Macrocyclic Diterpene Polyesters of the Jatrophane Type from *Euphorbia esula*

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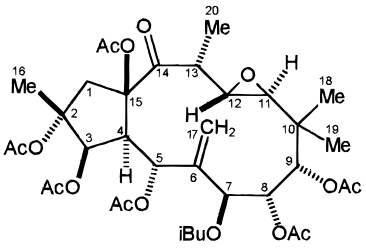
Three new jatrophane diterpenes, esulatins A−C (**1**−**3**) were isolated and characterized from the whole, undried plant of *Euphorbia esula*. By means of spectral analysis, the structures were established as pentaesters and heptaesters of hitherto unknown, polyfunctional diterpene parent alcohols. Esulatins A (**1**) and C (**3**) are the diterpenoids with the highest degree of esterification identified to date from the family Euphorbiaceae.

Euphorbia esula L. or leafy spurge (Euphorbiaceae) is distributed worldwide and contains a skin-irritant, toxic, milky latex.[1] Extracts of the plant have been widely used in folk medicine to treat various cancers, swellings, and warts.[2] Previous phytochemical and pharmacological studies demonstrated the proinflammatory, tumor-promoting, and antitumor activity of the plant extracts, and three ingenane diterpenoids, ingenol 3,20-dibenzoate, ingenol 3-dodecanoate, and ingenol 3-$\Delta^{2,4,6,8,10}$-pentene tetradecanoate, were found to be responsible for these activities.[3−5]

Besides ingenol esters, macrocyclic diterpenes have also been isolated from *E. esula*, namely, the jatrophane esters, esulons A, B, and C, with moderately toxic and mildly inflammatory effects from leafy spurge roots collected in North Dakota, and lathyrane and jatrophane triesters from seeds collected in Canada.[6−8] All previous studies suggested a high variation of the diterpene compounds in *E. esula*, and significant differences were found between North American and European accessions.[7]

As part of our studies on biologically active compounds from the family Euphorbiaceae, we have examined a Hungarian population of *E. esula* for its diterpene constituents. This paper deals with the isolation and structure elucidation of three new jatrophane esters, named esulatins A, B, and C (**1**−**3**).



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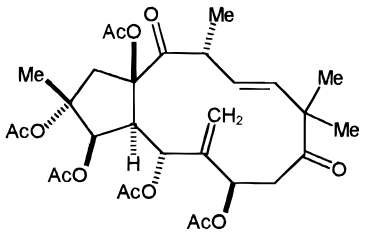

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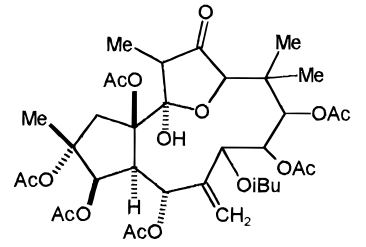

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Results and Discussion

The dichloromethane phase of a MeOH extract of the whole, undried plant of *E. esula* was fractionated by column chromatography on polyamide, then on Si gel, and further purified by preparative TLC and HPLC to afford esulatins A, B, and C (**1**−**3**).

