Isolation and Structural Characterization of New, Highly Functionalized Diterpenes from *Euphorbia serrulata*

by **Dóra Rédei***[a]), **Judit Hohmann**[a]), **Ferenc Evanics**[b]), **Peter Forgo**[c]), **Pál Szabó**[d]), and **Imre Máthé**[a])

[a]) Department of Pharmacognosy, University of Szeged, Eötvös u. 6, H-6720 Szeged (tel.: 3662545558; fax: 3662545704; e-mail: redei@pharma.szote.u-szeged.hu)
[b]) Department of Pharmaceutical Analysis, University of Szeged, H-6720 Szeged
[c]) Department of Organic Chemistry, University of Szeged, H-6720 Szeged
[d]) Institute of Chemistry, Chemical Research Centre, Hungarian Academy of Sciences, H-1525 Budapest

Five new diterpene polyesters, **1**–**5**, with jatrophane skeletons were isolated from the fresh whole plants of *Euphorbia serrulata*. The structure elucidation was performed by means of UV/VIS spectroscopy, HR-ESI-MS, and advanced two-dimensional NMR methods, including ^{1}H-NMR, JMOD, ^{1}H,^{1}H-COSY, NOESY, HMQC, and HMBC experiments. The relative configurations of **1**–**5** and their conformations in solution were analyzed on the basis of NOESY measurements. As a result of detailed NMR studies, complete ^{1}H and ^{13}C chemical-shift assignments of the compounds were possible. The isolated compounds differ stereochemically and do not comprise a uniform series regarding the configurations at C(2), C(6), and C(13). Compound **5** possesses the new structural feature of a double bond with (*Z*)-configuration in the macrocyclic ring of the jatrophane skeleton, while compound **2** has a C=C bond in the five-membered ring, this being the first observation of this structural feature in the type of macrocyclic Euphorbiaceae diterpenes.

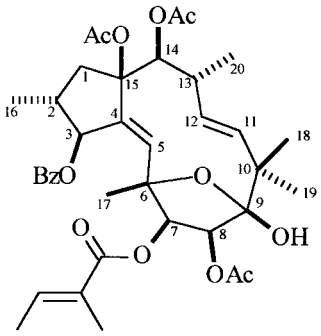
1. Introduction. – Plants of the Euphorbiaceae family display a high diversity of structurally unique diterpenoids, which have attracted great interest from biogenetic, synthetic, biological, and toxicological points of view. These diterpenoids are found exclusively within the Euphorbiaceae and Thymelaeaceae families and can be derived from the cembrene cation *via* transannular intramolecular cyclizations [1–5].

In the course of our search for biologically active compounds from Hungarian Euphorbiaceae, we have examined *Euphorbia serrulata* THUILL. (nom. illegit. *E. stricta* L.), an annual herb found in southern, central, and western countries in Europe [6]. We recently reported the isolation and structure determination of jatrophane diterpenes from the hexane-soluble extract of the fresh whole plants [7][8]. Biological studies on these compounds revealed that some of them exhibit significant multidrug-reversing activity on mouse lymphoma cells, moderate cytotoxicity on *Vero* cells, and a pronounced antiviral effect against *herpes simplex* virus type 2 [8][9].

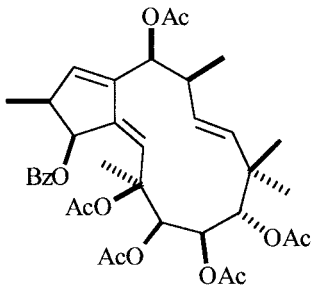
In continuation of our investigations on the chemical constituents of *E. serrulata*, we now report on the isolation and structural characterization of five diterpene polyesters, **1**–**5**, by high-resolution NMR spectroscopy and mass spectrometry. We also discuss the conformational behavior of the compounds, with an interpretation of the results of NOESY experiments.

2. Results and Discussion. – The MeOH extract of fresh whole plants of *E. serrulata* was subjected to solvent–solvent partitioning to furnish a hexane-soluble fraction. The

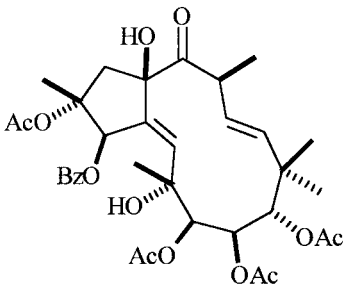
organic phase was chromatographed on an open polyamide column, and selected fractions from this chromatography were then further fractionated on NP and RP silica gel by vacuum liquid chromatography (VLC) and HPLC to afford compounds **1**–**5**.



