

New Diterpenoids from *Euphorbia teheranica*

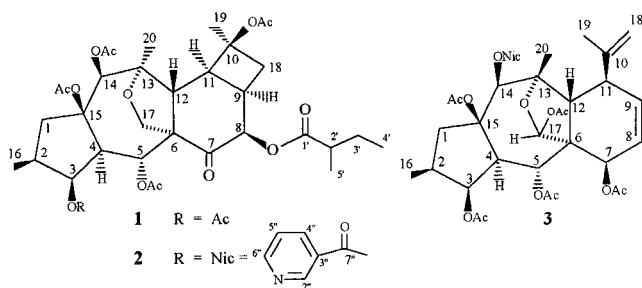
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Two novel pentacyclic diterpenoid esters of the cyclomyrsinane type (**1**, **2**), and one tetracyclic diterpenoid ester of the myrsinane type (**3**) were isolated from the aerial parts of the plant *Euphorbia teheranica*. The structures of the novel compounds were determined by spectral data interpretation.

Euphorbia teheranica Boiss. (Euphorbiaceae) is a plant widespread in the surroundings of Tehran and the semi-desert area of the central parts of Iran.¹ To the best of our knowledge, except for a publication on the mild skin-irritant activity of this plant,² there has been no report of any other investigation in the literature. An extract of the plant gave three diterpenoid esters of the myrsinane type (**1**–**3**).



Compound **1** was assigned the molecular formula $C_{35}H_{48}O_{14}$ on the basis of CIMS, m/z 691 $[M - 1]^+$, and HREIMS m/z 633.2889 ($C_{33}H_{45}O_{12}$) $[M + 1 - 60]^+$. The EIMS and CIMS both showed several losses of 60 mass units, indicating acetate groups leaving the molecule as acetic acid, and also a peak at m/z 85, indicating the presence of a C_4H_9CO moiety in the molecule.

The IR spectrum of **1** indicated the presence of the carbonyl groups of the ester moieties from a prominent absorption at 1740 cm^{-1} . Its ^1H NMR spectrum showed signals due to four oxymethine groups at δ 5.86 (dd, $J = 1.6, 11.0$ Hz, H-5), 5.40 (t, $J = 4.3$ Hz, H-3), 5.25 (d, $J = 6.9$ Hz, H-8), and 5.02 (s, H-14), five singlets for acetates at δ 1.90, 1.95, 2.07, 2.08, and 2.18; signals for two methyls at δ 0.88 (t, $J = 7.5$ Hz, H-4') and 1.29 (d, $J = 6.9$ Hz, H-5') of a 2-methylbutanoate ester group; and three methyls at δ 0.85 (d, $J = 6.8$ Hz, H-16), 1.18 (s, H-20), and 1.62 (s, H-19). Also, informative signals at δ 4.21 (d, $J = 9.7$ Hz, H-17'), 3.58 (dd, $J = 1.6, 9.7$ Hz, H-17) indicated the presence of a tetrahydrofuran ring of a myrsinane-type skeleton, which was recognized from its long-range coupling constant with H-5 ($J = 1.6$ Hz) and the relatively small geminal J value (9.7 Hz).^{3–5} Other indicative ^1H NMR signals for the presence of a myrsinane-type skeleton were the peak at δ 4.03 (d, $J = 12.4$ Hz, H-12) and a doublet of doublets at δ 2.90 ($J = 3.9, 11.0$ Hz, H-4).^{3–8}

In the ^{13}C NMR spectra (BB and DEPT) of **1**, 35 signals were observed, in which 10 were CH_3 , four CH_2 , 10 CH,