Esulatin A (**1**) was shown by elemental analysis and ESIMS to have the molecular formula $C_{36}H_{50}O_{16}$. The ^{1}H- and ^{13}C-NMR spectra of **1** revealed the presence of six acetate groups [δ_H 2.16 s, 2.12 s, 2.11 s, 2.11 s, 2.04 s, 2.03 s; δ_C 170.0, 169.7, 169.5, 169.1, 168.6, 168.3 (*C*O) and 22.2, 21.3, 21.2, 21.2, 20.8, 20.5 (*C*H$_3$)] and one isobutanoate group [δ_H 2.60 sept (C*H*), 1.21 d, 1.18 d (C*H*$_3$); δ_C 175.0 (*C*O), 33.8 (*C*H), 19.1, 18.4 (*C*H$_3$)] (Table 1). The ^{13}C-NMR and DEPT spectra suggested that the skeleton consisted of 20 carbons: four methyls, two methylenes, nine methines, and five quaternary carbons, including one ketone (δ_C 210.3). The ^{1}H-NMR spectrum contained 17 signals due to the parent skeleton, which were assigned with the aid of HMQC and ^{1}H−^{1}H COSY experiments. The ^{1}H−^{1}H COSY spectrum defined two structural fragments with correlated protons: −CH$_2$−CR$_2$−CHR−CHR−CHR−C(=CH$_2$)−CHR−CHR−CHR− (A) and −CH(CH$_3$)−CHR−CHR− (B). Their connectivities were determined from the long-range C−H correlations observed in an HMBC spectrum (Table 1). The long-range correlations of the quaternary carbons (C-6, C-10, C-2, and C-15) with protons of the two fragments established fragment A

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

atom	^{1}H	^{13}C	^{1}H−^{1}H COSY	HMBC	NOESY
1a	3.73 d (16.2)	46.2	H-1b, H-3	C-2, C-3, C-4, C-14	H-16
1b	2.04 d (16.2)		H-1a, H-3	C-15	H-16
2		86.7			
3	5.58 br d (3.3)	78.0	H-1a,b, H-4	C-1, C-2, C-15, Ac δ 168.6	H-7, H-16, H-17a, H-4
4	2.98 dd (3.3, 1.7)	49.8	H-3, H-5	C-3, C-14	H-3, H-7
5	6.06 br s	67.6	H-4, H-17a	C-3, C-4, C-6, C-15, C-17, Ac δ 168.3	H-7, H-8, H-12, H-13, H-17a
6		141.7			
7	5.38 br s	68.5	H-8, H-17b	C-1′, C-6, C-9, C-17, i-Bu-CO	H-3, H-4, H-5, H-8, H-9, H-11
8	5.51 d (4.1)	69.0	H-7, H-9	C-6, C-9, C-10, Ac δ 169.5	H-4, H-5, H-11, H-12, H-19
9	4.95 d (4.1)	77.3	H-8	C-8, C-10, C-11, C-18, C-19, Ac δ 169.1	H-7, H-11
10		39.6			
11	3.00 d (2.1)	58.4	H-12	C-10, C-18, C-19	H-7, H-8, H-9, H-18, H-19, H-20
12	3.32 dd (4.7, 2.1)	57.1	H-11, H-13	C-13, C-14	H-5, H-8
13	3.67 dq (4.7, 6.9)	37.0	H-12, H-20	C-14, C-20	H-5
14		210.3			
15		92.6			
16	1.52 s	18.1		C-1, C-2, C-3	H-1a,b, H-3
17a	5.09 s	111.9	H-5	C-7	H-3, H-5
17b	5.05 s		H-7		
18	0.99 s[d]	23.5		C-9, C-10, C-11, C-19	H-11
19	0.71 s[d]	17.5		C-9, C-10, C-11, C-18	H-8, H-11
20	1.18 d (6.9)	15.3	H-13	C-12, C-13, C-14	H-11
i-Bu					
1′		175.0			
2′	2.60 sept (7.0)	33.8	H-3′,4′	C-1′, C-3′, C-4′	
3′	1.21 d (7.0)	19.1	H-2′	C-1′, C-2′, C-4′	
4′	1.18 d (7.0)	18.4	H-2′	C-1′, C-2′, C-3′	
Acetyls					
2-CO		169.7[b]			
2-COMe	2.11 s[a]	22.2[c]		2-CO	
3-CO		168.6			
3-COMe	2.11 s[a]	21.2[c]		3-CO	
5-CO		168.3			
5-COMe	2.16 s	21.3		5-CO	
8-CO		169.5			
8-COMe	2.03 s	20.5		8-CO	
9-CO		169.1			
9-COMe	2.04 s	20.8		9-CO	
15-CO		170.0[b]			
15-COMe	2.12 s[a]	21.2		15-CO	