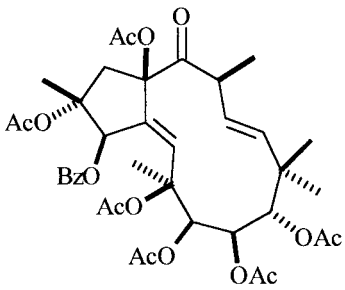
1



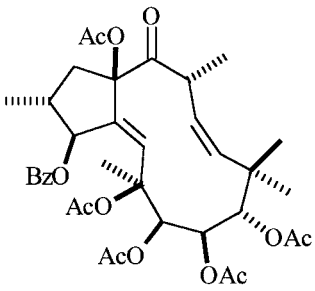
2



3



4



5

Compound **1** was obtained as an amorphous solid, with $[\alpha]_D^{25} = +88$ ($c = 0.05$, $CHCl_3$). The molecular formula $C_{38}H_{48}O_{12}$ was assigned by HR-ESI-MS from the *m*/*z* 829.2205 $[M + Cs]^+$ ion (calc. 829.2200, $\Delta = -0.6$ ppm). The ^{1}H-NMR and JMOD

spectra of **1** unequivocally indicated the presence of one benzoate, one tigliate, and three acetate groups (*Table 1*). Further analysis of the ^{1}H- and ^{13}C-NMR resonances revealed that **1** contains one disubstituted (δ(C) 139.5 and 134.5, and δ(H) 5.43 (*d*) and 5.36 (*dd*)) and one trisubstituted (δ(C) 133.5 and 139.8, and δ(H) 5.55 (*d*)) C=C bond. From the molecular formula, a degree of unsaturation of 11 was deduced, which (excluding the ester and olefin groups) required the presence of a tricyclic skeleton. The JMOD experiment confirmed a C_{20} diterpene skeleton involving four Me, one CH_2, and ten CH groups, and five quaternary C-atoms. The gradient ^{1}H,^{1}H-COSY and HMQC spectra showed one CH_2 group and four sequences of correlated protons: δ(H) 1.24 (*d*), 2.53 (*m*), 5.74 (*dd*), and 5.55 (*d*) ([–CH(Me)–CH(OR)–C=CH–]; unit A)[1]), δ(H) 5.70 (*d*) and 5.61 (*d*) ([–CH(OR)–CH(OR)–]; unit B), δ(H) 5.43 (*d*) and 5.36 (*dd*) ([–CH=CH–]; unit C), δ(H) 3.18 (*m*), 0.98 (*d*), and 4.91 (*d*) ([–CH(Me)–CH(OR)–]; unit D; R = acyl; *Fig.*). One signal at δ(H) 3.20, which did not exhibit any correlation in the HMQC spectrum, suggested one OH group in the molecule.

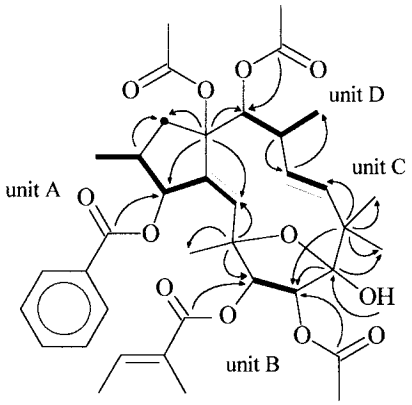


Fig. 1. *Selected ^{1}H,^{1}H COSY* (▬) *and HMBC* (C → H) *Correlations for* **1**

The partial structures A–D, three tertiary Me groups, four sp^3 and one sp^2 quaternary C-atoms were connected by inspection of the long-range C,H correlations observed in a gradient HMBC spectrum as presented in the *Figure*. The correlations of H–C(1)/C(2), H–C(1)/C(15), H–C(3)/C(15), and H–C(5)/C(15) suggested that structural fragment A and the CH_2(1) group are connected, and, together with C(15), are joined to a Me-substituted five-membered ring in **1**. The long-range correlations H–C(5)/C(6), H–C(17)/C(6), and H–C(7)/C(6) led to the assembly of units A and B, and one Me group (Me(17)) through a quaternary C-atom (C(6)). Similarly, the connection of units B and C, and two Me groups through C(9) and C(10) was evident from the observation of ^2J(C,H) and ^3J(C,H) couplings H–C(8)/C(9), H–C(7)/C(9), H–C(8)/C(10), H–C(11)/C(10), H–C(18)/C(10), and H–C(19)/C(10). The presence of cross-peaks between H–C(12) and C(13), H–C(20) and C(12), and H–C(12) and

[1]) The ^{1}H,^{1}H-COSY spectrum showed long-range allylic couplings for fragment A between the H-atoms at δ(H) 5.74 (H–C(3)) and 5.55 (H–C(5)).

Table 1. *NMR Data of* **1** (500 MHz, $CDCl_3$, δ [ppm] (multipl., J [Hz]))

