Triterpenes from *Euphorbia rigida*

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ABSTRACT

Phytochemical studies of the aerial parts of *Euphorbia rigida* afforded three triterpenes: betulin (1), cycloart-23Z-ene-3, 25-diol (2) and cycloartan-3, 24, 25-triol (3), firstly isolated from this plant. The structures and relative stereochemistry were determined on the basis of extensive spectroscopic analyses, including 1D and 2D NMR experiments (1H NMR, 13C NMR, COSY, NOESY, HMQC and HMBC).

Key words: *Euphorbia rigida*, Euphorbiaceae, cycloartan triterpene

INTRODUCTION

Euphorbia genus belongs to the family Euphorbiaceae. This family comprises about 300 genus and 5000 species distributed mainly in America and tropical Africa.[1] *Euphorbia* species have been used in folk medicine to treat skin diseases, migraines, intestinal parasites and warts.[2] The biological activities of the genus, including antitumor, antiviral, cytotoxic properties and different vascular effects, are generally attributed to the presence of specific types of diterpenes, both macrocyclic and polycyclic derivatives.[3-5] The skin irritant and tumor-promoting properties of tigliane, ingenane and dephanane diterpenes of this plant are well known. Considerable attention has recently been given to the macrocyclic diterpenes because of their high chemical diversity and therapeutically relevant bioactivity.[6-8] Jatrophane and modified jatrophane diterpenoids, which are rare in the genus *Euphorbia,* are potent inhibitors of a membrane protein (so-called P-glycoprotein) pumping cytotoxic drugs out of cells and conferring upon the cells the ability to resist high doses of these drugs.[9] Therefore, the genus has been subjected to numerous chemical studies and these have led to the isolation of diterpenes[10,11] dimeric diterpenoid[12] diterpene polyesters[11,13] triterpenes[14] and pentacyclic triterpenes.[15] Few sesquiterpenoids and flavonoids have been isolated from the genus.[16,17]

Spurges *Epuhorbia* species are a common constituent of many ancient treatments of mouse leukemia and diseases such as cancer, swelling and warts.[18]

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MATERIALS AND METHODS

Plant material

The aerial parts of *Euphorbia rigida* were collected from Greek in August 2004, by Dr. Olga Tzakou, Department of pharmacy and Chemistry of Natural products, Faculty of Pharmacy, University of Athens, Greece.

Extraction and isolation

The air-dried plant (1 kg) was crushed and extracted with CH_2Cl_2–MeOH (1:1) at room temperature. The extract was concentrated *in vacuo* to obtain a residue (30 g). The residue was fractionated by silica gel CC (6 × 120 cm) eluted with *n*-hexane (3 l), followed by a gradient of *n*-hexane–CH_2Cl_2 up to 100% CH_2Cl_2 and CH_2Cl_2–MeOH up to 15% MeOH (2 l of each solvent mixture) with increasing degree of polarity. The *n*-hexane–CH_2Cl_2 (1:1) was pre-fractionated by CC using Sephadex LH-20 (2 × 40 cm) and eluted with *n*-hexane–CH_2Cl_2 (7:4) to give compound **1** (80 mg). Compound **2** (60 mg) was isolated from *n*-hexane–CH_2Cl_2 (2:3) fraction and the latex was pre-fractionated by CC on Sephadex LH-20 (1 × 30 cm) and eluted with *n*-hexane–

CH_2Cl_2–MeOH (7:4:0.25). Compound **3** (30 mg) isolated from 100% CH_2Cl_2 fraction, was pre-fractionated by CC on Sephadex LH-20 (1 × 30 cm) and eluted with *n*-hexane–CH_2Cl_2–MeOH (7:4:0.5).

RESULTS AND DISCUSSION

Repetitive chromatographic steps of the methylenechloride/methanol (1:1) extract of the air-dried aerial parts of *E. rigida* yielded three known triterpenes [Figure 1].