[a−d] δ values are interchangeable.

and B to be C-1−C-9 (with an exomethylene C-17 on C-6) and C-13−C-11 of a jatrophane diterpene, respectively. The $^2J_{CH}$ and $^3J_{CH}$ correlations between H-13, H-12, H-1β, and the carbon signal at δ_C 210.3 placed the keto group at C-14. The positions of ester groups were also established via an HMBC experiment. The correlation of the carbonyl signal at δ_C 175.0 (isobutanoyl *C*O) with the proton signals at δ_H 5.38 (H-7) and δ_H 1.21, 1.18 (methyl signals of isobutanoyl) indicated the presence of the isobutanoyl group at C-7. Similarly, the long-range couplings of the carbonyl carbon signals at δ_C 169.5, 169.1, 168.6, and 168.3 with the proton signals at δ_H 5.51 (H-8), 4.95 (H-9), 5.58 (H-3), and 6.06 (H-5) and the acetyl methyl signals at δ_H 2.03, 2.04, 2.11, and 2.16 demonstrated the presence of acetyl groups on C-8, C-9, C-3, and C-5, respectively. The two remaining acetyl groups did not show any long-range correlations, and it was therefore supposed that they were attached to quaternary carbons. When the ^{1}H-NMR data of **1** were compared with those of analogous compounds having 2,3,15-triacyl substitution on the five-membered ring, similar δ values for H-1 and H-3 were found.[9] Thus, the two acetyl groups (δ_H 2.11 and 2.12) were assigned to positions C-2 and C-15. With regard to the molecular formula and substituents mentioned above, the presence of a further epoxy group in the molecule was concluded. Chemical shift values of H-11, H-12 (δ_H 3.00 d and 3.32 dd), C-11, and C-12 (δ_C 58.4, 57.1) indicated that the epoxy group must be at position C-11, C-12.

The stereochemistry and absolute configuration of esulatin A were studied by NOESY measurements (Table 1) and X-ray analysis. On the basis of crystallographic investigations esulatin A was elucidated as (2*R*,3*R*,4*S*,5*R*,7*S*,8*R*,9*S*,11*R*,12*S*,13*R*,15*R*)-2,3,5,8,9,15-hexaacetoxy-11,12-epoxy-7-(isobutanoyloxy)jatroph-6(17)-en-14-one (**1**); data are published elsewhere.[10] The structure of **1** is very similar to that of kansuinin B, isolated from *Euphorbia kansui*, which has been shown to possess analgesic and anti-writhing activities.[11]

Esulatin B had a molecular formula of $C_{30}H_{40}O_{12}$, obtained from HRMS and NMR analyses. Its EIMS spectrum exhibited fragment ion peaks due to the sequential loss of HOAc and ketene units, indicating the presence of five acetyl groups in the molecule (see Experimental Section). Accordingly, the ^{1}H- and ^{13}C-NMR spectra of esulatin B contained signals corresponding to five acetyl groups [δ_H 2.16 s, 2.14 s, 2.13 s, 2.11 s, and 2.05 s, δ_C 170.4, 170.1, 169.7, 169.6, 169.0 (*C*O), and 22.1, 21.4, 21.2, 20.9, and 20.8 (*C*H$_3$)]. Additionally, the ^{13}C-NMR and DEPT spectra exhibited resonances for four methyls, three methylenes, seven methines, and six quaternary carbons, including two keto groups (δ_C 211.1 and 209.4) (Table 2). The ^{1}H-NMR and ^{1}H−^{1}H COSY spectra revealed the presence of three tertiary methyls (δ_H 1.48 s, 1.17 s, 1.16 s) and the structural elements −CH$_2$CR$_2$CHRCHRCHRC-(=CH$_2$)CHRCH$_2$− (A) and *trans* −CH(CH$_3$)CH=CH (B). Fragment A was elucidated with the aid of the ^4J couplings observed between H-1 and H-3, and between