C-Atom	^{1}H	^{13}C	HMBC (H-Atoms)	NOESY
C(1)(β)	2.71 (*dd*, 14.4, 9.7)	38.6	14, 16	1α, 2
C(1)(α)	1.54 (*dd*, 14.4, 5.3)			1β, 3, 14
C(2)	2.53 (*m*)	38.1	1α, 1β, 16	1β, 3, 16
C(3)	5.74 (*dd*, 3.9, 2.4)	79.5	1α, 1β, 5, 16	1α, 2, 16
C(4)	–	133.5	1β, 3, 5	–
C(5)	5.55 (*d*, 2.4)	139.8	3, 17	13, 17, 15-Ac
C(6)	–	82.3	5, 7, 17	–
C(7)	5.70 (*d*, 4.0)	73.0	5, 8, 17	17
C(8)	5.61 (*d*, 4.0)	70.6	7, 9-OH, 8-CO*Me* [a])	9-OH, 19
C(9)	–	104.8	7, 8, 9-OH, 18, 19	–
C(10)	–	42.1	8, 9-OH, 11, 12, 18, 19	–
C(11)	5.43 (*d*, 16.2)	139.5	12, 18, 19	13, 18
C(12)	5.36 (*dd*, 16.2, 9.0)	134.5	11, 14, 20	13, 19, 20
C(13)	3.18 (*m*)	39.0	11, 12, 20	5, 11, 12, 14, 20
C(14)	4.91 (*d*, 9.3)	79.1	1α, 1β, 12, 13, 20, 14-CO*Me* [a])	1α, 13, 16, 20
C(15)	–	91.2	1α, 1β, 3, 5, 14, 15-CO*Me* [a])	–
C(16)	1.24 (*d*, 7.4)	19.6	1α, 1β, 3	2, 3, 14
C(17)	1.28 (*s*)	21.2	–	5, 7, 3″
C(18)	1.18 (*s*)	23.5	11, 19	11
C(19)	0.90 (*s*)	20.3	11, 18	8, 12
C(20)	0.98 (*d*, 7.0)	19.9	–	12, 13, 14
9-OH	3.20 (*s*)	–	–	3″, 8
3-OBz				
CO	–	165.8	3, 2′, 6′	
C(1′)	–	130.8	3′, 5′	
C(2′, 6′)	8.09 (‘*d*’, 7.1)	129.9	2′–6′	
C(4′)	7.49 (‘*t*’, 7.4)	132.4	2′, 6′	
C(3′, 5′)	7.39 (‘*t*’, 7.6)	128.0	3′, 5′	
7-OTig				
C(1″)	–	165.3	7, 3″, 4″	
C(2″)	–	127.6	4″, 5″	
C(3″)	6.59 (*dq*, 7.0, 1.4)	137.9	4″, 5″	9-OH, 17
C(4″)	1.66 (*dd*, 7.0, 1.0)	14.3	3″	
C(5″)	1.59 (*d*, 1.0)	12.0	3″	
8-OCO*Me*	2.02 (*s*)	20.6	–	
8-O*C*OMe	–	169.2	8, 8-CO*Me*	
14-OCO*Me*	2.15 (*s*)	20.7	–	
14-O*C*OMe	–	170.4	14, 14-CO*Me*	
15-OCO*Me*	2.19 (*s*)	22.4	–	5
15-O*C*OMe	–	168.6	15-CO*Me*	

[a]) ^4J(C,H) Couplings.

C(14) indicated that units C and D were connected. Finally, the linkage of unit D and C(15) was substantiated by the HMBC correlation between H–C(14) and C(15). The positions of the ester groups were determined on the basis of the long-range correlations between the ester C=O C-atoms and oxymethine H-atoms. Thus, the BzO group was placed at C(3), the tigloyl at C(7), and Ac groups at C(8) and C(14) (*Table 1*). Additionally, in the HMBC spectrum, weak ^4J(C,H) couplings were observed between the Me H-atoms of Ac and skeletal C-atoms, corroborating the

positions of Ac groups at C(8), C(14), and C(15). The HMBC cross-peak between the OH group and C(9) clearly indicated the location of the OH group at C(9). As required by the tricyclic skeleton of the molecule, an epoxy group was established between C(6) and C(9) (δ(C) 82.3 for C(6), and δ(C) 104.8 for C(9)). The above structure elucidation resulted in the same planar structure for **1** as that of serrulatin A [7]. Accordingly, the difference must lie in their configuration.

The relative configurations of the nine stereogenic centers were investigated by a phase-sensitive NOESY experiment, aided by consideration of the coupling constant values. Starting from H_α–C(3), it was found that an α-oriented Me group is present at C(2) and an α-H-atom at C(14) with regard to the nuclear *Overhauser* effects observed between H–C(3) and H_α–C(1), H_α–C(1) and H–C(14), H_β–C(1) and H–C(2), and H–C(14) and H–C(16). The coupling constant $J(13,14) = 9.3$ Hz is consistent with H_β–C(13) [7]. Regarding the configuration of the C(8)–C(12) part of the molecule, the NOESY correlations between H–C(13) and H–C(11), H–C(11) and H–C(18), H–C(12) and H–C(19), and H–C(19) and H–C(8) indicated that H–C(12) is directed below, and H–C(11) above the plane of the macrocycle, and that H–C(8) and H–C(19) are in the α, and H–C(18) in the β position. In the absence of appreciable NOEs, the orientation of H–C(7) was concluded from the J(7,8) value of 4.0 Hz to be α, in view of the similar value in serrulatin A [7]. The NOE interactions between H–C(3″)(tigloyl) and 9–OH, H–C(3″) and H–C(17), H–C(17) and H–C(5), and H–C(5) and 15-OAc are compatible with β-oriented 9-OH, 17-Me, 7-tigloyloxy, and 15-OAc groups. The configuration of the C(4)=C(5) bond was assigned from the NOESY correlations H–C(5)/15-OAc and H–C(5)/H–C(13) as (*E*). As a result of the above NMR study, the structure of this compound was elucidated as **1**, and complete and unambiguous ^{1}H and ^{13}C chemical-shift assignments were determined as listed in *Table 1*.