Compound **1** was obtained as a white powder. The structure of **1** was determined from careful investigation of the 1D and 2D NMR measurements. The ^{1}H-NMR spectrum (600 MHz, $CDCl_3$) [Table 1] showed the triterpenoid pattern with six methyl singlets in the up-field at δ_H 0.74 (Me-24), 0.79 (Me-25), 0.92 (Me-23), 0.94 (Me-27), 0.99 (Me-26) and the one methyl group of Me-30 appeared as a sharp singlet at δ_H 1.68 (Me-30). The down-field shift for Me-30 indicated the presence of a double bond between C-20 and C-29. In the down-field of spectrum, there were two broad singlets: at δ_H 4.65 (1H, br s, H-29$_a$) and 4.55 (1H, br s, H-29$_b$), suggesting the presence of an olefinic proton. The HMQC spectrum showed correlations between H-29$_a$ at δ_H 4.65 (1H, br s) and H-29$_b$ at δ_H 4.55 (1H, br s) with carbon signal at δ_C 109.72. Additionally, the hydroxylated methylene protons at δ_H 3.75 (1H, d, J = 9 Hz, H-28$_a$), coupled in ^{1}H–^{1}H COSY spectrum with a signal at δ_H 3.32 (1H, d, J = 9 Hz, H-28$_b$), giving a doublet. The HMQC spectrum showed correlation between H28$_a$ at δ_H 3.75 (1H, d, J = 9 Hz) and H-28$_b$ at δ_H 3.32 (1H, d, J = 9 Hz) with carbon at δ_C 60.39. The ^{1}H NMR also revealed a secondary hydroxyl group placed at C-3, inferred from the down-field shift of methine proton which appeared at δ_H 3.18 (1H, dd, J = 8, 3.2 Hz, H-3) which showed correlation in HMQC with carbon signal at δ_C 78.86.

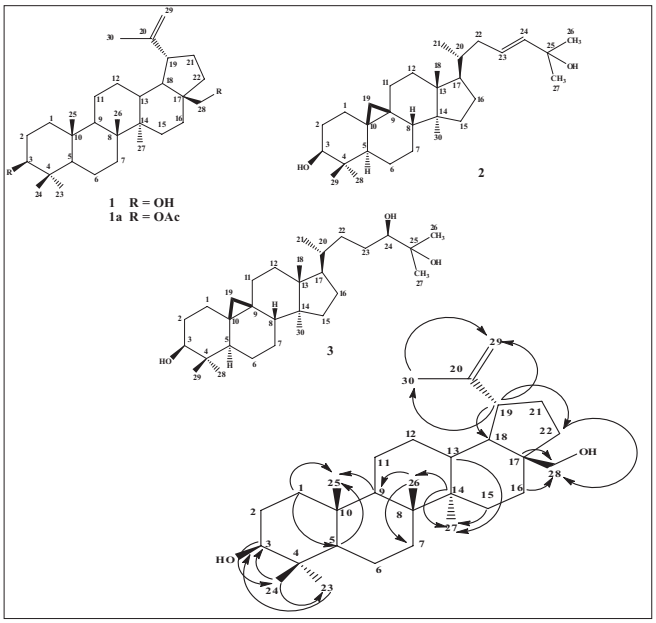

Figure 1: Selected HMBC correlations of compound 1

The ^{13}C-NMR spectrum (125 MHz, $CDCl_3$) [Table 2] displayed 30 carbon signals and DEPT experiments indicated these signals corresponding to 6 methyl groups, 12 methylene groups, including one attached to oxygen appearing at δ_C 60.42 for C-28. Six methine groups including one attached to oxygen appeared at δ_C 78.86 for C-3 and six quaternary carbon atoms. The olefinic carbons C-20 and C-29 appeared at δ_C 15.46 and 109.77, respectively. HMQC and HMBC were used to determine the position of the hydroxylated methyl carbons; the two proton signals at δ_H 3.75 (H-28$_a$) and 3.32 (H-28$_b$) seen in the HMBC experiment show clear

Table 1: 1H NMR spectroscopic data for compounds 1–3 (600 MHz, CDCl3)

