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JATROPHANE DITERPENOIDS FROM *EUPHORBIA ESULA*

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Key Word Index—*Euphorbia esula*; Euphorbiaceae; jatrophane polyesters; macrocyclic diterpenes.

Abstract—Two new jatrophane diterpenoids, esulatin D and E, have been isolated from the dichloromethane extract of the whole, undried plant of *Euphorbia esula*. The structures have been assigned on the basis of spectral analysis, including 2D NMR experiments.

INTRODUCTION

Plants of the Euphorbiaceae are known to produce highly irritant, carcinogenic, tumour-promoting diterpenoids with bi-, tri- or tetracyclic skeletons [1]. The jatrophane diterpenoids comprise a small group of the constituents of this family; they are known to occur only in a few species: *Jatropha gossypiifolia*, *J. macrorhiza*, *Euphorbia maddeni*, *E. kansui*, *E. helioscopia*, *E. characias*, *E. latheriflora* and *E. esula* L. [1–3]. These compounds have aroused interest because of their antitumour, antiwrithing, analgesic and phytotoxic activities [4–6].

As part of our studies on biologically active compounds from Hungarian *Euphorbia* species, *E. esula* was examined. Our earlier work yielded three new jatrophane polyesters, esulatin A–C, from the whole undried plant [7]. Further investigations, reported here, have led to the isolation of the related compounds esulatin D (**1**) and E (**2**), which have been established to be tetra- and hexaesters of hitherto unknown, polyfunctional diterpene parent alcohols.

RESULTS AND DISCUSSION

The dichloromethane-soluble fraction obtained from a methanolic extract of the fresh, whole plant of *E. esula* was subjected to polyamide column chromatography and eluted with mixtures of water and methanol. The methanol–water (4:1) eluate was further purified by CC, TLC and HPLC to afford esulatin D and E.

Esulatin D (**1**) was obtained as colourless crystals. Its molecular formula ($C_{32}H_{44}O_{13}$) was derived from the HR mass spectrum and NMR analyses. The EI-mass spectrum displayed fragment peaks due to the sequential loss of HOAc and a ketene unit (see Experimental). The ^1H and ^{13}C NMR spectra of esulatin D showed signals corresponding to six acetyl groups [δ_H 2 × 2.14 *s*, 2.12 *s*, 2.11 *s*, 2 × 2.00 *s*; δ_C 170.4, 170.3, 169.9, 2 × 169.4 and 168.9 (*C*O) and 22.1, 2 × 21.3, 2 × 21.0 and 20.8 (*C*H$_3$)]. Additionally, the ^{13}C NMR and DEPT spectra exhibited resonances of one ketone, two oxygen-substituted and one alkyl-substituted quaternary carbons, one single unsaturated quaternary carbon, one unsaturated methylene carbon (exomethylene), two tertiary unsaturated carbons, two alkyl methylenes, four oxygen-substituted tertiary carbons, two alkyl-substituted tertiary carbons, one secondary and three tertiary methyl groups (Table 1). Thus, esulatin D was a derivative of the bicyclic diterpenoid jatrophane [1].

The ^1H NMR spectrum of esulatin D contained 17 signals due to the parent skeleton. These signals were assigned on the basis of ^1H-^1H COSY and HMQC spectra, as listed in Table 1. The ^1H NMR and ^1H-^1H COSY spectra revealed the presence of one secondary and three tertiary methyls (δ_H 1.14 *d*, 1.49 *s*, 1.06 *s* and 1.02 *s*) and four sequences of correlated protons: δ_H 3.80 *br d* and 1.93 *d* (—C*H*$_2$—) (A); δ_H 5.42 *d*, 2.85 *d*, 5.94 *s*, 5.21 *s* and 5.15 *s* [—C*H*R—C*H*—C*H*R—C(=C*H*$_2$)—] (**B**); δ_H 4.96 *t*, 2.00 *m* and 4.74 *dd* [—C*H*R—C*H*$_2$—C*H*R—] (C); δ_H 5.85 *d*, 5.54 *dd*, 3.48 *dq* and 1.14 *d* [*trans*-C*H*=C*H*—C*H*(C*H*$_3$)—] (D) (R = acetyl). The connection of these partial structures was determined from two- or three-bond long-range correlations observed in an HMBC spectrum.