Compound **2**, an amorphous solid, with $[\alpha]_D^{25} = +43$ ($c = 0.025$, $CHCl_3$), has the molecular formula $C_{37}H_{46}O_{12}$, determined *via* the quasimolecular ion peak at *m*/*z* 815.2043 ($[M + Cs]^+$; calc. 815.2044, $\Delta = +0.1$ ppm) in the HR-ESI-MS and supported by the H- and C-atom counts in the NMR spectra. The ^{1}H-NMR spectrum indicated the presence of one Bz and five Ac groups in the molecule (*Table 2*). The skeletal C-atoms and directly bonded H-atoms were assigned by means of HMQC, ^{1}H,^{1}H-COSY, TOCSY, and HMBC experiments. Excluding the resonances of ester moieties, the signals of three tertiary and two secondary Me groups, five O-substituted and two alkyl-substituted CH groups, four tertiary unsaturated C-atoms, one O-substituted, one alkyl-substituted, and two unsaturated quaternary C-atoms were observed in the spectrum. Thus, compound **2** is based on a diterpenoid skeleton with three C=C bonds and six ester functions. Interpretation of the ^{1}H,^{1}H-COSY and TOCSY spectra revealed the existence of three partial structures with regard to the cross-peaks between the signals at δ(H) 5.87 (*s*), 3.29 (*dq*), 1.02 (*d*), and 6.15 (*d*) (fragment A; [=CH–CH(CH_3)–CH(OR)–]), 5.30 (*d*), 5.49 (*d*), and 5.41 (*s*) (fragment B; [–CH(OR)–CH(OR)–CH(OR)–]), and 5.40 (*d*), 5.64 (*dd*), 2.42 (*dq*), 1.14 (*d*), and 5.64 (*s*) (fragment C; [–CH=CH–CH(CH_3)–CH(OR)–]). After the chemical shifts of the protonated C-atoms had been assigned *via* the HSQC spectrum, the connection of these three fragments and four quaternary C-atoms was performed on the basis of the HMBC spectrum. The long-range correlations listed in *Table 2* led to the conclusion

Table 2. *NMR Data of* **2** (500 MHz, $CDCl_3$, δ[ppm] (multipl., *J* [Hz]))

C-Atom	^{1}H	^{13}C	HMBC (H-Atoms)	NOESY
C(1)	5.87 (*s*)	139.0	3, 16	14
C(2)	3.29 (*dq*, 5.4, 7.3)	42.5	1, 16	3, 16
C(3)	6.15 (*d*, 5.4)	75.1	1, 16	2, 7, 17
C(4)	–	142.0	1, 3, 5	
C(5)	6.04 (*s*)	130.0	17	8, 9, 12, 17
C(6)	–	82.0	17	
C(7)	5.30 (*d*, 3.4)	77.7	17	3, 8, 11, 17
C(8)	5.49 (*d*, 3.4)	70.0	–	5, 7, 9, 11, 14
C(9)	5.41 (*s*)	75.9	18, 19	5, 8
C(10)	–	40.0	9, 11, 12, 18, 19	
C(11)	5.40 (*d*, 15.9)	138.0	9, 18, 19	7, 8, 12, 18, 19
C(12)	5.64 (*dd*, 15.9, 9.8)	133.0	20	5, 11, 18, 20
C(13)	2.42 (*dq*, 9.8, 6.9)	45.5	11, 12, 14, 20	14
C(14)	5.64 (*s*)	77.5	1, 20	1, 8, 13
C(15)	–	141.5	1, 3, 5, 14	
C(16)	1.02 (*d*, 7.3)	13.4	–	2
C(17)	1.50 (*s*)	23.9	5	3, 5, 7
C(18)	1.00 (*s*)	28.5	11, 19	9, 11, 12
C(19)	0.88 (*s*)	23.2	11, 18	8, 11
C(20)	1.14 (*d*, 6.9)	19.4	–	12
3-OBz				
CO	–	165.9	3, 2′, 6′	
C(1′)	–	131.0	–	
C(2′, 6′)	7.99 (‘*d*’, 7.1)	130.4	2′, 3′, 5′, 6′	
C(4′)	7.52 (‘*t*’, 7.5)	133.6	2′, 6′	
C(3′, 5′)	7.40 (‘*t*’, 7.5)	129.0	3′–5′	
6-OCO*Me*	2.05 (*s*)[a])	21.4[b])	–	
6-O*C*OMe	–	171.0[c])	6-CO*Me*	
7-OCO*Me*	2.02 (*s*)	20.8	–	
7-O*C*OMe	–	170.2	7, 7-CO*Me*	
8-OCO*Me*	2.13 (*s*)[a])	22.3[b])	–	
8-O*C*OMe	–	170.5[c])	8-CO*Me*	
9-OCO*Me*	2.13 (*s*)	21.5	–	
9-O*C*OMe	–	169.8	9, 9-CO*Me*	
14-OCO*Me*	2.18 (*s*)[a])	21.0[b])	–	
14-O*C*OMe	–	171.0[c])	14-CO*Me*	

[a]) [b]) [c]) Interchangeable signals.

that **2** is a jatrophane triene substituted with ester groups at C(3), C(6), C(7), C(8), C(9), and C(14). From the HMBC correlation between H–C(3) and the C=O C-atom of the Bz group, it was evident that, in **2**, the benzoate group is located at C(3), and, consequently, Ac groups are connected in all other positions.

