Chemical Constituents of *Euphorbia marschalliana* Boiss.

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Acetone extract of aerial parts of *Euphorbia marschalliana* collected from Iran has been subjected to different chromatography techniques for fractionation and purification. The stereo-structures of the myrsinol esters 15-*O*-acetyl-3-*O*-propionyl-5-*O*-butanoyl-7-*O*-nicotinoylmyrsinol (**1**) and 15-*O*-acetyl-3,5-*O*-dibutanoyl-7-*O*-nicotinoylmyrsinol (**2**) have been probed using ROESY spectroscopy and modified for the stereochemistry at C-6, C-12 and C-13. β-Sitosterol (**3**), 29-norcycloart-5-ene (**4**), 5,8-lanostadiene-3β-ol (**5**), 3β,24(*S*),25-trihydroxycycloartane (**6**), 3β,24(*R*),25-trihydroxycycloartane (**7**) and 24-methylenecycloartan-3β-ol (**8**) were identified for the first time in this plant.

Key words: Euphorbia marschalliana Boiss., Myrsinol Ester Diterpenoids, Cycloartane Triterpenoids

Introduction

The irritant ingenol and non-irritant myrsinol diterpene esters have been separated from *Euphorbia myrsinites* (Rentzea *et al.*, 1982; Rentzea and Hecker, 1982; Öksüz *et al.*, 1995).

Recently a new diterpenoid ester was isolated from *E. decipiens*, which showed inhibitory activity against prolyl endopeptidase, a serine protease, and analgesic activity (Ahmad *et al.*, 2002). Investigation of the constituents of *E. myrsinites* of Turkey yielded four myrsinol esters with moderate anti-HIV-1 reverse transcriptase (RT) inhibitory activity (Öksüz *et al.*, 1995). The above biological activities and the inhibitory activity of cheiradone, a myrsinol-type diterpenoid isolated from *E. cheiradenia*, against α-glucosidase (Abbas *et al.*, 2000) prompted us to study the constituents of *Euphorbia marschalliana* of Iran. Determination of the stereochemistry of the myrsinane-type diterpenoids was other objective of this research. These types of research seem to be essential since in different articles opposite stereochemistry is reported for myrsinol-type diterpenoids (Rentzea *et al.*, 1982; Rentzea and Hecker, 1982; Öksüz *et al.*, 1995). Recently our group determined the absolute configuration of decipinone, a myrsinane-type diterpenoid, by NMR spectroscopy (Jassbi *et al.*, 2002).

This article reports the isolation of two myrsinol esters, 15-*O*-acetyl-3-*O*-propionyl-5-*O*-butanoyl-7-*O*-nicotinoylmyrsinol (**1**) and 15-*O*-acetyl-3,5-*O*-dibutanoyl-7-*O*-nicotinoylmyrsinol (**2**), and the determination of their relative configuration. β-sitosterol (**3**), 29-norcycloart-5-ene (**4**), 5,8-lanostadiene-3β-ol (**5**), 3β,24(*S*),25-trihydroxycycloartane (**6**), 3β,24(*R*),25-trihydroxycycloartane (**7**) and 24-methylenecycloartan-3β-ol (**8**) were identified for the first time in the acetone extract of *E. marschalliana.*

Results and Discussion

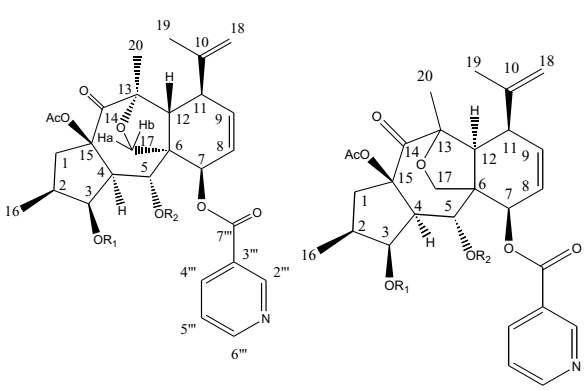
The acetone extract of the aerial parts of the plant *E. marschalliana* was subjected to column chromatography on silica gel (70–230 mesh).