Position	1	2	3
H-1	1.65 dd (3.6, 12.6)	1.30 m	1.60 m
	0.89 dd (3.6, 12.6)	0.90 m	
H-2	1.58 m	1.4 m	1.65 m
		1.23 m	
H-3	3.18 dd (3.2, 8)	3.10 m	3.35 t (3.2)
H-5	0.33 br d (18)	0.91 m	1.25 m
H-6	1.50 m	1.26 m	1.50 m
	1.38 m	0.45 dd (12.5, 8)	
H-7	1.37 m	1.65 m	1.72 m
		1.40 m	
H-8		1.16 dd (5,13)	1.16 dd (3, 8)
H-9	1.29 m		
H-11	1.40 m	0.77 m	0.11 m
	1.19 m		
H-12	1.62 m	0.93 m	1.30 m
	1.05 m		
H-13	1.63 m		
H-15	1.63 m	1.27 m	0.15 m
	1.69 m		
H-16	1.89 m	1.60 m	1.88 m
	1.28 m		
H-17		1.24 m	1.50 m
H-18	1.58 m	0.62 s	0.97 s
H-19	1.36 m	0.22 d (4.5)	0.53 d (4.5)
		0.01d (4.5)	0.30 d (4.5)
H-20		1.10 m	1.35 m
H-21	1.98 m	0.53 d (3.5)	0.87 d (3.2)
	1.29 m		
H-22	1.03 m	1.88 m	2.22 m
	1.85 m		
H-23	0.92 s	5.26 d (8)	3.10 m
H-24	0.74 s	5.26 d (8)	3.25 m
H-25	0.79 s		
H-26	0.99 s	0.98 s	1.12 s
H-27	0.94 s	0.98 s	1.24 s
H-28	3.30 d (9)	0.62 s	0.97 s
	3.75 d (9)		
H-29	4.65 br s	0.42 s	0.75 s
	4.55 br s		
H-30	1.68 s	0.56 s	0.88 s

Table 2: ^{13}C NMR spectroscopic data for compounds 1–3 (125 MHz, $CDCl_3$)[a]

Position	1	2	3
C-1	38.64 t	31.93 t	31.97 t
C-2	27.03 t	30.33 t	30.38 t
C-3	78.86 d	78.80 d	78.83 d
C-4	38.82 s	40.45 s	40.48 s
C-5	55.26 d	47.10 d	47.11 d
C-6	18.28 t	21.08 t	21.11 t
C-7	34.21 t	28.04 t	28.36 t
C-8	40.89 s	48.00 d	47.97 d
C-9	50.36 d	20.00 s	19.93 s
C-10	37.13 s	25.96 s	26.00 s
C-11	20.80 t	26.07 t	26.02 t
C-12	25.17 t	35.55 t	35.54 t
C-13	37.28 d	45.29 s	45.42 s
C-14	42.69 s	48.80 s	48.83 s
C-15	27.01 t	32.76 t	33.01 t
C-16	29.15 t	26.42 t	26.46 t
C-17	47.74 s	52.00 d	52.29 d
C-18	48.72 d	18.04 q	17.99 q
C-19	47.77 d	30.00 t	29.88 t
C-20	150.46 s	36.36 d	36.35 d
C-21	29.72 t	18.25 q	18.12 q
C-22	33.95 t	39.00 t	33.31 t
C-23	27.96 q	139.31 d	28.41 t
C-24	15.35 q	125.57 d	79.63 d
C-25	16.08 q	70.75 s	76.74 s
C-26	15.95 q	29.66 q	23.24 q
C-27	14.74 q	29.83 q	26.54 q
C-28	60.42 t	19.26 q	19.26 q
C-29	109.72 t	13.9 q	13.98 q
C-30	19.06 q	25.41 q	25.42 q

[a]Multiplicity was determined by DEPT experiments (s, quaternary; d, methine; t, methylene; q, methyl)

long-range correlations between the carbon signals at δ_C 29.15 (C-16), 33.95 (C-22) and 47.74 (C-17), while the carbon signal at δ_C 60.39 (C-28) showed a correlation with the proton signal at δ_H 1.03 (H-22$_a$), 1.85 (H-22$_b$), 1.28 (H-16$_a$). Other important correlations were observed between the carbon signals at δ_C 15.35 (C-24), 27.96 (C-23) and 38.64 (C-1) with the proton signal at δ_H 3.18 (H-3). Therefore, the hydroxylated methyl was placed at C-3. The assignment of all proton signals and their connectivity to adjacent protons and carbon signals were established from the results of 2D ^{1}H-^{1}H COSY and HMQC experiments.