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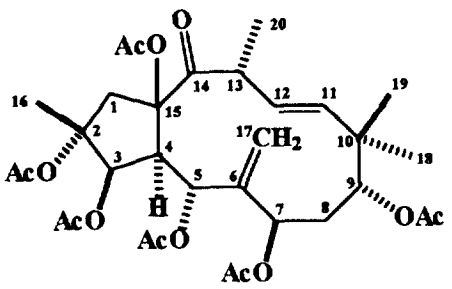

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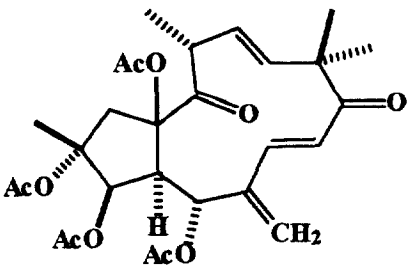

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Structural elements were identified as C-1 (A), C-3–C-6 with an exomethylene on C-6 (B), C-7–C-9 (C) and C-11–C-13–C-20 (D) as follows. The quaternary carbon signal at δ_C 86.6 (C-2) showed correlation peaks with the proton signals at δ_H 3.80 and 1.93 (H-1) and 5.42 (H-3), which confirmed the linkage of structural elements A and B. Cross-peaks between the carbon signal at δ_C 146.0 (C-6) and the proton signals at δ_H 5.94 (H-5) and 4.96 (H-7) and between δ_C 111.7 (C-17) and H-5, H-7 revealed the connection of partial structure B to C. The $^2J_{CH}$ and $^3J_{CH}$ correlations between the carbon signal at δ_C 40.4 (C-10) and the proton signals at δ_H 2.00 (H-8), 4.74 (H-9), 5.85 (H-11) and 5.54 (H-12) suggested that molecular moiety C is adjacent to D. The quaternary carbon signal at δ_C 92.6 was assigned on the basis of its long-range couplings with H-1, H-3, H-4 and H-5 as C-15. Cross-peaks between the carbon signal at δ_C 210.9 and H-

Table 1. NMR Spectral Data of **1** [$CDCl_3$, TMS, δ (ppm), (J = Hz)]