The relative configuration of **2** was assessed by analysis of the coupling-constant pattern and the results of the NOESY experiment. As reference point, the position of H–C(3) was chosen to be α. The observed NOESY correlations between H–C(3) and H–C(2), H–C(3) and H–C(7), and H–C(3) and H–C(17) proved the β position of C(16) and the BzO group and the β orientation of the Ac groups at C(6) and C(7). The NOE interaction of the ^{1}H signal at 5.30 (H–C(7)) with the ^{1}H signal at 5.40

(H–C(11)) indicated that H–C(11) is oriented below the plane of the macrocyclic ring. The cross-peaks of H–C(11) with H–C(8), and H–C(13) and H–C(19) suggested the α position of these H-atoms and the Me group. H–C(9) displayed a cross-peak with H–C(18), and, therefore, an α-oriented Ac group must be present at C(9). The coupling constant between H–C(11) and H–C(12) ($J = 16$ Hz) required the (*E*)-configuration of the C(11)=C(12) bond. The C(4)=C(5) bond also adopts an (*E*)-configuration, as deduced from the NOESY correlation of H–C(5) with H–C(11). All of the above data are compatible with structure **2** for this compound.

Compound **3**, obtained as an amorphous solid, with $[\alpha]_D^{25} = -8$ ($c = 0.05$, $CHCl_3$) and with the molecular formula $C_{35}H_{44}O_{13}$, was shown to be a polyacylated jatrophane derivative. Signals of one Bz and four Ac groups were observed in the ^{1}H-NMR and JMOD spectra (*Table 3*). Analysis of the ^{1}H,^{1}H-COSY, HSQC, and HMBC spectra of

Table 3. *NMR Data of* **2**, **3**, *and* **4** ($CDCl_3$, δ [ppm] (multipl., *J* [Hz]))

C-Atom	**3**[a])		**4**[b])		**5**[c])	
	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C
C(1a)	3.12 (*d*, 15.5)	45.3	3.25 (*d*, 16.0)	46.4	2.47 (*m*)	41.1
C(1b)	2.34 (*d*, 15.5)		2.91 (*d*, 16.0)		2.24 (*t*, 12.1)	
C(2)	–	89.2	–	89.2	2.47 (*m*)	39.3
C(3)	6.93 (*d*, 1.7)	76.7	6.38 (br. *s*)	77.7	5.74 (*dd*, 4.8, 1.9)	77.9
C(4)	–	142.6	–	137.0	–	
C(5)	5.48 (*d*, 1.7)	136.7	5.86 (br. *s*)	138.4	6.05 (br. *s*)	139.3
C(6)	–	76.1	–	82.1	–	82.0
C(7)	4.60 (*d*, 4.0)	78.8	5.19 (*d*, 4.0)	77.1	5.29 (*d*, 2.6)	76.5
C(8)	5.37 (*d*, 4.0)	70.3	5.33 (*d*, 4.0)	68.5	5.70 (br. *s*)	71.2
C(9)	4.83 (*s*)	75.1	5.19 (s)	74.7	5.60 (*s*)	70.3
C(10)	–	39.9	–	41.7	–	42.0
C(11)	5.59 (*d*, 16.0)	139.6	5.51 (*d*, 16.2)	140.7	5.25 (d, 12.8)	136.2
C(12)	5.68 (*dd*, 16.0, 8.4)	131.5	5.73 (*dd*, 16.2, 8.8)	130.1	5.62 (*dd*, 12.8, 10.2)	131.7
C(13)	3.79 (*dq*, 6.8, 8.4)	44.2	3.68 (*dq*, 8.8, 6.8)	43.8	4.22 (*dd*, 10.2, 6.6)	41.8
C(14)	–	211.6	–	204.6	–	205.0
C(15)	–	85.9	–	92.0	–	
C(16)	1.72 (*s*)	20.6	1.59 (*s*)	21.0	1.43 (*d*, 7.3)	16.9
C(17)	1.00 (*s*)	25.7	1.59 (*s*)	22.9	1.45 (*s*)	22.9
C(18)	0.96 (*s*)	25.6	0.94 (*s*)	27.1	1.09 (*s*)	20.0
C(19)	0.86 (*s*)	20.5	0.85 (*s*)	19.7	0.96 (*s*)	28.7
C(20)	1.27 (*d*, 6.8)	19.5	1.28 (*d*, 6.8)	19.6	1.24 (*d*, 6.6)	19.8
3-OBz						
CO		165.5		165.5	–	
C(1′)		129.9		130.3	–	
C(2′, 6′)	8.08 (‘*d*’, 7.1)	130.0	8.06 (‘*d*’, 7.1)	130.7	8.02 (‘*d*’, 7.3)	129.6
C(4′)	7.56 (‘*t*’, 7.7)	133.2	7.57 (‘*t*’, 7.6)	133.9	7.54 (‘*t*’, 7.4)	132.9
C(3′, 5′)	7.44 (‘*t*’, 7.7)	128.5	7.44 (‘*t*’, 7.6)	129.2	7.42 (‘*t*’, 7.8)	129.2

