyl groups, establishing the linkage of the benzoate, the angelate, and the acetate at C-3, C-5, and C-17, respectively.

The relative configuration of compound **1**, as depicted in the formula, was drawn by measurement of coupling constants (Table 1), interpretation of ROESY cross-peaks (Figure 2), and comparison with literature data.[12−15] The

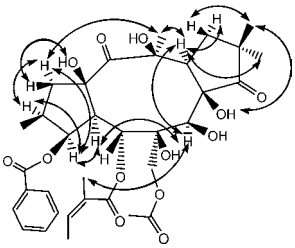

Figure 2. Selected ROESY correlations exhibited by **1**.

relative configuration was therefore identical to that of all the members of the segetane family isolated to date. This configuration can be confidently upgraded to the absolute one, taking into account data coming from the X-ray analysis performed for some of these compounds.

Pre-segetanin represents a diastereoisomer with opposite configuration at C-8 and C-15 of a diterpene compound previously isolated from *E. segetalis*[15] as inner acetal. In our case, epimerization at C-8 does not provide the stereostructural elements for the acetal formation.

Compound **2,** named segetanin A, showed a pseudomolecular ion peak at *m/z* 657.2909 $[M + H]^+$ corresponding to the molecular formula of $C_{35}H_{44}O_{12}$ by positive HR-FABMS. Both ^1H and ^{13}C NMR spectra of **2** (Tables 1 and 2) closely resembled those recorded for compound **4**. Therefore, the structural elucidation of **2**, based on inspection of 2D NMR spectra (COSY, HSQC, and HMBC), was guided by comparison with parallel spectral data of **4**. In particular, in agreement with the molecular formula, it was

clear that the skeleton of **2** differed from **4** only by the lack of C/H resonances attributable to one acetate group. This was easily identified as the acetate at C-11 by the substitution of the oxymethine proton at C-11 (δ 5.59) in **4** with upfield-shifted methylene proton signals (δ 1.82 and δ 2.01) in **2**.

As for pre-segetanin, stereochemical details of segetanin A were investigated through ROESY correlations. Diagnostic interactions were detected for H-3/H-1α, H-3/H-2, H-16/H-1β, H-1α/H-4, H-18/H-12, and H-5/15-OH, in accordance with the same relative configuration reported for compound **4**.

Compound **3**, named segetanin B, showed a pseudomolecular ion peak at *m*/*z* 773.3018 $[M + H]^+$, corresponding to the molecular formula $C_{39}H_{48}O_{16}$ (calcd for $C_{39}H_{49}O_{16}$ 773.3021) in the HR-FABMS. ^{1}H and ^{13}C NMR spectra of **3** (Tables 1 and 2), analyzed through inspection of 2D NMR experiments (COSY, HSQC, and HMBC), revealed the segetane nature of compound **3**. In particular, one benzoate and four acetate ester groups were found to be attached to the diterpenoid core of **3**, and their location has been argued, as for **1** and **2**, through the series of HMBC and ROESY cross-peaks reported in Figure 3.

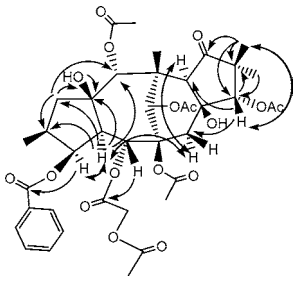

Figure 3. Selected HMBC (H→C) and ROESY (H↔H) correlations exhibited by **3**.

However, the molecular formula of compound **3** contains one additional oxygen atom compared to that of compound **5**. Deeper investigation of NMR features showed that the most striking differences between the spectra of **3** and **5** were concentrated in the C ring signals. In particular, the characteristic signals in the ^{1}H NMR spectrum of compound **5** at δ 3.75 (ddd, H-8) and δ 5.57 (d, H-11) were absent in the NMR spectra of **3** and replaced by a hydroxyl (^{1}H: s, δ 5.02) and a keto group (^{13}C: s, δ 208.23). Their location at C-8 and C-11, respectively, has been secured by the HMBC cross-peaks (Figure 3).

Further comparison of the ^{1}H NMR spectra of **3** and **5** evidenced the presence in **3** of an additional oxymethine proton signal. It has been located at C-9 by HMBC correlations of its carbon with H_3-18, H_3-19, H-12, and H_2-7 and by the correlations of H-9 with C-10, C-18, C-19, C-12, and C-7. Taken together, these results pointed to the assignment of compound **3** as depicted in formula. The relative configurations of the chiral centers of **3** through ROESY and coupling constant analysis have been found to be identical to those described for the other segetanes. In particular, the ROESY cross-peak of H_3-19/OH-8 indicated the β orientation of the OH group at C-8, whereas the ROESY cross-peak of H-18/H-12 indicated an α configuration for both.

From the biogenetic point of view, compounds **1**−**3** seem to be closely related. In fact, as reported in Scheme 1, the unprecedented tricyclic skeleton found for pre-segetanin could be considered a possible intermediate in the biogenesis of the segetane skeleton that originates, as supposed by Jakupovic,[15] by cyclization steps of an appropriate jatrophane compound. Subsequent oxidation and esterification steps on both skeletons give rise to the isolated polyester derivatives **1**−**7** (Scheme 1).

Finally, the cytotoxicity and the anti-MDR activity of compounds **1**−**7** were tested on human ovarian cancer cells A2780. In a range of concentrations between 0.1 and 10 000 nM, none of the tested compounds showed significant activity as compared to controls (data not shown).

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Supporting Information Available: Detailed description of the experimental procedures, IR and MS data of **1**−**3**, Scheme 1, and 1D and 2D NMR spectra of **1**−**3**. This material is available free of charge via the Internet at http://pubs.acs.org.

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