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

atom	^{1}H	^{13}C	^{1}H−^{1}H COSY	HMBC	NOESY
1a	3.82 dd (16.0, 1.0)	46.6	H-1b, H-3	C-2, C-3, C-4, C-14, C-15	H-1b
1b	1.91 d (16.0)		H-1a	C-2, C-14, C-15, C-16	H-1a
2		86.2			
3	5.51 d (3.3)	78.3	H-1a, H-4	C-2, C-15, Ac δ 169.0	H-16, H-4, H-17
4	2.91 dd (3.5, 1.0)	49.7	H-3, H-5	C-3, C-6, C-14, C-15	H-3, H-5, H-7
5	5.80 s	68.2	H-4, H-17	C-3, C-4, C-6, C-7, C-15, C-17, Ac δ 169.7[b]	H-4, H-7, H-8b, H-11, H-13
6		147.0			
7	4.94 d (9.6)	69.5	H-8b, H-8a, H-17	C-5, C-6, C-8, C-9, C-17, Ac δ 169.6[b]	H-4, H-5, H-19
8b	3.21 dd (14.0, 1.0)	45.4	H-7, H-8a	C-6, C-7, C-9	H-8a, H-5, H-11
8a	2.28 dd (14.0, 9.6)		H-7, H-8b	C-7, C-9	H-8b
9		209.4			
10		49.3			
11	6.07 d (15.9)	137.2	H-12	C-9, C-10, C-13, C-18, C-19	H-5, H-8b, H-13, H-18
12	5.52 dd (15.9, 9.6)	133.2	H-11, H-13	C-10, C-14	H-19, H-20
13	3.56 dq (9.6, 6.6)	44.7	H-12, H-20	C-11, C-12, C-14	H-11, H-5, H-20
14		211.1			
15		92.1			
16	1.48 s	17.8		C-1, C-2, C-3	H-3, H-1b
17	5.10 d (1.0)	110.1	H-5, H-7	C-5, C-6, C-7	H-3
	5.11 d (1.0)			C-5, C-6, C-7	H-3
18	1.17 s[d]	26.7		C-9, C-10, C-11, C-19	
19	1.16 s[d]	23.0		C-9, C-10, C-11, C-18	
20	1.12 d (6.6)	20.2	H-13	C-12, C-13, C-14	H-12, H-13
Acetyls					
2-CO		170.1			
2-COMe	2.13 s	20.9			
3-CO		169.0			
3-COMe	2.16 s	21.4		3-CO	
5-CO		169.7[b]			
5-COMe	2.05 s[a]	20.8[c]		5-CO	
7-CO		169.6[b]			
7-COMe	2.14 s[a]	21.2[c]		7-CO	
15-CO		170.4			
15-COMe	2.11 s	22.1			

[a−d] δ values are interchangeable.

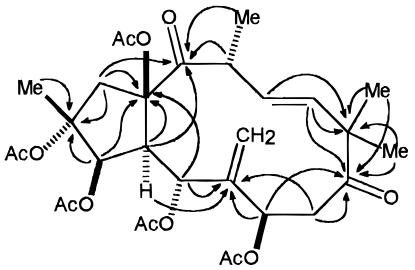

Figure 1. HMBC correlations of quaternary carbons of esulatin B (**2**) (H→C).

H-5, H-7 and the exomethylene (H-17). The connection of the partial structures was clarified by means of an HMBC experiment. The correlations of the quaternary carbons (see Figure 1) to proximate protons supported the structure of esulatin B as 2,3,5,7,15-pentaacetoxy-jatropha-6(17),11-diene-9,14-dione.

