Notes

Euphoportlandols A and B, Tetracylic Diterpene Polyesters from *Euphorbia portlandica* and Their Anti-MDR Effects in Cancer Cells

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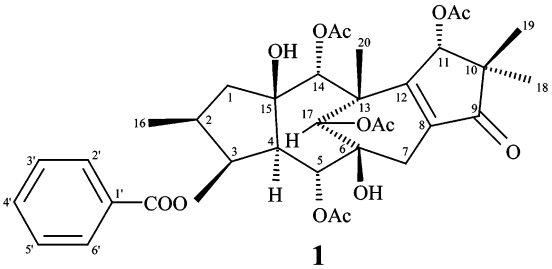
Two new tetracyclic diterpene polyesters, euphoportlandols A (**1**) and B (**2**), have been isolated along with 12 known tetracyclic triterpenes from an acetone extract of *Euphorbia portlandica*. Their structures were established as 5α,11α,-14α,17-tetraacetoxy-3*β*-benzoyloxy-6*β*,15*β*-dihydroxy-9-oxoseget-8(12)-ene (**1**) and 5α,11α,14α,17-tetraacetoxy-3*β*-benzoyloxy-6*β*,15*β*-dihydroxy-9-oxosegetane (**2**), respectively, by spectroscopic data interpretation. Compounds **1** and **2** were evaluated for their ability to inhibit multidrug resistance in cancer cells. Both compounds were found to be inhibitors of P-glycoprotein activity.

The genus *Euphorbia*, with over 2000 species, is widespread and has been used since ancient times to treat warts and tumors.[1] The discovery of macrocyclic jatrophane diterpenes characteristic of *Euphorbia* species as a new class of potent inhibitors of P-glycoprotein (P-gp) has led to an increasing interest in research of this genus.[2] P-gp is a transmembrane glycoprotein overexpressed in most multidrug-resistant cancer cells and is responsible for insensitivity to a host of chemotherapeutic agents unrelated in terms of structure, mechanism of action, and metabolism. P-gp seems to reduce the intracellular concentration of hydrophobic antitumor drugs from target cells by accelerating their efflux by an ATP-dependent process and is thereby associated with treatment failure in cancer.[3,4]

Previously, we have described the isolation and structure characterization of rearranged jatrophane derivatives from *Euphorbia portlandica* L. (Euphorbiaceae), which proved to be inhibitors of P-gp.[5] Continuing our evaluation of jatrophane diterpenes as effective multidrug resistance (MDR) inhibitors, and the elucidation of their structure−activity relationships,[6−8] the present study reports the isolation and characterization from *E. portlandica* of two new jatrophane diterpenoids (**1** and **2**) belonging to the segetane group,[9] as well as the evaluation of their ability as MDR modulators. The isolation of 12 known tetracyclic triterpenes is also reported.

Compound **1**, euphoportlandol A, was isolated as a white amorphous powder whose LDI-FTICR-HRMS exhibited a pseudomolecular ion at *m*/*z* 693.2511 $[M + Na]^+$, in agreement with a molecular formula of $C_{35}H_{42}O_{13}$. Its IR spectrum displayed absorption bands for hydroxyl (3478 cm^{-1}), ester (1744 cm^{-1}), and ketone (1715 cm^{-1}) groups, as well as for an aromatic ring (1640, 1601, 713 cm^{-1}). The ^1H NMR spectrum of **1** showed characteristic



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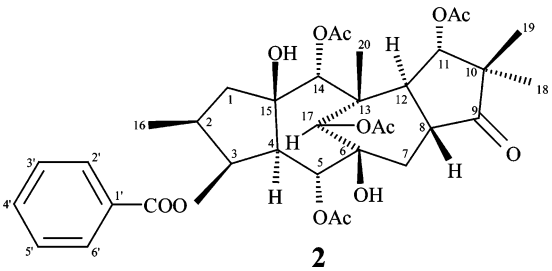