Two myrsinol esters (**1**, **2**), five triterpenes and β-sitosterol were separated from semi-polar fractions. The planar structures of **1** and **2** were determined by EI and FD mass and ^{1}H and ^{13}C NMR spectra and confirmed by comparison with those reported for these compounds previously (Öksüz *et al.*, 1995). The stereochemistry of myrsinol esters isolated from *E. myrsinites* were determined by X-ray crystallography but the orientation of H-5 was determined as alpha despite the large coupling constant between H-4 and H-5 (J = 11.5 Hz) (Rentzea *et al.*, 1982). Table I shows the ^{1}H NMR and ROESY spectral data for compound **1**. The carbon skeletons of **1** and **2** (Fig. 1) were suggested by Öksüz *et al.* (1995). Besides ^{1}H NMR and ^{13}C NMR, selective INEPT spectroscopy ap-

Table I. ^{1}H NMR and ROESY spectral data for compound **1**.

Position	^{1}H NMR (in $CDCl_3$)	NOE correlations using ROESY spectrum
1α	3.50 dd (9.6, 14.8)	
1β	1.4 m	
2	2.2 m	
3	5.21 t (3.7)	H-2′, H-4, H-16
4	2.29 dd (3.5, 11.1)	H-3, H_a-17
5	6.07 d (11.1)	H-7, H-12
7	5.11 d (6.4)	H-5, H-8, H-9, H_b-17, H_a-17
8	6.21 ddd (1.5, 6.4, 9.6)	H-7, H-11, H_b-17, H-18
9	5.91 dd (5.7, 9.6)	H-7, H-11, H_b-17
11	3.36 brs	H-9, H-18, H-20,
12	3.26 d (4.6)	H-5, H-18, H-19,
16	0.88 d (6.9)	
17_a	3.97 d (9.6)	H-4, H-7, H_b-17
17_b	3.60 d (9.6)	
18_a	4.80 brs	H-11, H-19, H-20
18_b	4.78 brs	
19	1.73 brs	
20	1.56 s	
OAc	2.19 s	
Propionyl		
2′	2.22 q (7.4)	
3′	1.03 t (7.4)	
Butanoyl		
2″	2.14 t	
3″	1.43 m	
4″	0.83 t (7.4)	
Nicotinoyl		
2‴	9.14 s	H-5
4‴	8.21 ddd (1.7, 1.7, 8.0)	H-5
5‴	7.37 dd (5.0, 8.0)	
6‴	8.74 dd (1.7, 5.0)	

plied to determine the relative positions of the esters. The small coupling constant between H-12 and H-11 (J = 3.5 Hz) may be the reason of suggestion of alpha orientation for H-12 for compounds **1** and **2** (Fig. 1, right) (Öksüz *et al.*, 1995). In this article we determined the structures of **1** and **2** using ^{1}H NMR, ^{13}C NMR, HMQC and HMBC spectral data (Fig. 1, left). In order to investigate the stereochemistry of **1** isolated from *E. marschalliana* we additionally used 2D ROESY spectroscopy. In the ROESY spectrum of **1** the strong cross peaks between H-12 and H-5, H-5 and H-2″, H-5 and H-4″ in the nicotinoyl moiety and between H-12 and H-19 suggested that these protons have the same orientation in the molecule. On the other hand the NOE correlations between H_a-17 and H-4 and H-7, H-8 and H-9 with H_b-17 and between H-20 and H-11 suggested that these protons are located on the other face of the molecule (Fig. 2).



1: R_1=$COCH_2CH_3$, R_2=$COCH_2CH_2CH_3$
2: R_1=R_2=$COCH_2CH_2CH_3$

Stereochemistry proposed for compounds **1** and **2** by Öksüz *et al.* (1995).