The stereochemistry of esulatin B was investigated by X-ray analysis and NOESY measurements. From crystallographic data the same configuration of C-2, C-3, C-4, C-5, C-7, C-13, C-15 was deduced as that of esulatin A (**1**), and the structure of esulatin B was formulated as **2**.[12] NOESY correlations were in accordance with the stereochemistry elucidated from X-ray investigations. The full ^{1}H- and ^{13}C-NMR chemical shift assignments of **2** were carried out through ^{1}H−^{1}H COSY, HMQC, HMBC, and NOESY spectral analysis, as listed in Table 2. The EIMS spectrum of esulatin B (**2**) revealed significant fragments, which seem to be characteristic of jatroph-11-ene-9,11-dione derivatives. Peaks at m/z 123 (52%) $C_8H_{11}O$ (found 123.0794, calcd 123.0810) and m/z 96 (70%) C_7H_{12} (found 96.0934, calcd 96.0939) corresponding to the ions $(CH_3)_2C{=}CH{-}CH{=}CCH_3C{=}O^+$ and $(CH_3)_2C{=}CH{-}CH{=}CHCH_3^+$, were originated from the part of the molecule between the two keto groups.

Esulatin C, a minor diterpenoid of *E. esula*, was revealed by HRMS and NMR analyses to have the molecular formula $C_{36}H_{50}O_{17}$. The ^{1}H-NMR spectrum indicated the presence of seven ester groups: six acetate groups (δ_H 2.16 s, 2.11 s, 2.06 s, 2.02 s, 2.00 s, and 1.99 s) and one isobutanoate group [(δ_H 2.61 sept (C*H*), 1.23 d, 1.16 d (CH_3)]. Additionally, the ^{1}H-NMR spectrum exhibited signals attributed to four methyls (δ_H 1.56 s, 1.24 d, 1.24 s, 1.12 s), one methylene (δ_H 3.10 d, 2.80 d), one methyl-bearing methine (δ_H 2.17 dq) and, in the range δ_H 6.49−3.28, seven singlets and three doublets with coupling constants 3.5 and 1.9 Hz (Table 3). Correlations between these latter signals were detected in the ^{1}H−^{1}H COSY spectrum. Relationships between the signals at δ_H 6.02 (H-3), 3.28 (H-4), and 6.20 (H-5) and at δ_H 6.49 (H-7), 5.65 (H-8), and 4.94 (H-9) were clearly defined. Further, in the ^{1}H−^{1}H COSY spectrum ^4J couplings were detected between the signals at δ_H 3.10 and 2.80 (H-1) and 6.02 (H-3); at δ_H 6.20 (H-5), 6.49 (H-7), 5.04, and 4.95 (H-17a,b); at δ_H 4.94 (H-9) and 4.13 (H-11); at δ_H 4.13 (H-11) and 2.17 (H-13); and at δ_H 2.17 (H-13) and 3.99 (14-OH). These data are compatible with a jatrophane diterpene substituted on C-2, C-3, C-5, C-7, C-8, C-9, C-11, C-12, C-14, and C-15.

The structure of esulatin C was further studied by means of HMQC and HMBC experiments. From the HMQC spectrum the chemical shifts of protonated carbons were assigned as listed in Table 3. On the basis of the HMBC experiment, the presence of six quaternary carbons attributed to the parent skeleton was detected. Cross-peaks between H-1, H-3, H-16, and the signals at δ_C 88.5 (C-2) and between H-1, H-4, H-5, and δ_C 89.9

Table 3. NMR Spectral Data of Esulatin C (**3**) [$CDCl_3$, TMS, δ (ppm) (J = Hz)]