and 11 quaternary carbon atoms. Using ^1H – ^1H COSY 45 and HMQC spectra, it was determined that the partial structures $-\text{CH}_2-\text{CH}(\text{CH}_3)-\text{CH}(\text{OR}_1)-\text{CH}-\text{CH}(\text{OR}_2)-$ and $-\text{CH}(\text{OR}_3)-\text{CH}(\text{CH}_2)-\text{CH}-\text{CH}-$ were present, which were connected to each other through the quaternary carbons at C-6, C-7, C-10, C-13, and C-15. The relative positions of the ester groups were deduced from the cross-peaks observed in the HMBC spectrum. To confirm the ^1H – ^1H connectivities in the cyclobutane ring and the 2-methylbutanoate moiety, compound **1** was subjected to a decoupling experiment, in which irradiation of the signal at δ 2.60 (H-2') collapsed the doublet at δ 1.29 (Me-5') to a singlet, and irradiation of H-11 at δ 2.40 changed the H-12 signal to a doublet with a small J value. Irradiation of H-18 was not informative, however, so the relative configuration of **1** was determined according to the NOESY spectrum and also by comparison with ^1H NMR spectral data of related compounds based on the myrsinane-type skeleton.^{3–8} The cross-peaks between H-4/H-3, H-14; H-8/H-9, H-17; H-11/H-14, H-19; and H-20/H-14, H-19, in the NOESY spectrum, indicated that these protons all lie on one face of the molecule. A cross-peak between H-5 and H-12, together with the observed coupling constant ($J = 11.0$ Hz) between H-4 and H-5, confirmed the configuration of **1**.

Compound **2** exhibited a molecular ion at m/z 755.3092 in the HREIMS, indicating a molecular formula of $C_{39}H_{49}O_{14}N$. In the EIMS, the ions at m/z 85 $[\text{C}_4\text{H}_9\text{CO}]^+$ and 57 $[\text{C}_4\text{H}_9]^+$, together with that of m/z 106 $[\text{C}_6\text{H}_4\text{ON}]^+$ and 124 $[\text{C}_6\text{H}_5\text{O}_2\text{N}+1]^+$, established the presence of $\text{C}_4\text{H}_9\text{CO}_2\text{R}$ and nicotinoate ester units in the molecule. The IR spectrum gave signals for unsaturation (1580 and 740 cm^{-1}), besides signals for carbonyl absorption at 1740 cm^{-1} .

The ^1H and ^{13}C NMR spectra of **2** (Tables 1 and 2) were similar to those recorded for **1**, except for the signals that appeared at δ 9.09 (br s, H-2''), 8.79 (br s, H-6''), 8.26 (br d, $J = 8$ Hz, H-4''), and 7.45 (br s, H-5''), due to a nicotinoate moiety. The positions of the ester groups were determined by HMBC cross-peaks between H-8 and the carbonyl carbon of the 2-methylbutanoate unit at δ 174.5 and between H-14 and the carbonyl carbon of an acetate. The positions of the other acetate groups and the nicotinoate ester functionalities were deduced from the downfield shift of H-3 (0.32 ppm) in comparison to **1**, which was the result of substitution of the nicotinoate at C-3 in compound **2**, and the upfield shift of H-16 (0.23 ppm), which can be considered as a consequence of the existence of Me-16 in the shielding anisotropic field of the nicotinoate aromatic ring. To confirm the relative configuration of **2**, it was subjected to a NOE difference experiment. Irradiation of the signal at δ 5.11 (H-14) gave a significant enhancement of the Me-19 and Me-20 signals, while irradiation of H-8

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Table 1. ¹H NMR Spectral Data of Compounds **1–3** (in CDCl₃)