Fig. 1. Structure of myrsinane diterpenoids from *E. marschalliana* (left) and *E. myrsinites* (right).

Isolation of cycloartane triterpenes and myrsinol esters from both *E. marschalliana* of Iran and *E. myrsinites* of Turkey (Öksüz *et al.*, 1995) indicates the close chemical relationship between these two plants. Identification of compounds **3**–

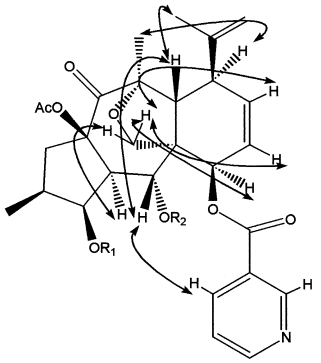


Fig. 2. NOE correlations using ROESY spectral data for compound **1**.

8 was confirmed by their mass spectra, ^{1}H NMR and in some cases ^{13}C NMR spectra.

Experimental

General

^{1}H and ^{13}C NMR spectra (broad band and DEPT experiments) were measured on a JEOL, JNM-EX270 instrument. ^{1}H NMR, HMQC, HMBC and ROESY spectra of compound **1** were measured on a 500 MHz Bruker AMX500. MS spectra were recorded on a JEOL JMS-SX102A spectrometer. Analytical TLC experiments were performed on Merck silica gel 60 F_{254} and DIOL F_{254} HPTLC pre-coated glass plates. Column chromatographies were performed, on silica gel (63–210 μm and 40–60 μm) and LiChroprep Diol (40–60 μm).

Plant material

The aerial parts of the plant *Euphorbia marschalliana* Boiss. were collected from Taleghan area near Karaj, Iran in April 2000. The plant was identified by Mr. Bahram Zehzad at the Department of Biological Sciences, Shahid Beheshti University, Evin, Tehran, Iran. The voucher specimens were deposited in the herbarium of the same department (herbarium no. 2000–913, 98–104).

Extraction of the plant material and purification of the compounds

After grinding, the shade dried plant material (2 kg) was extracted with acetone for one week. The acetone extract was concentrated under reduced pressure and partitioned between water/chloroform and water/*n*-butanol. The chloroform layer (54.5 g) was subjected to column chromatography using silica gel (63–210 μm, 550 g). The column was eluted with *n*-hexane with increasing polarity up to ethyl acetate followed by methanol. Compounds **1** and **2** were purified from fractions 12 and 13 eluted with about 30% ethyl acetate in hexane. 1.4 g of the above fractions were subjected to column chromatography using diol stationary phase (80 g), the column was eluted with hexane followed by increasing the polarity using chloroform and 10% methanol in chloroform. The fractions were eluted with 70% chloroform in hexane were subjected to preparative silica gel TLC using ethyl acetate/toluene (3.5:6.5) and chloroform/acetone/acetic acid (96:3:1 v/v/v) to afford **1** (6 mg) and **2** (41 mg), respectively. The fractions were eluted (from the first column) with hexane/ethyl acetate (predominant in hexane) were mixed (2.08 g) and load (0.70 g) on a column containing 8% AgNO3 on silica gel (40–60 μm, 28 g). The column was eluted with hexane/chloroform (1:1) with increasing the polarity from chloroform to 10% acetone in chloroform. Compounds **4** and **5** (4 mg) were semi-purified as a mixture. Fraction 11 (2.24 g) eluted with 15% ethyl acetate in hexane was subjected to column chromatography (40–60 μm, 66 g) using hexane with increasing the polarity up to chloroform and 10% acetone in chloroform. Repeated preparative silica gel TLC of chloroform rich fractions using 5% acetone in chloroform and 20% ethyl acetate in chloroform yielded compounds **3** (3.5 mg) and **8** (5.2 mg). Compound **7** (534 mg) was purified from more polar fractions prominent in ethyl acetate using column chromatography over flash silica gel using 80% chloroform in hexane with increasing the polarity to pure acetone.