atom	^{1}H	^{13}C	^{1}H−^{1}H COSY	HMBC	NOESY
1a	3.10 d (16.5)	43.6	H-1b, H-3	C-2, C-3, C-4, C-14	H-1b, H-20
1b	2.80 d (16.5)		H-1a, H-3	C-14, C-15, C-16	H-1a, H-16
2		88.5			
3	6.02 d (3.5)	74.2	H-1b, H-4	C-2	H-4, H-16
4	3.28 d (3.5)	49.7	H-3, H-5	C-3, C-5, C-6, C-14, C-15	H-3, H-5, H-7, 14-OH, H-16
5	6.20 s	69.4	H-4, H-7, H-17a,b	C-3, C-4, C-6, C-7, C-15	H-4, H-8
6		147.0			
7	6.49 s	67.8	H-5, H-8, H-17a,b	C-6, C-8, C-9, C-17, C-1′	H-4, H-8, H-11
8	5.65 s	70.9	H-7, H-9	C-6, C-9, Ac δ 170.5	H-5, H-7, H-9, H-18
9	4.94 s	82.6	H-8, H-11	C-8, C-10, C-11, C-19, Ac δ 169.6	H-8, H-18, H-19
10		41.4			
11	4.13 s	77.1	H-9, H-13	C-9, C-10, C-12, C-18	H-7, H-19
12		213.6			
13	2.17 dq (7.0, 1.9)	21.6	H-11, 14-OH, H-20	C-12, C-14, C-20	
14		105.7			
14-OH	3.99 d (1.9)		H-13		H-4
15		89.9			
16	1.56 s	18.3		C-1, C-2, C-3	H-1b, H-3
17a	5.04 s	107.9	H-5, H-7, H-17b	C-5, C-6, C-7	
17b	4.95 s		H-5, H-7, H-17a	C-5, C-6, C-7	
18	1.24 s[a]	18.9		C-10, C-11, C-19	H-8, H-9
19	1.12 s[a]	22.4		C-9, C-10	H-11
20	1.24 d (7.0)	9.6	H-13	C-12, C-14	H-1a, H-5
i-Bu					
1′		176.6			
2′	2.61 sept (7.0)	34.2		C-1′, C-4′	
3′	1.16 d (7.0)	18.8		C-1′, C-4′	
4′	1.23 d (7.0)	19.8		C-1′, C-3′	
Acetyls					
8-CO		170.5			
8-COMe	2.06 s	21.2		8-CO	
9-CO		169.6			
9-COMe	1.99 s	22.2		9-CO	
2,3,5,15-CO		169.6			
		168.9			
		168.6			
		167.5			
2,3,5,15-COMe	2.02 s	21.1		Ac δ 169.6	
	2.16 s	22.8		Ac δ 168.9	
	2.11 s	21.8		Ac δ 168.6	
	2.00 s	21.2		Ac δ 167.5	

[a] δ values are interchangeable.

(C-15) suggested that a similarly substituted five-membered ring is present in the molecule as in esulatin A (**1**). Furthermore, in the HMBC spectrum a series of correlations was seen between H-5−H-11 and the surrounding carbons via two or three bonds, which confirmed an identical C-5−C-11 partial structure and esterification in positions C-5, C-7, C-8, and C-9 as in **1**. A difference between the two compounds was found in the sequence C-12−C-14. Correlations between H-11, H-13, H-20, and the carbon signal at δ_C 213.6 indicated that a keto group must be located at C-12. On the basis of its long-range couplings with H-1, H-4, H-13, and H-20, the quaternary carbon signal at δ_C 105.7 was assigned to C-14. A hydroxyl group was placed on C-14 because its doublet signal (δ_H 3.99) coupled to H-13 (J = 1.9 Hz). Judging from the quaternary carbon signal at δ_C 105.7 (C-14) and the methine signal at δ_H 4.13 (H-11), which was observed at higher field than for esterified methines,[13] an ether functionality (deduced from the molecular formula and substituents discussed above) must be sited between C-11 and C-14.