	1	2	3	3 (in C ₆ D ₆)
1 α	2.85 dd (9.8, 16.0)	2.94 dd (11.2, 16.1)	2.66 m	2.79 dd (9.1, 15.9)
1 β	2.49 dd (9.7, 16.0)	2.4 m	2.66 m	2.95 dd (11.0, 15.9)
2	2.3 m	2.2 m	2.2 m	1.5 m
3	5.40 t (4.3)	5.72 t (3.8)	5.18 t (3.6)	5.05 t (3.7)
4	2.90 dd (3.9, 11.0)	3.10 dd (3.6, 11.1)	3.05 dd (3.5, 11.1)	2.92 dd (4.0, 11.2)
5	5.86 dd (1.6, 11.0)	5.86 dd (1.1, 11.1)	6.04 d (11.1)	6.22 d (11.2)
7			5.04 d (5.9)	5.41 d (5.9)
8	5.25 d (6.9)	5.19 d (7)	6.01 ddd (10.0, 6.0, 2.2)	6.14 ddd (2.2, 5.9, 9.9)
9	2.73 dddd (6.9, 9.2, 9.2, 9.4)	2.70 m	5.73 dd (4.2, 10.0)	5.58 dd (4.3, 9.9)
11	2.40 m	2.40 m	3.3 m	3.34 m
12	4.03 d (12.4)	4.09 d (12.3)	3.3 m	3.48 d (5.3)
14	5.02 s	5.11 s	5.21 s	5.45 s
16	0.85 d (6.8)	0.62 d (6.8)	0.82 d (6.7)	0.72 d (6.7)
17	3.58 dd (1.6, 9.7)	3.60 dd (1.5, 9.8)	6.36 s	6.78 s
17'	4.21 d (9.7)	4.25 d (9.8)		
18	2.50 m	2.50 m	4.92 br s	4.9 br s
18'	2.50 m	2.50 m	5.14 br.s	5.1 br s
19	1.62 s	1.63 s	1.86 s	1.57 s
20	1.18 s	1.21 s	1.39 s	1.49 s
2-Methylbutanoyl				
2'	2.6 q (6.9)	2.70 m		
3'	1.70 m	1.60 m		
4'	0.88 t (7.5)	0.78 t (7.4)		
5'	1.29 d (6.9)	0.93 d (6.7)		
Acetyl				
	1.90 s	1.93 s	1.98 s	1.84 s, 6H
	1.95 s	2.10 s	1.99 s	1.87 s
	2.07 s	2.12 s	2.00 s	1.91 s
	2.08 s	2.31 s	2.01 s	1.93 s
	2.18 s		2.07 s	
Nicotinoyl ^a				
2''		9.09 br s	9.02 br s	9.48 br d (2.2)
4''		8.26 br d (8)	8.40 br s	8.26 dt (1.8, 8.0)
5''		7.45 br s	7.54 br s	6.67 ddt (0.9, 4.8, 7.9)
6''		8.79 br s	8.80 br s	8.51 dd (1.8, 4.8)

enhanced the Me-19 signal. Finally, H-5 showed a correlation with H-12, and a correlation between H-16 and H-4'' confirmed the location of the nicotinoate moiety.

Compound **3** was obtained as a white powder and assigned the molecular formula C₃₆H₄₃O₁₃N on the basis of its HREIMS (*m/z* 697.2758). Its IR spectrum showed strong absorptions for ester carbonyl (1730 cm⁻¹) and C=C bonds (1590 cm⁻¹). In the EIMS, the peaks at *m/z* 637 [M - HOAc]⁺ and 517 [M - 3 × HOAc]⁺, and the prominent peaks at *m/z* 106 [C₆H₄ON]⁺ and 124 [C₆H₅O₂N+1]⁺, suggested the presence of acetate and nicotinoate esters in the molecule. The ¹H NMR spectrum of **3** was similar to those of known myrsinol esters,^{5,7} with the major differences being the presence of two additional oxymethines and the lack of the signals for the diastereotopic C-17 methylene. The spectrum also showed signals for five acetate groups and one nicotinoate unit at δ 1.98, 1.99, 2.00, 2.01, and 2.07, and δ 9.03 (s, H-2''), 8.80 (br s, H-6''), 8.40 (br s, H-4''), and 7.45 (br s, H-5''), respectively.

In the ¹³C NMR spectrum of **3**, signals at δ 97.8 and 81.6 were observed for C-17 and C-14, respectively. For the determination of the relative positions of the esters in **3**, it was subjected to a HMBC experiment (in CDCl₃). The cross-peaks between H-3, H-5 and the carbonyl carbons of the acetates and between H-14 and C-7'' established the positions of the acetate groups at C-3 and C-5 and the nicotinoate moiety at C-14 in **3**. The correlation between H-7 and an acetyl moiety was observed when the HMBC experiment was carried out in C₆D₆. The relative configuration of **3** was assigned from the interpretation of the ¹H NMR coupling constants and NOESY spectra. The configuration at C-17 was assigned by NOE difference experiments (enhancement of the signal of H-17 upon irradiation

of H-4) and further supported by the lack of detection of a *W*-type long-range coupling between H-5 and the oxymethine H-17.