Spectral data for the identified compounds

Compound **1**: EIMS: *m/z* (rel. int., %) = 638 (3) $[M+1]^{+}$ ($C_{35}H_{43}NO_{10}$), 609 (16), 578 (16), 567 (22), 549 (14), 521 (44), 166 (83), 124 (100), 106 (26). – ^{1}H NMR (500 MHz, $CDCl_3$) see the Table I. – ^{13}C NMR (67.5 MHz, $CDCl_3$): δ = 42.2 (C-1), 36.8 (C-2), 77.1 (C-3), 52.9 (C-4), 66.3 (C-5), 55.4 (C-6), 66.0 (C-7), 121.8 (C-8), 134.8 (C-9), 146.0 (C-10), 41.8 (C-11), 42.9 (C-12), 90.0 (C-13), 203.1 (C-14), 90.4 (C-15), 14.4 (C-16), 69.9 (C-17), 113.3 (C-18), 21.1 (C-19), 20.8 (C-20), CH_3CO: 20.0, CH_3CO: 169.7, CH_3CH_2CO: 173.4 (C-1′), 27.8 (C-2′), 8.8

(C-3′), CH_3CH_2 CH_2CO: 170.8 (C-1″), 35.8 (C-2″), 17.9 (C-3″), 13.6 (C-4″), C_6H_4NO: 150.5 (C-2‴), 126.3 (C-3‴), 136.8 (C-4‴), 122.9 (C-5‴), 152.8 (C-6‴), 164.2 (C-7‴).

Compound **2**: EIMS: *m/z* (rel. int., %) = 652 (2) $[M+1]^+$ ($C_{36}H_{45}NO_{10}$), 581 (9), 535 (18), 405 (12), 282 (14), 264 (14), 124 (100). – FDMS *m/z* = 651 $[M]^+$, 623 $[M^+-CO]$. – ^1H NMR (270 MHz, $CDCl_3$): δ = 3.51 (H-1α, dd, *J* = 9.5, 14.8 Hz), 5.22 (H-3, t, *J* = 3.8 Hz), 2.30 (H-4, dd, *J* = 3.6, 11.2 Hz), 6.09 (H-5, d, *J* = 11.2 Hz), 5.13 (H-7, d, *J* = 6.4 Hz), 6.23 (H-8, ddd, *J* = 2.0, 6.4, 9.8 Hz), 5.93 (H-9, dd, *J* = 5.8, 9.8 Hz), 3.38 (H-11, brs), 3.27 (H-12, d, *J* = 3.5 Hz), 0.83–0.92 (m, 9H, H-16, H-4′, H-4″), 3.99 (H_a-17, d, *J* = 9.6 Hz), 3.62 (H_b-17, d, *J* = 9.6 Hz), 4.83 (H_b-18, brs), 4.80 (H_b-18, brs), 1.76 (H-19, s), 1.58 (H-20, s), CH_3CO: 2.21 (s), $CH_3CH_2CH_2CO$: 2.13–2.19 (H-2′, H-2″, m), 1.38–1.54 (H-3′, H-3″, m), C_6H_5NO: 9.16 (H-2‴, s), 8.23 (H-4‴, dt, *J* = 2.0, 6.2 Hz), 7.39 (H-5‴, dd, *J* = 5.0, 8.0 Hz), 8.75 (H-6‴, brs).

Compound **3**: EIMS: *m/z* (rel. int., %) = 414 (100) $[M]^+$ ($C_{29}H_{50}O$), 399 (17), 396 (25), 381 (12), 329 (17), 303 (15). – ^1H NMR (270 MHz, $CDCl_3$): δ = 5.35 (brd, *J* = 5.4 Hz), 3.52 (m, 1H), 1.00 (s, 3H), 0.92 (d, *J* = 6.6 Hz, 3H), 0.83 (d, *J* = 6.0 Hz, 6H), 0.81 (t, *J* = 6.0 Hz, 3H), 0.68 (s, 3H). – ^{13}C NMR (67.5 MHz, $CDCl_3$): δ = 140.0, 121.6, 71.8, 56.8, 56.1, 50.2, 45.9, 42.4, 42.4, 39.8, 37.3, 36.6, 36.2, 34.0, 31.9, 31.9, 31.8, 29.2, 28.3, 26.2, 24.4, 23.2, 21.1, 19.9, 19.5, 19.1, 18.9, 12.1, 11.9.

