Isolation and Structure Determination of New Jatrophane Diterpenoids from *Euphorbia platyphyllos* L.

by **Judit Hohmann***[a]), **Peter Forgo**[b]), **Dezsö Csupor**[a]), and **Gitta Schlosser**[c])

[a]) Department of Pharmacognosy, University of Szeged, Eötvös u. 6, H-6720 Szeged (phone: 3662545558; fax: 3662545704; e-mail: hohmann@pharma.szote.u-szeged.hu)

[b]) Department of Organic Chemistry, University of Szeged, H-6720 Szeged

[c]) Institute of Chemistry, Chemical Research Centre, Hungarian Academy of Sciences, H-1525 Budapest

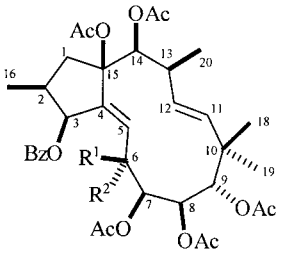
Three new, highly functionalized jatrophane diterpenes (**1**–**3**) have been isolated from the whole, dried plants of *Euphorbia platyphyllos* L., together with one known jatrophane polyester (**4**). The structures were established by UV/VIS spectroscopy, HR-ESI-MS, and advanced two-dimensional NMR, including ^{1}H-NMR, JMOD, ^{1}H,^{1}H-COSY, HMQC, and HMBC experiments. Stereochemical studies and conformational analyses were performed by means of NOESY experiments. Interestingly, compounds **1**–**4** do not represent a stereochemically uniform series because they differ in the orientations of the Me groups at C(2), C(6), and C(13). A similar observation was reported earlier for *Euphorbia serrulata*, whose diterpenes are related to the compounds obtained from *E. platyphyllos*. This chemical relationship is of taxonomic importance and supports the botanical similarity of the two species.

Introduction. – Diterpenes based on the jatrophane skeleton are found in nature in various oxygenated forms, mainly as polyesters. Such macrocyclic diterpenes occur exclusively in the Euphorbiaceae and Thymelaeaceae families. Some of these compounds are of considerable interest because of their antitumor, cytotoxic, antiviral, NADH-oxidase-inhibitory, and multidrug resistance-reversing activities [1–5]. As a continuation of our current interest in the chemistry and pharmacology of the genus *Euphorbia*, we have now investigated the secondary metabolites of *E. platyphyllos*.

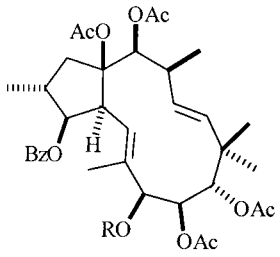
E. platyphyllos L. (broad-leaved spurge) is a glabrous or pubescent annual plant that occurs mainly in southern parts of Europe. The plant produces a white milky latex characteristic of Euphorbiaceae species. The latex and other plant parts of *E. platyphyllos* have been used in traditional medicine for the treatment of warts, wens, and hangnails [6]. In spite of these medicinal benefits, no data have been reported previously on the pharmacology and chemistry of the species.

The present paper describes the isolation and structure determination of three new, highly functionalized jatrophane polyesters **1**–**3** from *E. platyphyllos*, together with the known compound **4**. The structure elucidation, including relative configurations and conformations, was performed by means of HR-ESI-MS, ^{1}H-NMR, JMOD, ^{1}H,^{1}H-COSY, NOESY, HMQC, and HMBC experiments.

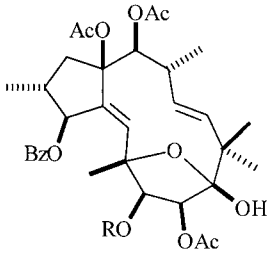
Results and Discussion. – The $CHCl_3$ extract of the whole, dried plants of *E. platyphyllos* was subjected to polyamide column chromatography (CC). Fractions obtained with a MeOH/H_2O 3:2 mixture were fractionated by vacuum liquid chromatography (VLC) and by preparative thin-layer chromatography (TLC) on



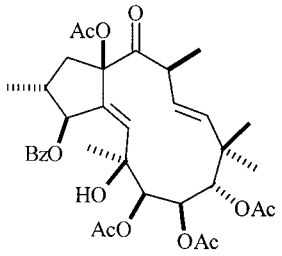
	R^1	R^2
1	CH_3	OH
5	OH	CH_3



	R
2	Ac
6	H



	R
3	Bz
7	Tig



4

Ac = acetyl, Bz = benzoyl, Tig = tigloyl

silica gel, and finally purified by normal- (NP) and reversed-phase (RP) HPLC to afford the pure compounds **1**–**4**.