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signals for four acetyls and one benzoyl group (Table 1) and a two-proton broad singlet at δ 2.60 without any correlations in the HMQC spectrum, which suggested the presence of two hydroxyl groups in the molecule. Additionally, its NMR and DEPT spectra revealed the presence of three tertiary methyl groups (δ 1.00, 1.07, and 1.21) and one secondary methyl group (δ 0.92, d, $J = 6.8$ Hz), five oxymethines geminal to ester functions (δ 5.26, 5.35, 5.70, 5.75, 6.07; δ_C 69.5, 73.7, 75.6, 79.5, 80.6), two methylene carbons, and seven quaternary carbons (one keto group at δ_C 208.6, two carbons bearing oxygen at δ_C 83.2 and 73.8, and two olefinic carbons at δ_C 138.2 and 164.0). The marked downfield olefinic carbon resonance at δ_C 164.0 together with the carbonyl carbon suggested the presence of an enone system in the molecule, which was confirmed by long-range correlations observed in the HMBC spectrum between the olefinic carbons and H-11 and between C-8 and H-7α,*β* (see Supporting Information). The above data indicated the molecular formula $C_{20}H_{30}O_8$ for the parent polyol structure of

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Table 1. NMR Data of Compounds **1** and **2** (400 MHz, $CDCl_3$)

	euphoportlandol A (**1**)		euphoportlandol B (**2**)	
position	δ_C, mult.	δ_H (*J* in Hz)	δ_C, mult.	δ_H (*J* in Hz)
1α	47.8, CH_2	2.33 dd (10.4; 15.2)	50.2, CH_2	2.38 dd (4.0; 15.0)
1*β*		1.53 dd (10.4; 15.2)		1.65 m[b]
2	37.0, CH	2.15 m	37.1, CH	2.08 m
3	80.6, CH	5.70 m	80.7, CH	5.78 t (3.4)
4	47.0 CH	2.83 dd (3.4; 11.4)	47.7, CH	2.80 dd (3.4; 12.0)
5	69.5, CH	5.26 d (11.4)	69.5, CH	5.21 d (12.0)
6	73.8, C		74.6, C	
7α	30.3, CH_2	2.39 dd (1.8; 16.9)	32.1, CH_2	1.65 m[b]
7*β*		2.60 br s (16.9)		2.24 dd (3.4; 12.9)
8	138.2, C		45.0, CH	3.89 td (3.6; 14.0)
9	208.6, C		215.2, C	
10	48.1, C		49.6, C	
11	79.5, CH	6.07 br s	77.4, CH	5.56 d (11.2)
12	164.0, C		46.4, CH	2.09 m
13	47.3, C		45.6, C	
14	75.6, CH	5.35 s	75.4, CH	5.28 s
15	83.2, C		82.2, C	
16	14.5, CH_3	0.92 d (6.8)	14.2, CH_3	0.96 d (6.3)
17	73.7, CH	5.75 s	74.0, CH	5.42 s
18	19.2, CH_3	1.00 s	18.4, CH_3	0.95 s
19	23.4, CH_3	1.21 s	25.2, CH_3	1.20 s
20	21.8, CH_3	1.07 s	26.9, CH_3	1.05 s
OH-6[a]		2.60 br s		2.80 br s
OH-15[a]		2.60 br s		2.80 br s
OBz-3				
C=O	165.7, C		165.8, C	
1′	129.4, CH		129.4, CH	
2′, 6′	129.1, CH	7.74 d (7.2)	129.2, CH	7.86 d (7.2)
3′, 5′	128.7, CH	7.41 t (7.8)	128.8, CH	7.46 t (7.7)
4′	133.5, CH	7.56 t (7.5)	133.4, CH	7.59 t (7.4)
OAc				
	169.2, C	1.95 s	169.5, C	2.02 s
	169.8, C	2.10 s	169.8, C	2.09 s
	170.4, C	2.10 s	170.5, C	2.12 s
	171.0, C	2.20 s	171.2, C	2.13 s
	20.5, CH_3		20.5, CH_3	
	3 × 20.8, CH_3		3 × 20.9, CH_3	

[a,b] Signals interchangeable.