Atom	^{1}H	^{13}C	^{1}H-^{1}H COSY	HMBC	NOESY
1a	3.80 *br d* (16.1)	46.8		H-1b	C-2, C-3, C-4, C-14, C-15
1b	1.93 *d* (16.1)	H-1a	C-2, C-14, C-15, C-16	H-16	
2		86.6			
3	5.42 *d* (3.1)	78.2	H-4	C-1, C-2, C-15, 3-*C*OMe	H-4, H-17b
4	2.85 *d* (3.1)	49.2	H-3, H-5	C-3, C-5, C-6, C-14, C-15	H-3, H-5, H-7
5	5.94 *s*	68.8	H-4, H-17a,b	C-3, C-4, C-6, C-15, C-17, 5-*C*OMe	H-4, H-8, H-11, H-13
6		146.0			
7	4.96 *t* (5.6)	68.0	H-8	C-5, C-6, C-8, C-9, C-17, 7-*C*OMe	H-4, H-8
8	2.00 *m*	35.2	H-7, H-9	C-6, C-7, C-9, C-10	H-5, H-7, H-9, H-17a
9	4.74 *dd* (7.2, 4.4)	74.6	H-8	C-8, C-10, C-11, C-18, C-19, 9-*C*OMe	H-8, H-19, H-11
10		40.4			
11	5.85 *d* (16.0)	139.3	H-12	C-9, C-10, C-13, C-18, C-19	H-5, H-9, H-13, H-19
12	5.54 *dd* (16.0, 9.3)	130.0	H-11, H-13	C-10, C-13, C-14, C-20	H-18
13	3.48 *dq* (9.3, 6.6)	43.7	H-12, H-20	C-11, C-12, C-14, C-20	H-5, H-11, H-20
14		210.9			
15		92.6			
16	1.49 *s*	18.0		C-1, C-2, C-3	H-1b
17a	5.21 *s*	111.7	H-5	C-5, C-6, C-7	H-8
17b	5.15 *s*		H-5	C-5, C-6, C-7	H-3
18	1.06 *s*	21.8		C-9, C-10, C-11, C-19	H-12
19	1.02 *s*	26.8		C-9, C-10, C-11, C-18	H-9, H-11
20	1.14 *d* (6.6)	19.5	H-13	C-12, C-13, C-14	H-13
Acetyls					
2-CO		170.4[a]			
2-CO*Me*	2.11 *s*[a]	21.0		2-*C*OMe	
3-CO		168.9			
3-CO*Me*	2.14 *s*	21.3[b]		3-*C*OMe	
5-CO		169.4			
5-CO*Me*	2.00 *s*[b]	20.8[d]		5-*C*OMe	
7-CO		169.4			
7-CO*Me*	2.12 *s*[b]	22.1[e]		7-*C*OMe	
9-CO		169.9			
9-CO*Me*	2.14 *s*	21.3[c]		9-*C*OMe	
15-CO		170.3[c]			
15-CO*Me*	2.00 *s*[d]	21.0		15-*C*OMe	

[a–e] δ values are interchangeable.

12, H-13 and H-20 indicated that the keto group must be sited at C-14. In addition, in the HMBC spectrum, correlations were observed between the carbonyl carbon signals of the acetyl groups and H-3, H-5, H-7 and H-9. Unfortunately, unambiguous assignment of the ^1H and ^{13}C NMR signals of the acetyl groups was not possible because the carbon signals of 5-*C*OMe and 7-*C*OMe and the proton signals of 3-CO*Me* and 9-CO*Me* were the same, and the acetyl groups attached to the quaternary carbons (2-OAc and 15-OAc) could not be differentiated in the absence of long-range correlations.

The relative stereochemistry of esulatin D was studied by means of the NOESY spectrum. A convenient point of reference was H-4, which was assumed to be α. Cross-peaks between H-4 and H-3 proved the β orientation of the acetyl group on C-3. The zero coupling constant between H-4 and H-5 required that H-5 was β as in esulatin A–C. In the NOESY spectrum, correlation signals between H-4 and H-7 revealed the presence of a β-oriented ester group at C-7. The proton signal at δ_H 5.94 (H-5) correlated with the proton signal at δ_H 5.85 (H-11), which indicated that H-11 is oriented above the plane of the macrocyclic ring. The cross-peak observed between H-11 and H-13 dictates the α orientation of the methyl group on C-13. H-12, whose orientation is the opposite of that of H-11, showed an NOE interaction with one of the geminal methyl groups on C-10 (δ_H 1.06). Thus, this methyl group (C-18) is in the α position and the other (C-19) is in the β position. Correlative signals between H-19 and H-9 indicated the presence of an α-oriented acetyl group on C-9. In the NOESY spectrum, no correlation was observed between H-4 and 15-OAc, which suggested the β orientation of the acetyl group on C-15. With regard to the above data, the structure of esulatin D is formulated as **1**. The stereochemistry and absolute configuration of esulatin D were also studied by means of X-ray analysis [8]. The crystallographic data corroborated the structure elucidated above.