Compounds **4**, **5**: FDMS: *m/z* (rel. int., %) = 426 $[M]^+$. – ^1H NMR (270 MHz, $CDCl_3$): δ = 5.09 (t, *J* = 6.7 Hz), 3.25 (ddd, *J* = 4.3, 11.4, 11.4 Hz), 1.68 (s), 1.60 (s), 0.96 (s), 0.89 (s), 0.80 (s), 0.55 (d, *J* = 4.1 Hz), 0.32 (d, *J* = 4.1 Hz). – ^{13}C NMR (67.5 MHz, $CDCl_3$): δ = 134.4, 134.0, 133.5, 130.9, 125.3, 125.2, 79.0, 78.8, 52.3, 50.9, 50.4, 50.0, 49.6, 48.8, 47.9, 47.1, 36.3, 36.2, 35.8, 35.6, 35.4, 35.2, 32.8, 31.9, 30.9, 30.4, 29.9, 29.8, 29.7, 28.1, 27.9, 26.5, 26.1, 25.7, 25.4, 24.9, 24.7, 24.5, 21.5, 21.1, 20.1, 20.0, 19.3, 18.9, 18.2, 18.0, 17.7, 15.7, 15.6, 15.3, 15.4, 14.0.

Compounds **6**, **7**: FDMS: *m/z* (rel. int., %) = 460 $[M]^+$. – ^1H NMR (270 MHz, $CDCl_3$): δ = 3.30 (m), 1.21 (s), 1.16 (s), 0.96 (s, 6H), 0.89 (s), 0.80 (s), 0.33 (d, *J* = 4.1 Hz), 0.55 (d, *J* = 4.1 Hz). – ^{13}C NMR (67.5 MHz, $CDCl_3$): δ = 79.6, 78.8, 78.7, 73.2, 73.1, 52.5, 52.3, 48.5, 48.0, 47.1, 45.4, 45.3, 40.5, 36.4, 35.9, 35.6, 33.6, 33.2, 32.9, 32.0, 30.4, 29.9, 28.8, 28.5, 28.3, 28.2, 26.7, 26.6, 26.5, 26.1, 26.0, 25.5, 23.3, 23.2, 21.2, 20.0, 19.4, 18.5, 18.2, 18.1, 14.1.

Compound **8**: EIMS: *m/z* (rel. int., %) = 440 (69) $[M]^+$ ($C_{31}H_{52}O$), 426 (30), 425 (53), 422 (100), 407 (73), 379 (31), 300 (58). – ^1H NMR (270 MHz, $CDCl_3$): δ = 4.71 (brs), 4.66 (brs), 3.28 (dd, *J* = 4.3, 10.7 Hz), 2.26 (m), 1.02 (d, *J* = 6.7 Hz), 1.03 (d, *J* = 6.7 Hz), 0.96 (s, 6H), 0.90 (s), 0.89 (d, *J* = 6.4 Hz), 0.81 (s), 0.32 (d, *J* = 4.1 Hz), 0.55 (d, *J* = 4.1 Hz). – ^{13}C NMR (67.5 MHz, $CDCl_3$): δ = 105.0, 78.8, 52.2, 48.8, 47.9, 47.1, 45.3, 40.4, 36.1, 35.5, 35.0, 35.8, 32.9, 31.9, 31.3, 30.4, 29.8, 29.6, 28.1, 26.4, 26.1, 26.0, 25.4, 21.9, 21.8, 21.1, 20.0, 19.3, 18.3, 18.0, 13.9.

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