1, with six degrees of unsaturation, corresponding to a tetracyclic diterpenoid skeleton. Analysis of the HMQC and $^1H-^1H$ COSY spectra provided evidence for the sequences $-CH_2CH(CH_3)CH(OR)CHCH(OR)-$ and $-CH_2C(R)=C(R)CH(OR)-$. The ^2J and ^3J HMBC correlations allowed the assignment of C-17 as a substituted methane bridge between C-6 and C-13 and the identification of the remaining quaternary carbons bridging the structural fragments of **1**, as well as the placement of functional groups.

The relative configuration of **1** was deduced from the NOESY NMR spectrum, assuming an α-orientation for the angular H-4, characteristic of all jatrophane derivatives isolated to date.[10,11] NOE effects were observed between H-4α/H-17 (very strong), H-4α/H-2, and H-4α/H-3, supporting an α-orientation for these protons. Moreover, the *ortho*-protons of the aromatic ring showed NOE correlations with H-5, Me-16, and one of the two overlapped hydroxyl protons at *δ* 2.60. Furthermore, from the NOE correlations between H-14/H-11 (very strong), H-14/Me-20, Me-20/Me-19, H-11/Me-19, H-11/H-7*β*, and H-7*β*/H-5 (very strong), the *β*-orientation of these protons was concluded. Strong NOE enhancements were also detected between H-5 and the signal at *δ* 2.60 (OH-15, OH-6), but no effects were observed for H-4α and other α-oriented protons, which confirmed the *trans*-linked cyclopentane ring and the *β*-configuration at C-6. The calculated conformation of **1**, generated with the Gaussian 03 program[12] (Figure 1), agreed well with the spectroscopic results. Accordingly, from the above-mentionated data, a modified jatrophane skeleton containing a bicyclo[4.3.1]undecane ring system could be proposed for **1**. A similar structure, segetene A, has been previously isolated from *E. paralias*,[13] which differs from **1** in the substitution at C-6, where the hydroxyl is replaced by an acetyl group, and in the ester residue at C-5 and in the configuration of the stereocenters at C-6, C-13, and C-14.

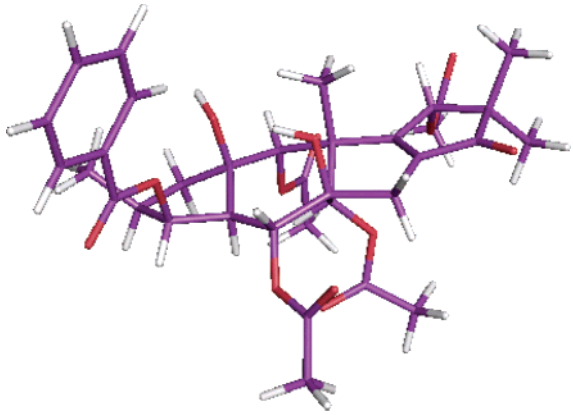

Figure 1. Calculated conformation of compound **1**.

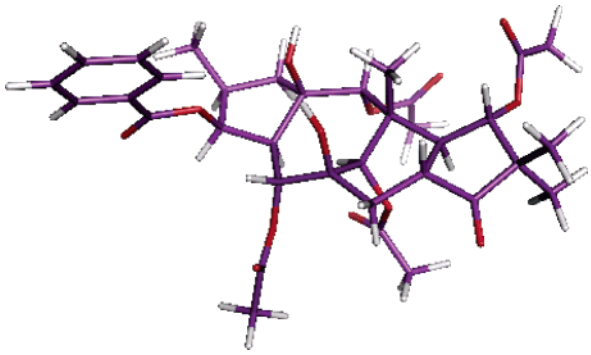

Figure 2. Calculated conformation of compound **2**.