Diterpenoids containing the same cyclomyrsinol skeleton as **1** and **2** have already been reported from *E. prolifera*, and their structures were confirmed by X-ray crystallography.³ An ester with similar structure was isolated from *E. seguieriana*.⁴ The lithium aluminum hydride reduction of myrsinol produced the 14 β -hydroxy derivative as the major product with the same configuration at C-14 as **3**.⁵ Recently, five esters with similar structures to **3**, with opposite configuration at C-14, together with a diterpene ester with the same carbon skeleton as **1** and **2** were isolated from *E. seguieriana*.⁶

Experimental Section

General Experimental Procedures. Melting points were uncorrected and measured by a Büchi 535 instrument. Optical rotations were measured on a JASCO DIP-360 polarimeter. The UV spectra were recorded on a Hitachi U-3200 instrument. The IR spectra were recorded on Shimadzu IR-460 and JASCO IR-302 instruments. ¹H NMR spectra were taken in CDCl₃ at 500 and 400 MHz with TMS as internal standard on Bruker AM-400 and AM-500 NMR spectrometers. The mass spectra were recorded on a Finnigan MAT-312 double-focusing mass spectrometer.

Plant Material. *Euphorbia teheranica* Boiss. was collected in Tehran, Iran, in July 1996, and identified by Prof. Dr. M. Sanei Chariat Pannahi, Karaj Agriculture College, University of Tehran, Iran. A voucher specimen (no. 2256) is deposited in the herbarium of that college.

Extraction and Isolation. The ground, air-dried aerial parts of *E. teheranica* (1.2 kg) were extracted with MeOH. Removal of the solvent under reduced pressure gave a syrup that was partitioned between a mixture of MeOH-H₂O and

Table 2. ^{13}C NMR Spectral Data for **1–3** (in CDCl_3)

carbon	1	2	3	3 (in C_6D_6)
1	43.1 t	43.6 t	43.9 t	44.1 t
2	35.7 d	36.4 d	36.9 d	36.9 d
3	77.6 d	77.5 d	76.4 d	76.4 d
4	51.5 d	51.4 d	52.0 d	52.1 d
5	68.7 d	68.4 d	69.4 d	69.8 d
6	62.2 s	62.0 s	56.6 s	57.2 s
7	204.4 s	204.2 s	63.3 d	63.6 d
8	71.2 d	71.0 d	123.2 d	123.9 d
9	30.1 d	30.0 d	133.6 d	133.9 d
10	77.6 s	78.0 s	147.4 s	147.6 s
11	41.3 d	41.4 d	43.8 d	44.0 d
12	41.9 d	41.9 d	37.2 d	37.8 d
13	89.3 s	89.5 s	89.6 s	89.9 s
14	81.9 d	81.8 d	81.6 d	81.2 d
15	90.5 s	90.1 s	91.0 s	91.6 s
16	14.2 q	14.2 q	14.2 q	14.1 q
17	67.2 t	67.3 t	97.8 d	98.2 d
18	35.1 t	35.1 t	114.1 t	113.6 t
19	24.5 q	24.4 q	18.8 q	19.0 q
20	22.3 q	22.4 q	24.9 q	25.0 q
COCH_3				
	170.4 s	170.6 s	170.7 s	170.1 s
	170.1 s	169.7 s	170.0 s	169.4 s
	169.6 s	168.8 s	169.5 s	169.2 s
	168.8 s	168.2 s	168.9 s	168.9 s
	168.0 s		168.5 s	167.7 s
COCH_3				
	20.6 q	20.8 q	22.5 q	22.1 q
	20.8 q	21.3 q	21.3 q	21.1 q
	21.3 q	21.7 q	21.2 q	20.8 q
	21.6 q	23.9 q	21.0 q	20.7 q
	23.4 q		20.7 q	20.5 q
2-Methylbutanoyl				
1'	174.5 s	174.5 s		
2'	41.0 d	40.9 d		
3'	27.0 t	26.9 t		
4'	11.1 q	11.0 q		
5'	15.9 q	15.5 q		
Nicotinoyl				
2''			151.1 d	151.5 d
3''			126.1 s	126.3 s
4''			138.2 d	137.3 d
5''			123.6 d	123.2 d
6''			153.7 d	153.8 d
7''			163.8 s	164.7 s

hexane, EtOAc, and *n*-butanol. The EtOAc fraction, on concentration (20 g), was subjected to column chromatography over Si gel (400 g, 70–230 mesh), using hexane and hexane– Me_2CO mixtures of increasing polarity up to 100% Me_2CO , and then MeOH. The fractions eluted with 20–30% Me_2CO in hexane were subjected to repeated flash chromatography (Si gel 230–400 mesh) with hexane– CHCl_3 gradient mixtures, beginning with 50% hexane in CHCl_3 leading to 100% CHCl_3 and then Me_2CO . Repeated preparative TLC (Si gel F_{254}) of CHCl_3 -rich fractions, obtained from flash chromatography, using 1% MeOH in CHCl_3 , permitted the purification of **1** (18 mg, 0.0015%), **2** (4 mg, 0.0003%), and **3** (14 mg, 0.0012%).