[a]) ^{1}H-NMR Signals of OH groups: 4.01 (*s*, 6-OH); 4.58 (*s*, 15-OH). NMR Signals of Ac groups: δ(H) 2 × 2.04 (*s*), 2.11 (*s*) and 2.16 (*s*). δ(C) 20.8, 21.3, 21.5, 22.3, 169.8, 170.3, 170.4, and 171.8. [b]) NMR Signals of Ac groups: δ(H) 1.98 (*s*), 2.05 (*s*), 2.08 (*s*), 2.09 (*s*), 2.16 (*s*), and 2.20 (*s*). δ(C) 21.5, 2 × 21.6, 21.9, 22.3, 23.0, 169.4, 169.9, 170.1, 170.2, 171.0, and 171.2. [c]) NMR Signals of Ac groups: δ(H) 2.05 (*s*), 2.09 (*s*), 2.12 (*s*), 2.16 (*s*), and 2.17 (*s*). δ(C) 20.3, 20.6, 20.9, 21.3, 21.4, 168.4, 169.0, 2 × 169.3, and 170.6. ^{13}C-NMR Signals of C(4), C(15), BzCO, and C(1′) could not be determined.

3 led to the identification of a jatrophane-4,11-diene structure containing a keto group (δ(C) 211.6) and seven additional O-substituted C-atoms: C(2), C(3), C(6), C(7), C(8), C(9), and C(15) (δ(C) 89.2, 85.9, 78.8, 76.7, 76.1, 75.1, and 70.3). The presence of two OH groups in the molecule was concluded from the missing ^{13}C correlations of two ^{1}H signals (4.01 (*s*) and 4.58 (*s*)) in the HSQC spectrum. The positions of the OH groups were determined on the basis of the ^{2}J(C,H) couplings observed in the HMBC spectrum between C(6) and the ^{1}H signal at 4.01 and between C(15) and the H-atom at δ(H) 4.58. The keto group was located at C(14), as dictated by the HMBC correlations between the C-atom at 211.6 and H–C(13), H–C(20), and 15-OH. The cross-peak between the ester C=O C-atom at 165.5 and H–C(3) clearly demonstrated the presence of a BzO group at C(3). The remaining acyl groups (four Ac) were located at C(2), C(7), C(8), and C(9).

The relative configuration of **3** was elucidated by interpretation of a NOESY experiment. Starting from the α-position of H–C(3), the nuclear *Overhauser* effects between H–C(3) and 6-OH, 6-OH and H–C(8), H–C(8) and H–C(7), H–C(8) and H–C(19), and H–C(18) and H–C(9) were indicative of the β position of the ester groups at C(3), C(7), and C(8), and the Me group at C(6), and the α orientation of the Ac group at C(9). On the basis of the NOESY correlations between H–C(19) and H–C(11), and between H–C(18) and H–C(12), it was concluded that H–C(11) is directed above, while H–C(12) is directed below the plane of the twelve-membered ring. The β orientation of the Me groups CH_3(16) and CH_3(20) followed from the nuclear *Overhauser* effects between H–C(11) and H–C(13), H–C(13) and H_a–C(1), and H_b–C(1) and H–C(16). As H–C(5) exhibited NOEs with H–C(9) and with 15-OH, H–C(5) must be directed above and inwards the macrocyclic ring, and accordingly the C(4)=C(5) bond adopts (*E*)-configuration, and 15-OH is in the β position. All of the above data indicate structure **3** for this compound.

Compound **4** was isolated as an amorphous solid with $[\alpha]_D^{25} = -15$ ($c = 0.025$, $CHCl_3$) and the molecular formula $C_{39}H_{48}O_{15}$, as established by HR-ESI-MS. Its NMR spectral data were similar to those of **3**, differing only in the ester pattern (*Table 3*). In the ^{1}H- and ^{13}C-NMR spectra of **4**, the signals of two OH groups were missing, and the signals of two additional Ac groups appeared. After the chemical-shift assignment of all C- and H-atoms *via* the ^{1}H,^{1}H-COSY, HSQC, and HMBC spectra had been achieved, it was evident that in **4** Ac groups are present at C(7) and C(15) because of the observed paramagnetically shifted C(6) (δ(C(6) 76.1 (**3**) and 82.1 (**4**)) and C(15) signals (δ(C(15)) 85.9 (**3**) and 92.0 (**4**)). The relative configuration of **4**, determined on the basis of a NOESY experiment, proved to be very close to that of compound **3**. A difference was found in the configuration at C(6), since the NOE correlations H–C(3) and H–C(17), H–C(3) and H–C(7), and between H–C(17) and H–C(7) were indicative of the α orientation of the Me group at C(6).