Acetylating of **1** gave the diacetyl derivative (**1a**), for which the ^{1}H NMR spectrum displayed two new acetyl signals and confirmed the structure of compound **1**. The structure of compound **1** was deduced from the comparison of its spectral data with those of literature and identified as betulin.[19,20]

Compound **2** was isolated as colorless needles. The ^{1}H-NMR spectrum (600 MHz, $CDCl_3$) [Table 1] of compound 2 displayed two doublets at δ_H 0.22 (1H, d, J = 4.5 Hz, H-19a) and at 0.01 (1H, d, J = 4.5 Hz, H-19b) which are characteristic of the presence of a C-9/C-10 cyclopropyl methylene group of a cycloartan-3-one triterpenoid. Cycloartane-type triterpenes possess a cyclopropane bridge between C-9 and C-10, and protons attached to cyclopropyl rings characteristically appear as a pair of doublets in the high-field ^{1}H-NMR region with gem-coupling constant (J = 4.5 Hz). The 1H-NMR spectrum showed the presence of an olefinic proton double bond at δ_H 5.26 (2H, d, J = 8, H-23, H-24). The low coupling constant (J = 8 Hz) between H-23 and H-24 indicate the stereochemistry "*Z*" at C-23. The HMQC spectrum showed correlation between H-23 at δ_H 5.26 (1H, d, J = 8) with carbon signal at δC 139.31 and H-24 at δ_H 5.26 (1H, d, J = 8) with carbon signal at δ_C 125.57. Additionally, δ_H 2.95 (1H, m, H-3) which suggested the existence of secondary hydroxyl group. The ^{1}H-NMR spectrum showed the presence of six tertiary methyl groups as a singlet at δ_H 0.42 (3H, s, Me-29), 0.56 (3H, s, Me-30), a sharp signal that integrated for six protons at δ_H 0.62 (6H, s, Me-18, Me-28), 0.98 (6H, s, Me-26, Me-27) and one methyl group at δ_H 0.53 (3H, d, J = 6.5 Hz, Me-21) coupled with H-20 (methine proton) gave a doublet.

The ^{13}C-NMR spectrum (125 MHz, $CDCl_3$) [Table 2] of compound 2 showed the presence of 30 carbon signals. Determination of the multiplicity was carried out by DEPT experiments which indicated the presence of 6 quaternary carbon atoms, 7 methine groups, 10 methylene groups and 7 methyl groups. It also showed the presence of two olefinic carbons C-23 and C-24 appearing at δ_C 139.31 and 125.57, respectively. Two oxygenated carbons appeared at δ_C 78.80 for C-3 and at 70.75 for C-25. The structure of compound 2 was deduced from the comparison of its spectral data with those of literature and identified as cycloart-23*Z*-ene-3, 25-diol.[21,22]

Compound **3** was isolated as colorless needles. The ^{1}H-NMR spectrum (500 MHz, $CDCl_3$) [Table 1] of compound **3** displayed two doublets at δ_H 0.55 (1H, d, J = 4.5 Hz, H-19$_a$) and at 0.3 (1H, d, J = 4.5 Hz, H-19$_b$) which are characteristic of a C-9/C-10 cyclopropyl methylene group of a cycloartan-3-one triterpenoid. Cycloartane-type triterpenes possess a cyclopropane bridge between C-9 and C-10, and protons attached to cyclopropyl rings characteristically appear as a pair of doublets in the high-field ^{1}H-NMR region with a gem-coupling constant (J = 4.5 Hz). Additionally, the presence of triplet bonds at δ_H 3.35 (1H, t, J = 3.2 Hz, H-3) and multiplet bands at δ_H 3.25 (1H, m, H-24) suggested the existence of secondary hydroxyl group. The ^{1}H-NMR spectrum showed the presence of six tertiary methyl groups at δ_H 0.75 (3H, s, Me-29), 0.88 (3H, s, Me-30), a sharp signal appearing at δ_H 0.97 (6H, s, Me-18, Me-28), 1.12 (3H, s, Me-26), 1.24 (3H, s, Me-27) and one methyl group at δ_H 0.87 (3H, d, J = 3.2 Hz, Me-

21) coupled with H-20 (methine proton) gave a doublet.

The ^{13}C-NMR spectrum (125 MHz, $CDCl_3$) [Table 1] of compound **3** showed the presence of 30 carbon signals. Determination of the multiplicity was carried out by DEPT experiments which revealed the presence of 7 methyl groups, 11 methylene groups, 6 methine groups with two oxygenated carbons at δ_C 78.83 for (C-3), 79.63 for (C-24) and 6 quaternary carbon signals with 1 oxygenated at δ_C 76.74 for (C-25).

The structure of compound **3** was deduced from the comparison of its spectral data with those of literature and was identified as cycloartan-3, 24, 25-triol.[23-26]

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