The relative stereochemistry of esulatin C was studied by NOESY measurements. NOE interactions and coupling constants of H-1, H-3, and H-4 are very similar to that of esulatins A and B, suggesting the same configuration of C-2, C-3, C-4, and C-15. The stereochemistry of C-4 and C-15 was also followed from the fact that all jatrophane diterpenes subjected to X-ray analysis exhibit a *trans* ring junction[6,11,14,15] and no NOE was observed between H-4 and 15-OAc. The zero coupling constant between H-4 and H-5 required that, similar to **1**, **2**, and kansuinin A, H-5 be β.[13] The NOE effect observed between OH-14 and H-4 indicated the presence of an α-hydroxyl group on C-14. The stereochemistry of C-7, C-8, C-9, C-11, and C-13 on the basis of NOESY correlations could not be determined because of the high flexibility of this part of the molecule. Thus, the structure of esulatin C was elucidated as shown in formula **3**. Esulatin C (**3**) displayed a close relationship to a diterpene constituent of *E. kansui*, kansuinin A.[13]

Esulatins A (**1**) and C (**3**) are the most highly esterified Euphorbiaceae diterpenoids identified to date. Presumably the isolation of such highly esterified compounds is associated with the use of fresh plant material. The parent diterpene alcohol found in **1**−**3** has not been described earlier. The isolated compounds are additional members of the small group of jatrophane diterpenoids, which are considered to be the most important taxonomic members in this family.[6−8] Jatrophane diterpenoids from North American accession of *E. esula* were reported earlier,[6] but this type of compound was isolated for the first time from a European accession. It may be of chemotaxonomic significance that compounds found in these two collections of leafy spurge are different.

In vitro primary antitumor screening on esulatin A (**1**) by the National Cancer Institute (Bethesda, MD) showed it to be inactive.

Experimental Section

General Experimental Procedures. Melting points were determined on a Boetius apparatus and are uncorrected. IR spectra were run as KBr disks on a Perkin-Elmer Paragon 1000 PC FTIR spectrometer. UV spectra in MeOH were obtained on a Shimadzu UV-2101 spectrophotometer. Mass spectral measurements were carried out on a Finnigan MAT 8430 spectrometer operating at 70 eV ionizing energy. The NMR spectra were recorded on a Bruker Avance DRX 400 spectrometer at 400 MHz (^{1}H) and 100 MHz (^{13}C), using $CDCl_3$ as solvent and TMS as internal standard. Optical rotations were determined in $CHCl_3$ at ambient temperature using a Perkin-Elmer 341 polarimeter. For column chromatography polyamide (ICN) and Si gel (Kieselgel GF_{254} 15 μm, Merck) were used. HPLC was carried out on a Waters Millipore instrument with RI detection on a normal-phase column (LiChrospher Si 100 5 μm, Merck). TLC was performed on Si gel 60 F_{254} plates using $CHCl_3$−Me_2CO (19:1), C_6H_6−$CHCl_3$−Et_2O (1:1:3) and cyclohexane−EtOAc−EtOH (20:10:1) as developing systems with visualization using 1% vanillin−H_2SO_4 spray reagent.

Plant Material. *Euphorbia 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 temperature. The crude extract was concentrated *in vacuo* and partitioned between CH_2Cl_2 (7 × 1.5 L) and H_2O. On evaporation, the organic-phase residue (130 g) was obtained, which was chromatographed over a polyamide column (600 g) with mixtures of H_2O−MeOH (4:1, 3:2, 2:3, 1:4) as eluents. Fractions 5−21 obtained with H_2O−MeOH (4:1) afforded a crystalline material upon standing, which was recrystallized from MeOH to yield esulatin A (**1**) (300 mg). The combined fractions 1−15 (15 g) were subjected to Si gel vacuum liquid chromatography (VLC) using a gradient system of cyclohexane−Me_2CO (19:1, 9:1. 4:1, 7:3, 1:1, 3:7). Fractions from cyclohexane−Me_2CO (4:1) were transferred repeatedly to a Si gel VLC and successively eluted with $CHCl_3$−MeOH mixtures of increasing polarity. From fractions obtained with $CHCl_3$−MeOH (99.7:0.3), 40 mg of esulatin B (**2**) was obtained as crystals. Fractions obtained with $CHCl_3$−MeOH (99:1) were further purified by preparative TLC on Si gel using C_6H_6−EtOAc (7:3) as solvent and by HPLC using cyclohexane−EtOAc−EtOH (30:10:1) as eluent (flow 0.5 mL/min), to yield 1.2 mg of esulatin C (**3**).