The molecular formula of euphoportlandol B (**2**), $C_{35}H_{44}O_{13}$, was deduced from its LDI-FTICR-HRMS, which showed a pseudomolecular ion at *m/z* 695.2657 $[M + Na]^+$. The NMR spectra of euphoportlandol B (see Table 1) resembled those recorded for **1**, but its ^{13}CNMR spectrum lacked the two carbon resonances corresponding to the tetrasubstituted double bond. Instead, two high-field methine carbons at δ_C 45.0 and 46.4 were observed, bearing protons resonating at *δ* 3.89 (td, *J* = 3.6, 14.0 Hz) and 2.09 (m), respectively. Due to the absence of the enone system, the ketone resonance in compound **2** was shifted to a low field (δ_C 215.2). The relative configuration of **2** was also assessed from NOESY NMR experiments. The coupling constant of $^3J_{8,12}$ (14.0 Hz) indicated the antiperiplanar orientation of H-8 and H-12, which was confirmed by the NOE effects observed between H-8/H-5, H-8/H-11, and H-12/Me-18. The absence of any interaction between H-8/H-12 corroborated this conclusion. The stereochemistry of the remaining tetrahedral stereocenters of **2** was found to be identical to that of **1** (see Supporting Information). The calculated conformation of **2** is depicted in Figure 2.

To the best of our knowledge, compounds with a segetane skeleton have been isolated previously only from *Euphorbia paralias*[13−15] and *E. segetalis*.[9] These two species, as well as *E. portlandica*, belong to the same subgeneric taxonomic section, which reinforces the significance of these modified jatrophane diterpene derivatives in the chemotaxonomy of the genus *Euphorbia*.[5,16]

The triterpenes cycloart-23-ene-3*β*,25-diol, cycloartane-3*β*,26-diol, cycloartane-3*β*,24,25-triol, 24-hydroperoxycycloart-25-en-3*β*-ol, cycloart-25-ene-3*β*,24-diol, 24-methylenecycloartanol, 3*β*-hydroxycycloart-25-en-24-one, cycloart-25-ene-3*β*,24-diacetate, 27-*nor*-3*β*-hydroxy-25-oxocycloartane, (22*E*)-25,26,27-*trisnor*-3*β*-hydroxycycloart-22-en-24-al, 25,26,27-*trisnor*-3*β*-hydroxycycloartan-24-al, and (24*E*)-3*β*-hydroxycycloart-24-en-26-al were identified

Table 2. Effect of Compounds **1** and **2** on Reversal of Multidrug Resistance (MDR) in Human MDR1 Gene Transfected Mouse Lymphoma Cells

compound	concentration (μg/mL)	FL-1[a]	fluorescence activity ratio[a]
PAR[b]		914.8	
MDR[c]		21.8	
verapamil	10	212.5	9.7
1	4	49.8	2.3
	40	880.2	40.3
2	4	30.8	1.4
	40	670.5	30.7
DMSO		6.8	0.67

[a] FL-1: mean fluorescence intensity of the cells. Fluorescence activity ratio values were calculated by using the equation given in the Experimental Section. [b] PAR control: a parental cell without MDR gene. [c] MDR: a parental cell line transfected with human MDR1gene.

by comparison of their spectroscopic data with those reported in the literature.[17−27] Such *nor*-derivatives have been rarely isolated, and this is the first reported occurrence of 27-*nor*-3β-hydroxy-25-oxocycloartane and (22*E*)-25,26,27-*trisnor*-3β-hydroxycycloart-22-en-24-al in a *Euphorbia* species. All these compounds, except 27-*nor*-3β-hydroxy-25-oxocycloartane, have been evaluated for their effects as apoptosis inducers and as P-gp inhibitors in cancer cells.[28]

The evaluation of the inhibition of P-glycoprotein-mediated drug efflux from L5178 Y mouse T-lymphoma parental cells, by compounds **1** and **2**, was performed by the determination of the intracellular accumulation of rhodamine 123, using a standard assay. As can be observed in Table 2, at the highest concentration both compounds were found to be inhibitors of P-glycoprotein activity [fluorescence activity ratios $R = 40.3$ (**1**) and $R = 30.7$ (**2**) at 40 μg/mL]. Compound **2** exhibited no significant activity at 4 μg/mL (fluorescence activity ratio $R = 1.4$), suggesting that the enone system might be important for the anti-MDR activity within this compound class. Comparison of the present results with previous data[5,6,8] led to the conclusion that macrocyclic diterpenes are more active than their polycyclic rearranged derivatives. Therefore, the macrocycle and its substitution pattern seem to play a significant role in the modulation of MDR by these diterpenoids.