Methyl gallate (90 mg, 0.0075%) was separated from a 50% hexane– Me_2CO fraction obtained from the initial column chromatographic step and purified by flash chromatography using CHCl_3 – Me_2CO (4:1).

3,5,10,14,15-O-pentaacetyl-8-O-2'-(methylbutanoyl)-cyclomyrsinol (1): obtained as a white powder (MeOH); mp 233–234 °C; $[\alpha]_D^{27} +49.3^\circ$ (*c* 0.36, CHCl_3); UV (MeOH) λ_{max} (log ϵ) 276 (2.75), 230 (3.39), 201 (3.65) nm; IR (CHCl_3) ν_{max} 2980, 1740, 1370, 1260, 1170, 1140, 1025 cm^{-1} ; ^1H and ^{13}C NMR data, see Tables 1 and 2; EIMS m/z 693 $[\text{M} + 1]^+$, 633 (3), 573 (2), 513 (2), 488 (2), 471 (2), 343 (18), 250 (12), 249 (70), 247 (11), 235 (14), 207 (11), 205 (20), 191 (40), 181 (20), 148 (21), 105 (29), 85 (37), 71 (11), 57 (100); CIMS m/z 691 $[\text{M} - 1]^+$, 633 $[\text{M} + 1 - \text{HOAc}]^+$, 573 $[\text{M} + 1 - 2 \times \text{HOAc}]^+$, 531 $[\text{M} + 1 - \text{HOAc} - \text{C}_5\text{H}_{10}\text{O}_2]^+$; HREIMS m/z 633.2889 $[\text{M} + 1 - \text{HOAc}]^+$ (calcd for $\text{C}_{33}\text{H}_{45}\text{O}_{12}$, 633.2911).

5,10,14,15-O-tetraacetyl-3-O-nicotinoyl-8-O-2'-(methylbutanoyl)-cyclomyrsinol (2): obtained as a white powder (MeOH); mp 202–203 °C; $[\alpha]_D^{20} +25$ (*c* 0.08, CHCl_3); UV (MeOH) λ_{max} (log ϵ) 263 (3.28), 219 (3.78), 200 (3.83) nm; IR (KBr) ν_{max} 3450 (H_2O), 2980, 2950, 1740, 1580, 1460, 1420, 1370, 1280, 1220, 1160, 1130, 1110, 1080, 1020, 960, 920, 740, 700 cm^{-1} ; ^1H and ^{13}C NMR data, see Tables 1 and 2; EIMS m/z 755 $[\text{M}]^+$, 696 (14), 654 (17), 610 (8), 552 (3), 510 (4), 406 (15), 364 (17), 249 (51), 191 (44), 149 (14), 124 (100), 106 (41), 85 (19), 57 (62), 42 (79); HREIMS m/z 755.3092 (calcd for $\text{C}_{39}\text{H}_{49}\text{O}_{14}\text{N}$, 755.3153).

3,5,7,15,17-O-pentaacetyl-14-O-nicotinoyl-17-hydroxymyrsinol (3): obtained as a white powder (MeOH); mp 183–184 °C; $[\alpha]_D^{27} -48.8^\circ$ (*c* 0.09, CHCl_3); UV (MeOH) λ_{max} (log ϵ) 264 (2.20), 219 (2.74), 201 (2.93) nm; IR (CHCl_3) ν_{max} 2980, 2400, 1730, 1590, 1360, 1270, 1110, 1010, 980, 600, cm^{-1} ; ^1H and ^{13}C NMR data, see Tables 1 and 2; EIMS m/z 697 $[\text{M}]^+$, 596 (2), 407 (8), 364 (13), 291 (10), 264 (7), 147 (24), 124 (68), 106 (100), 85 (2), 78 (22); HREIMS m/z 697.2758 (calcd for $\text{C}_{36}\text{H}_{43}\text{O}_{13}\text{N}$, 697.2734).

Methyl gallate: obtained as a white precipitate (Me_2CO); mp 196–198 °C (lit. mp 202 °C),⁹ and exhibited closely comparable spectroscopic data (IR, ^1H NMR, EIMS) to literature values.^{9,10}

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