Compound **5** was obtained in a very small quantity as an amorphous solid with $[\alpha]_D^{25} = +34$ ($c = 0.05$, $CHCl_3$). The HR-ESI-MS suggested the molecular formula $C_{37}H_{46}O_{13}$ with the *m*/*z* 721.2838 $[M + Na]^+$ ion (calc. 721.2836, $\Delta = -0.3$ ppm). On evaluation of the ^{1}H-NMR and 2D-NMR spectra, the constitution of **5** proved to be 6,7,8,9,15-pentaacetoxy-3-benzoyloxyjatrophane-4,11-dien-14-one (*Table 3*). The relative configuration of **5** differs in some respects from those of **1**–**4**. The coupling constant of J(H–C(11), H–C(12)) 12.8 Hz revealed the (*Z*)-configuration of the

C(11)=C(12) bond. The chemical shifts of H–C(2), H–C(3), H–C(16), C(2), C(3), and C(16) are in good agreement with those of other compounds present in *E. serrulata:* serrulatin B and 7β,8β,9α,15β-(4*E*,11*E*)-tetraacetoxy-3β-benzoyloxy-6β-hydroxy-14-oxojatropha-4,11-diene [8], suggesting the α orientation of the 2-Me and the β position of the 3-BzO group. The nuclear *Overhauser* effects between H–C(3) and H–C(7), H–C(3) and H–C(17), H–C(7) and H–C(17), and H–C(7) and H–C(8) established the β configuration of the Ac groups at C(6), C(7), and C(8). The NOESY correlations of H–C(8) with H–C(19), H–C(18) with H–C(9), and H–C(9) with H–C(13) indicated the α position of the Ac group at C(9) and the Me group at C(13). The (*E*)-configuration of the C(5)=C(6) bond was derived from the NOESY correlation between H–C(5) and H–C(12). The above evidence led to the formulation of this compound as **5**.

Conclusions. The diterpenes isolated from *E. serrulata* display extreme stereochemical diversity. With respect to the configurations at stereogenic centers C(2), C(6), and C(13), the compounds differ: in **1** and **5**, an α-oriented Me group is present at C(2), but in **2**–**4** this Me group is in the β position; compound **1** contains an α-oriented Me group at C(13), while in **2**–**5** this Me group is in the β position; in compound **3**, the orientation of the 17-Me group is β, in contrast with **2**, **4**, and **5**, which contain an α-Me group at C(6). Compound **5** is an unusual jatrophane diterpene, because it possesses a C(11)=C(12) bond with (*Z*)-configuration in the macrocyclic ring. Compound **2** is the first known jatrophane diterpene with a C=C bond in the five-membered ring.

Experimental Part

General. For column chromatography (CC), polyamide (ICN), and, for VLC, silica gel (*Kieselgel GF$_{254}$*, 15 µm, *Merck*) was used. HPLC: *Waters Millipore* instrument, on *LiChrospher Si 100* and *LiChrospher RP-18* (5 µm, 250 × 4 mm) columns, with hexane/AcOEt/EtOH 70:10:1 as eluent for NP-HPLC and MeCN/H$_2$O 7:3 and 4:1 for RP-HPLC, at a flow rate of 0.5 ml/min, with detection at 254 nm. M.p.: uncorrected. Optical rotations: *Perkin-Elmer 341* polarimeter. UV Spectra: *Shimadzu UV-2101 PC* spectrometer. NMR Spectra: in $CDCl_3$ on a *Bruker Avance DRX 500* spectrometer at 500 MHz (^{1}H) and 125 MHz (^{13}C); the signals of the deuterated solvent were taken as the reference. 2D data were acquired and processed with standard *Bruker* software. In the ^{1}H,^{1}H-COSY, HSQC and HMBC experiments, gradient-enhanced versions were used. HR-ESI-MS: *Perkin-Elmer Q-STAR Pulsar Q-TOF* spectrometer equipped with an electrospray ion source.

Plant Material and Extraction. *Cf.* [7].

Isolation. The hexane extract of *E. serrulata* was chromatographed on a polyamide column, with mixtures of MeOH/H$_2$O as eluents. The fractions obtained with MeOH/H$_2$O 3:2 were fractionated in two steps by vacuum liquid chromatography (VLC) on silica gel, with the following gradient solvent systems: for VLC-1 petroleum ether/AcOEt, and for VLC-2 benzene/$CHCl_3$/Et$_2$O mixtures of increasing polarity. *Fractions 3–7* obtained from VLC-2 with the first eluent (10:5:1) were further purified by normal-phase HPLC, and then by reverse-phase HPLC, to afford pure compounds **1** (5.9 mg) and **2** (1.0 mg). *Fr. 8* from VLC-2 was chromatographed by RP-HPLC with MeCN/H$_2$O 7:3 at a flow rate of 0.5 ml/min. The compounds observed at r.t. with t_R 17.61, 25.75 and 17.61 min were purified on an NP-HPLC column to afford compounds **3** (2.3 mg), **4** (2.2 mg) and **5** (1.0 mg).

rel-*(2*R*,3*S*,6*R*,7*S*,8*S*,9*S*,13*R*,14*S*,15*S*)-(4*E*,11*E*)-8,14,15-Triacetoxy-3-(benzoyloxy)-6,9-epoxy-9-hydroxy-7-(tigloyloxy)jatropha-4,11-diene* (**1**): amorphous solid. $[\alpha]_D^{25} = +88$ ($c = 0.05$, $CHCl_3$). UV (MeOH): λ_{max} 225 (4.08), 272 (2.71), 281 (2.75). ^{1}H- and ^{13}C-NMR: *Table 1*. HR-ESI-MS: 829.2205 ($[M+Cs]^+$, $C_{38}H_{48}CsO_{12}$; calc. 829.2200, $\Delta = -0.6$ ppm). ESI-MS: 719 ($[M+Na]^+$), 659 ($[M+Na-AcOH]^+$), 537 ($[M+Na-AcOH-BzOH]^+$), 477 ($[M+Na-2\times AcOH-BzOH]^+$), 377 ($[M+Na-2\times AcOH-BzOH-TigOH]^+$).