Experimental Section

General Experimental Procedures. Optical rotations were obtained using a Perkin-Elmer 241-MC polarimeter. IR spectra were determined on a Perkin-Elmer 1310 instrument. The NMR spectra were recorded on a Bruker ARX-400 NMR spectrometer (^{1}H 400 MHz; ^{13}C 100.61 MHz) or a Varian Unity-300 NMR spectrometer (^{1}H 300 MHz; ^{13}C 75.4 MHz), with TMS as internal standard and $CDCl_3$ as solvent. EIMS were taken at 70 eV on a Kratos MS25RF spectrometer, and the LDI-FTICR-HRMS on a Finnigan-FT-2001. Column chromatography was carried out on silica gel (Merck 9385). TLC was performed on precoated silica gel F_{254} plates (Merck 5554 and 5744) and visualized under UV light and by spraying with sulfuric acid−acetic acid−water (1:20:4) followed by heating. HPLC was carried out on a Merck-Hitachi instrument with UV detection, using a Merck Lichrospher 100 RP-18 (10 μm, 250 × 10 mm) column.

Plant Material. *E. portlandica* was collected in September 2001 near Leiria, Portugal, and identified by Dr. Teresa Vasconcelos (plant taxonomist) of the Instituto Superior de Agronomia, University of Lisbon. A voucher specimen (No. 248) has been deposited at the herbarium (LISI) of Instituto Superior de Agronomia.

Extraction and Isolation. The air-dried whole plant (4.8 kg) was powdered and extracted exhaustively with acetone (7 × 8 L) at room temperature. This extract was filtered and concentrated under a vacuum, and the resulting residue (367 g) was suspended in a MeOH−H_2O solution and partitioned sequentially with hexane and Et_2O. The ether extract (114 g) was chromatographed on silica gel (1.5 kg), with mixtures of hexane−EtOAc and EtOAc−MeOH (1:0 to 0:1) as eluents, to give fractions A (4.5 g; *n*-hexane−EtOAc, 7:3); B (10.0 g; *n*-hexane−EtOAc, 7:3), and C (40.0 g; *n*-hexane−EtOAc, 7:3 to 13:7). Fraction A furnished 25,26,27-*trisnor*-3β-hydroxycycloartan-24-al (14 mg) and 24-methylenecycloartanol (4 mg), and from fraction B 25,26,27-*trisnor*-3β-hydroxycycloartan-24-al (10 mg), 24-hydroperoxycycloart-25-en-3β-ol (5 mg), cycloart-25-ene-3β,24-diol (5 mg), 3β-hydroxycycloart-25-en-24-one (6 mg), and cycloart-25-ene-3β,24-diacetate (13 mg) were obtained. Fraction C was chromatographed on silica gel (700 g) using *n*-hexane−EtOAc (1:0 to 0:1), yielding five fractions (C_1−C_5). Fraction C_1 afforded the compounds (22*E*)-25,26,27-*trisnor*-3β-hydroxycycloart-22-en-24-al (5 mg), 27-*nor*-3β-hydroxy-25-oxocycloartane (5 mg), and (24*E*)-3β-hydroxycycloart-24-en-26-al (3 mg). Cycloart-23-ene-3β,25-diol (150 mg), cycloartane-3β,26-diol (8 mg), and cycloartane-3β,24,-25-triol (85 mg) were obtained from fractions C_2, C_3, and C_4, respectively (see the Supporting Information for details of the isolation and identification of these known compounds).