Esulatin A (1): colorless crystals from MeOH; mp 218−219 °C; $[\alpha]^{25}$D −82° (*c* 0.1, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 202 (4.73), 219 (sh, 4.08) nm; IR (KBr) ν_{max} 2983, 2939, 1749, 1468, 1426, 1372, 1321, 1210, 1149, 1129, 1085, 1026, 994, 934, 911, 873, 833, 809, 746, 659, 634, 610 cm^{-1}; ^{1}H and ^{13}C NMR, see Table 1; ESIMS *m/z* $[M + K]^+$ 777 (36), $[M + Na]^+$ 761 (40), $[M + NH_4]^+$ 756 (100); *anal.* C 58.08%, H 7.43%, calcd for $C_{36}H_{50}O_{16}$ C 58.51%, H 6.83%.

Esulatin B (2): colorless crystals from MeOH; mp 248−249 °C; $[\alpha]^{25}$D −101° (*c* 0.1, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 210 (4.88), 284 (3.89) nm; IR (KBr) ν_{max} 2984, 2939, 1741, 1659, 1431, 1374, 1251, 1229, 1133, 1099, 1042, 995, 926, 877, 805, 740, 629, 609 cm^{-1}; ^{1}H and ^{13}C NMR, see Table 2; EIMS *m/z* $[M]^+$ 592 (14), $[M - AcOH]^+$ 532 (3), $[M - AcOH - CH_2CO]^+$ 490 (6), $[M - 2 \times AcOH]^+$ 472 (6), $[M - 2 \times AcOH - CH_2CO]^+$ 430 (8), $[M - 3 \times AcOH]^+$ 412 (8), $[M - 3 \times AcOH - CH_2CO]^+$ 370 (26), $[M - 4 \times AcOH]^+$ 352 (8), $[M - 3 \times AcOH - 2 \times CH_2CO]^+$ 328 (16), $[M - 4 \times AcOH - CH_2CO]^+$ 310 (32), $[C_8H_{11}O]^+$ 123 (52), $[C_7H_{12}]^+$ 96 (70); HREIMS *m/z* 592.2631 (calcd for 592.2520 $C_{30}H_{40}O_{12}$) $[M]^+$.

Esulatin C (3): white amorphous solid; $[\alpha]^{25}$D +11° (*c* 0.06, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) nm 209 (4.84), 273 (4.02); IR (KBr) ν_{max} 3743, 2930, 1743, 1679, 1540, 1515, 1456, 1427, 1372, 1241, 1155, 1063, 1041 cm^{-1}; ^{1}H and ^{13}C NMR, see Table 3; EIMS *m/z* $[M - AcOH]^+$ 694 (22), $[M - i\text{-}BuOH]^+$ 666 (20), $[M - i\text{-}BuOH - OAc]^+$ 607 (53), $[M - i\text{-}BuOH - AcOH - CH_2CO]^+$ 564 (17), $[M - i\text{-}BuOH - 2 \times AcOH]^+$ 546 (38), $[M - i\text{-}BuOH - 2 \times AcOH - CH_2CO]^+$ 504 (43), $[M - 3 \times AcOH - CH_2CO - C_4H_4O]^+$ 476 (71), $[M - i\text{-}BuOH - 3 \times AcOH - CH_2CO]^+$ 444 (100), $[M - i\text{-}BuOH - 4 \times AcOH - CH_2CO]^+$ 384 (31), $[M - i\text{-}BuOH - 5 \times AcOH - CH_2CO]^+$ 324 (20); HREIMS *m/z* 694.2839 (calcd for 694.2837 $C_{34}H_{46}O_{15}$) $[M - AcOH]^+$, 666.2778 (calcd for 666.2524 $C_{32}H_{42}O_{15}$) $[M - i\text{-}BuOH]^+$.

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