Esulatin E (**2**) was obtained as an amorphous white solid. Its molecular formula was assigned by HR mass spectrometry and NMR analyses as $C_{28}H_{36}O_{10}$. In the EI mass spectrum, a molecular ion was observed at *m/z* 532, with prominent fragment ions at *m/z* 490, 430, 370 and 310, representing losses of acetic acid and a ketene unit. Fragment peaks were also seen at *m/z* 123 and 96, corresponding to the ions $(CH_3)_2C{=}CH{-}CH{=}CCH_3C{=}O^+$ and $(CH_3)_2C{=}CH{-}CH{=}CHCH_3^+$, which were previously found to be characteristic of jatroph-11-ene-9,14-dione derivatives [7]. The ^1H and ^{13}C NMR spectra of esulatin E showed signals corresponding to four acetyl groups [δ_H 2.17 *s*, 2.15 *s*, 2.03 *s* and 2.02 *s*; δ_C 170.5, 170.1, 169.7 and 168.8 (*C*O) and 22.2, 21.4, 21.1 and 20.8 (*C*H$_3$)]. Additionally, the ^{13}C NMR spectrum exhibited resonances for six quaternary carbons, including two ketones (δ_C 208.3 and 202.8), four olefinic carbons, two ester-bearing and two alkyl-substituted methines, two methylenes and four methyls. The ^1H NMR and ^1H-^1H COSY spectra revealed the presence of three tertiary methyls (δ_H 1.64 *s*, 1.27 *s* and 1.23 *s*) and structural elements —C$^1H_2$— (δ_H 3.46 *d* and 2.13 *d*) (A), —C^3*H*R—C^4*H*—C^5*H*R—C^6(C$^{17}H_2$)—C^7*H*=C^8*H*— (δ_H 5.33 *d*, 2.21 *dd*, 6.03 *d*, 5.37 *s*, 6.71 *d* and 6.58 *d*) (B) and —C^{11}H=C^{12}H—C^{13}H(C$^{20}H_3$)— (δ_H 5.70 *d*, 5.42 *dd*, 3.60 *dq* and 1.16 *d*) (C) (R = acetyl). Fragment B was elucidated with the aid of the ^4J couplings observed between H-5, H-7 and the exomethylene (H-17). Connection of sequences A, B and C was performed by means of HMBC experiment (Table 2). The correlations of the quaternary carbons (Figure 1) supported the structure of esulatin E as 2,3,5,15-tetraacetoxyjatropha-6(17),7*E*,11*E*-triene-9,14-dione.

The relative configuration of esulatin E was investigated by means of NOESY measurements. The observation of an NOE effect between H-3 and H-4 revealed a β-oriented ester group on C-3. The relative small coupling constant between H-4 and H-5 indicated that, similarly as in esulatin A, B and D, H-5 is in the β position [7]. The coupling constants of H-7, H-8, H-11 and H-12 (16.4 and 15.9 Hz) proved that the C-7/C-8 and C-11/C-12 double bonds have the *E* configuration. The cross-peaks between H-4 and H-8 and between H-8 and H-11 suggested that H-8 and H-11 are oriented below the plane of the molecule. NOESY correlations concerning the stereochemistry of C-2, C-13 and C-15 were not found, but on the basis of the close relationship with esulatin A, B and D, the β orientation of H-13 and the 15-OAc and 16-methyl groups seems likely. The above data led to the formulation of esulatin E as **2**. The ^1H and ^{13}C NMR chemical shifts of **2** were assigned by means of ^1H-^1H COSY, HMQC and HMBC spectral analysis, as listed in Table 2.

Previous phytochemical studies on *E. esula* resulted in the isolation of diterpenes with ingenane, lathyrane and jatrophane skeletons [5, 9–13]. In the collection of Hungarian plants which we investigated, ingenane and lathyrane diterpenoids were not found and the isolated jatrophanes were different from those reported earlier from North American collections [5, 12, 13]. In the esulatin series obtained from the Hungarian collection, aromatic acyl residues were missing and the alcohol core of the compounds is new. This variation of jatrophane diterpenoids may be of chemotaxonomic significance.