Fraction C_5 (2.1 g; *n*-hexane−EtOAc, 7:13 to 2:8) was chromatographed repeatedly on silica gel columns with CH_2Cl_2−EtOAc mixtures. Fractions eluted with CH_2Cl_2−EtOAc (4:1 to 7:3) were pooled (150 mg) and submitted to preparative TLC (CH_2Cl_2−acetone, 9:1), yielding C_{5a} (20 mg, R_f 0.45) and C_{5b} (40 mg, R_f 0.5). Fraction C_{5a} was further purified by successive HPLC (MeOH−H_2O, 65:35, 5 mL/min, t_R 8 min) and preparative TLC ($CHCl_3$−MeOH, 9:1, R_f 0.70), to afford 14 mg of **1**. Fraction C_{5b} was also subjected to purification by HPLC (MeOH−H_2O, 1:1, 5 mL/min, t_R 25 min) and preparative TLC ($CHCl_3$−MeOH, 19:1, R_f 0.75), to afford 12 mg of **2**.

Euphoportlandol A [5α,11α,14α,17-tetraacetoxy-3β-benzoyloxy-6β,15β-dihydroxy-9-oxoseget-8(12)-ene] (1): white amorphous powder; $[\alpha]_D^{25}$ +13 (*c* 0.07, $CHCl_3$); IR (film) ν_{max} 3478, 1744, 1715, 1640, 1601, 1372, 1275, 1233, 1030, 713 cm^{-1}; ^{1}H NMR and ^{13}C NMR, see Table 1; EIMS *m*/*z* 652 $[M - H_2O]^+$ (2) , 610 $[M - AcOH]^+$ (>1), 550 $[M - 2 \times AcOH]^+$ (<1), 428 $[M - 2 \times AcOH - C_6H_5CO_2H]^+$ (<1), 368 $[M - 3 \times AcOH - C_6H_5CO_2H]^+$ (1), 308 $[M - 4 \times AcOH - C_6H_5CO_2H]^+$ (1), 189 (5), 105 $[C_6H_5CO]^+$ (33), 43 (100); LDI-fticr-hrMS *m*/*z* 693.2511 $[M + Na]^+$ (calcd for $C_{35}H_{42}O_{13}Na$, 693.2518).

Euphoportlandol B (5α,11α,14α,17-tetraacetoxy-3β-benzoyloxy-6β,15β-dihydroxy-9-oxosegetane) (2): white amorphous powder; $[\alpha]_D^{25}$ +12 (*c* 0.10, $CHCl_3$); IR (film) ν_{max} 3474, 1741, 1454, 1372, 1273, 1237, 1026, 714 cm^{-1}; ^{1}H NMR and ^{13}C NMR, see Table 2; EIMS *m*/*z* 654 $[M - H_2O]^+$ (1), 612 $[M - AcOH]^+$ (<1), 594 $[M - AcOH - H_2O]^+$ (3), 430 $[M - 2 \times AcOH - C_6H_5CO_2H]^+$ (3), 370 $[M - 3 \times AcOH - C_6H_5CO_2H]^+$ (2), 310 $[M - 4 \times AcOH - C_6H_5CO_2H]^+$ (4), 105 $[C_6H_5CO]^+$ (100), 43 (69); LDI-fticr-hrMS *m*/*z* 695.2657 $[M + Na]^+$ (calcd for $C_{35}H_{44}O_{13}Na$, 695.2674).

Assay for MDR-Reversal Activity. The reversion of MDR was evaluated in a rhodamine 123 exclusion experiment using L5178 mouse lymphoma cells transfected with the pHa MDR1/A retrovirus, as reported.[5−8] The fluorescence of the cell population was measured by flow cytometry with a Beckton Dickinson FACScan instrument. Verapamil was used as a positive control. The fluorescence activity ratio was calculated from the drug accumulation of treated MDR and untreated MDR cells related to parental treated per untreated drug-sensitive cells. An activity ratio (*R*) was calculated on the basis of the measured fluorescence values via the following equation.[29,30]

$$R = \frac{\text{MDR treated/MDR control}}{\text{parental treated/parental control}}$$

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Supporting Information Available: Details of the isolation and identification of the known compounds from *E. portlandica* and of the NMR experiments performed on **1** and **2**. Figure S1, showing ^{1}H NMR spin systems and selected HMBC correlations of **1**, and Figures S2 and S3, showing relevant NOE correlations observed for **1** and **2**. This information is available free of charge via the Internet at http://pubs.acs.org.

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