rel-*(2*S*,3*S*,6*S*,7*R*,8*R*,9*S*,13*S*,14*S*)-(4*Z*,11*E*)-6,7,8,9,14-Pentaacetoxy-3-(benzoyloxy)jatropha-1(15),4,11-triene* (**2**): amorphous solid. $[\alpha]_D^{25} = +43$ ($c = 0.025$, $CHCl_3$). UV(MeOH): λ_{max} 230 (3.67), 271 (2.91), 281 (2.89). ^{1}H- and ^{13}C-NMR: *Table 2*. HR-ESI-MS: 815.2043 ($[M+Cs]^+$; $C_{37}H_{46}CsO_{12}$; calc. 815.2044, $\Delta = +0.1$ ppm).

rel-*(2*R*,3*R*,6*R*,7*R*,8*R*,9*S*,13*S*,15*R*)-(4*E*,11*E*)-2,7,8,9-Tetraacetoxy-3-(benzoyloxy)-6,15-dihydroxy-14-oxojatropha-4,11-diene* (**3**): amorphous solid. $[\alpha]_D^{25} = -8$ ($c = 0.05$, $CHCl_3$). UV (MeOH): λ_{max} 229 (3.98), 273 (2.83), 281 (2.73). ^{1}H- and ^{13}C-NMR: *Table 3*. HR-ESI-MS: 695.2680 ($[M+Na]^+$, $C_{35}H_{44}NaO_{13}$; calc. 695.2679, $\Delta = +0.1$ ppm).

rel-*(2*R*,3*R*,6*S*,7*R*,8*R*,9*S*,13*S*,15*R*)-(4*E*,11*E*)-2,6,7,8,9,15-Hexaacetoxy-3-(benzoyloxy)-14-oxojatropha-4,11-diene* (**4**): amorphous solid. $[\alpha]_D^{25} = -15$ ($c = 0.025$, $CHCl_3$). UV (MeOH): λ_{max} 230 (3.96), 275 (2.92), 282 (2.89). ^{1}H- and ^{13}C-NMR: *Table 3*. HR-ESI-MS: 779.2900 ($[M+Na]^+$, $C_{39}H_{48}NaO_{15}$; calc. 779.2891, $\Delta = +1.2$ ppm).

rel-*(2*R*,3*S*,6*S*,7*R*,8*R*,9*S*,13*R*,15*R*)-(4*Z*,11*E*)-6,7,8,9,15-Pentaacetoxy-3-(benzoyloxy)-14-oxojatropha-4,11-diene* (**5**): amorphous solid. $[\alpha]_D^{25} = +34$ ($c = 0.05$, $CHCl_3$). UV (MeOH): λ_{max} 229 (3.65), 275 (2.94), 282 (2.94). ^{1}H- and ^{13}C-NMR: *Table 3*. HR-ESI-MS: 721.2838 ($[M+Na]^+$, $C_{37}H_{46}NaO_{13}$; calc. 721.2836, $\Delta = -0.3$ ppm).

This work was supported by grants OTKA T035200, OTKA M36478 M.E.-00050/2001 and FKFP 0024/2001. The authors thank *Tamás Rédei* (Hungarian Natural History Museum, Budapest, Hungary) for the identification and collection of plant material.

REFERENCES

[1] F. Evans, S. E. Taylor, *Prog. Chem. Org. Nat. Prod.* **1983**, *44*, 1.
[2] G. Günther, T. Martinek, Gy. Dombi, J. Hohmann, A. Vasas, *Magn. Reson. Chem.* **1999**, *37*, 365.
[3] G. Appendino, E. Belloro, G. C. Tron, J. Jakupovic, M. Ballero, *J. Nat. Prod.* **1999**, *62*, 1399.
[4] G. Vogg, E. Mattes, J. Rothenburger, N. Hertkorn, S. Achatz, H. Sanderman, *Phytochemistry* **1999**, *51*, 289.
[5] W. He, M. Cik, A. Lesage, I. Van der Linden, N. De Kimpe, G. Appendino, J. Bracke, S. G. Mathenge, F. P. Mudida, J. E. Leysen, L. Van Puyvelde, *J. Nat. Prod.* **2000**, *63*, 1185.
[6] T. G. Tutin, V. H. Heywood, N. A. Burges, D. M. Moore, D. H. Valentine, S. M. Walters, D. A. Webb, 'Flora Europaea', University Press, Cambridge, 1972, Vol. 2.
[7] J. Hohmann, D. Rédei, F. Evanics, A. Kálmán, Gy. Argay, T. Bartók, *Tetrahedron* **2000**, *56*, 3619.
[8] J. Hohmann, J. Molnár, D. Rédei, F. Evanics, P. Forgo, A. Kálmán, Gy. Argay, P. Szabó, *J. Med. Chem.* **2002**, *45*, 2425.
[9] I. Mucsi, J. Molnár, J. Hohmann, D. Rédei, *Planta Med.* **2001**, *67*, 672.

Received August 2, 2002