EXPERIMENTAL

General experimental procedures. Mp are uncorr. UV: MeOH; EIMS: 70 eV, direct inlet; ^1H and ^{13}C NMR and DEPT: 400 MHz (^1H) and 100 MHz (^{13}C), $CDCl_3$, with TMS as int. standard. NOESY and 2D ^1H-^1H and ^{13}C-^1H correlation spectra were obtained using standard Bruker software. Optical rotations were determined in $CHCl_3$ at ambient temp; CC: polyamide (ICN). VLC: silica gel (Kieselgel GF_{254} 15 μm, Merck). HPLC: LiChrospher RP-18 and LiChrospher

Table 2. NMR Spectral Data of **2** [$CDCl_3$, TMS, δ (ppm), (J = Hz)]

Atom	^1H	^{13}C	^1H-^1H COSY	HMBC	NOESY
1a	3.46 *d* (15.9)	46.2	H-1b	C-2, C-3, C-4, C-14	H-1b
1b	2.13 *d* (15.9)		H-1a	C-14, C-15, C-16	H-1a, H-16
2	—	86.5	—	—	—
3	5.33 *d* (6.9)	78.6	H-4	C-2, C-15, 3-*C*OMe	H-4
4	2.21 *dd* (6.9, 3.5)	48.1	H-3, H-5	—	H-3, H-5, H-8
5	6.03 *d* (3.5)	69.6	H-4, H-17	—	H-4, H-7, H-8, H-17
6	—	144.0	—	—	—
7	6.71 *d* (16.4)	136.5	H-8, H-17	C-5, C-8, C-9	H-5, H-17
8	6.58 *d* (16.4)	129.6	H-7	C-6, C-7, C-9	H-4, H-5, H-11, H-18
9	—	202.8	—	—	—
10	—	49.4	—	—	—
11	5.70 *d* (15.9)	139.2	H-12	C-10, C-13, C-18, C-19	H-8, H-13, H-18, H-19, H-20
12	5.42 *dd* (15.9, 9.0)	131.1	H-11, H-13	C-10	H-13, H-18, H-19, H-20
13	3.60 *dq* (9.0, 6.7)	45.5	H-12, H-20	—	H-11, H-12, H-20
14	—	208.3	—	—	—
15	—	89.9	—	—	—
16	1.64 *s*	19.8	—	C-1, C-2, C-3	H-1b
17	5.37 *s* (2 H)	118.6	H-5, H-7	C-5, C-6, C-7	H-5, H-7
18	1.27 *s*	24.4	—	C-9, C-10, C-11, C-19	H-11, H-12, H-8
19	1.23 *s*	23.0	—	C-9, C-10, C-11, C-18	H-11, H-12
20	1.16 *d* (6.7)	17.8	H-13	C-12, C-13, C-14	H-11, H-12, H-13
Acetyls					
2-CO	—	170.1[c]	—	—	—
2-CO*Me*	2.17 *s*[a]	21.4[b]	—	2-*C*OMe	—
3-CO	—	169.7	—	—	—
3-CO*Me*	2.02 *s*	20.8	—	3-*C*OMe	—
5-CO	—	170.5[c]	—	—	—
5-CO*Me*	2.03 *s*[a]	22.2[a]	—	5-*C*OMe	—
15-CO	—	168.8[c]	—	—	—
15-CO*Me*	2.15 *s*[a]	21.1[b]	—	15-*C*OMe	—

[a,b,c] δ values are interchangeable.

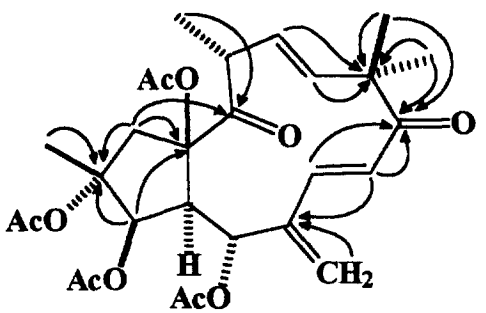

Fig. 1. HMBC correlations of quaternary carbons of esulatin E (**2**) (H → C).

Si 100 (5 μm), flow 0.5 ml/min^{-1}, with RI detection; TLC: Si gel 60 F_{254}.

Plant material. E. esula was collected in May 1994 in Szeged, Hungary, on the banks of the Tisza River and identified by Károly Penszka (Department of Botany and Plant Physiology, Agricultural University of Gödöllö, Hungary). A voucher specimen has been deposited at the Herbarium of the Museum of Natural Sciences in Budapest, Hungary.

Extraction and isolation. The fresh and entire plants of *E. esula* (11 kg) were extracted with MeOH (75 l) at room temp. The crude extract was concd *in vacuo* and partitioned between CH_2Cl_2 (7 × 1.5 l) and H_2O. On evapn, the organic phase residue (130 g) was obtained, which was chromatographed over a polyamide column (600 g) with mixts of H_2O–MeOH (4:1, 3:2, 2:3, 1:4) as eluents. The combined frs 1–15 (15 g) were subjected to silica gel VLC (VLC I) using a gradient system of cyclohexane–Me_2CO (19:1, 9:1, 4:1, 7:3, 1:1, 3:7). Frs eluted with cyclohexane–Me_2CO (4:1) were transferred repeatedly to a Silica gel VLC (VLC II) and successively eluted with $CHCl_3$–MeOH mixts of increasing polarity. Frs 22–23 obtained from VLC II with $CHCl_3$–MeOH (100:0.3) were further purified by prep. TLC on Si gel using n-hexane–THF–MeCN (20:5:1) as solvent and by HPLC using normal phase column and cyclohexane–EtOAc–EtOH (30:10:1) as eluent to yield 5.1 mg of esulatin E (**2**). Frs 28–30, which were eluted from VLC II with $CHCl_3$–MeOH (100:0.3), after HPLC purification on reverse phase column using MeOH–H_2O (7:3) as eluent afforded esulatin D (**1**) (10 mg).

Esulatin D (**1**). Colourless crystals from MeOH; mp 154–5°. $[\alpha]_D^{25}$ −99 (*c*, 0.6, $CHCl_3$). UV λ_{max} nm (log ε): 216 (3.26), 296 (2.19). EIMS, *m/z* (rel. int.): 636 $[M]^+$ (6), 576 $[M-HOAc]^+$ (3), 516 $[M-2\times HOAc]^+$ (3), 386 $[M-3\times HOAc-CH_2CO-CO]^+$ (13), 43 $[MeCO]^+$ (100). HRMS: *m/z*: 516.2406 $[M-2\times HOAc]^+$ $C_{28}H_{36}O_9$ required 516.2359. ^1H and ^{13}C NMR: see Table 1.

Esulatin E (**2**). Amorphous white solid. $[\alpha]_D^{25}$ −95

(c, 0.1, $CHCl_3$). UV λ_{max} nm (log ε): 204 (2.72), 267 (2.77). EIMS, m/z (rel. int.): 532 $[M]^+$ (0.3), 490 $[M-CH_2CO]^+$ (3), 430 $[M-HOAc-CH_2CO]^+$ (3), 370 $[M-2\times HOAc-CH_2CO]^+$ (6), 310 $[M-3\times HOAc-CH_2CO]^+$ (15), 123 $[C_8H_{11}O]^+$ (23), 96 $[C_7H_{12}]^+$ (27), 43 $[MeCO]^+$ (100). HRMS, m/z: 430.1972 $[M-HOAc-CH_2CO]^+$ $C_{24}H_{30}O_7$ required 430.1992. ^1H and ^{13}C NMR: see Table 2.

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