Compound **1** was obtained as an amorphous solid, with $[\alpha]_D^{29} = +10$ ($c = 0.025$, $CHCl_3$). The molecular formula was determined to be $C_{37}H_{48}O_{13}$ by HR-ESI-MS, which showed a quasimolecular ion peak at m/z 723.2958 ($[M + Na]^+$, $C_{37}H_{48}O_{13}Na^+$; calc. 723.2992), indicative of 13 degrees of unsaturation. Its UV spectrum exhibited absorption maxima at 234, 273, and 286 nm, characteristic of a benzoyl (Bz) group. The ^{1}H- and ^{13}C-NMR data corroborated the presence of one BzO group and clearly indicated five AcO groups in the molecule (*Table 1*). Detailed analysis of the ^{1}H and ^{13}C resonances with the aid of ^{1}H,^{1}H-COSY, HMQC, and HMBC revealed that **1** contains three quaternary C-atoms (C(6), C(10), (C(15)), one disubstituted (C(11)=C(12)) and one trisubstituted (C(4)=C(5)) olefin, five O-substituted methines (C(3), C(7), C(8), C(9), and C(14)), one methylene (C(1)), three tertiary (C(19), C(18), C(17)) and two secondary Me (C(16), C(20)) groups. The presence of an OH group was apparent from the ^{1}H-NMR spectrum, where a *singlet* at δ 6.07 was observed without any correlation in the HSQC spectrum. The above data pointed to a

jatrophane diterpene with two endocyclic double bonds, and bearing one OH and six acyl groups. When the ^{1}H- and ^{13}C-NMR data were compared with those of known jatrophane diterpenes, the presence of a fused, 2-methyl-3,15-diacyl-substituted five-membered ring was anticipated [7–10]. Regarding the macrocyclic part of the molecule, homonuclear ^{1}H,^{1}H-COSY was informative, revealing two isolated fragments of correlated H-atoms; –CH(OR)–CH(OR)– (fragment A, R = acyl) and –CH=CH–CH(Me)– (fragment B with an (*E*)-configured olefin (J = 16.0 Hz)). On the basis of the heteronuclear ^{1}H/^{13}C long-range correlations detected in the HMBC spectrum, these fragments could be connected with quaternary C-atoms (C_q) next to Me and methine groups. The HMBC cross-peaks between the C_q-atom at δ(C) 90.2 (C(15)) and the H-atoms at δ(H) 5.97 (H–C(14)) and 6.52 (H–C(5)), and between the signal at δ(C) 78.7 (C(14)) and that at δ(H) 1.04 (H–C(20)) indicated that fragment B represents the C(11)–C(12)–C(13)(C(20)) part of **1**, coupled *via* methine C(14) to C(15). The two- and three-bond correlations of the resonances at 139.1 (C(11)), 40.7 (C(10)), and 72.3 (C(9)) with the Me group at δ(H) 0.92 (H–C(18)) and 0.88 (H–C(19)) corroborated the linkage of fragment B, one oxymethine group (C(9)) and two Me groups (C(18), C(19)) through the quaternary C(10). Furthermore, the HMBC correlations of the signals at δ(H) 6.52 (H–C(5)) with those for C(3), C(4), and C(15) allowed the assignment of the trisubstituted olefin as C(4)=C(5). Finally, the HMBC signals of the C-atom at δ(C) 78.0 (C(6)) with H-atoms at δ(H) 6.52 (H–C(5)), 5.60 (H–C(8)), and 1.28 (H–C(17)) revealed the complete structure of **1**. The positions of the Ac groups at C(7), C(8), C(9), and C(14) were deduced from the observed long-range couplings between the oxymethine H-atoms and the corresponding C=O C-atoms. The HMBC cross-peak between the OH group and C(6) confirmed the location of the OH group. The BzO group was placed at C(3) on the basis of the downfield-shifted H–C(3) signal at δ(H) 6.36. Then, the remaining Ac group had to be located at C(15). The above structure elucidation of **1** resulted in the same planar (but isomeric) molecule as in the case of (2*S*,3*S*,4*E*,6*S*,7*R*,8*R*,9*S*,11*E*,13*S*,14*S*,15*R*)-7,8,9,14,15-pentaacetoxy-3-(benzoyloxy)-6-hydroxyjatropha-4,11-diene (**5**), isolated earlier from *E. serrulata* [5].

The relative configurations of the stereogenic centers were studied by means of NOESY experiments (see the *Figure*), and by comparison of the chemical shifts of **1** and **5**. Due to the similar ^{1}H and ^{13}C resonances of the five-membered ring in **1** and **5**, the same configuration was assumed for this part of the molecule. In the NOESY spectrum, correlation signals were detected between H–C(2) and H–C(13), indicating an α-oriented H–C(13). The coupling constant J(13,14) $\approx$ 0 Hz demonstrated the α-position of H–C(14) [7][11]. The *Overhauser* effect between H–C(13) and H–C(11) indicated that H–C(11) is oriented below the plane of the macrocyclic ring; with regard to the (*E*)-configuration of C(11)=C(12), H–C(12) must be above the plane of the molecule. Consequently, the NOE interactions between H–C(11) and H–C(18), between H–C(18) and H–C(8), and between H–C(8) and H–C(3) spoke for the α-positions, while the NOE between H–C(19) and H–C(9) indicated β-positions. A further important *Overhauser* effect was detected between H–C(5) and H–C(12), revealing that H–C(5) is pointing into the twelve-membered ring and that the C(4)=C(5) bond adopts an (*E*)-configuration. The AcO–C(15) and AcO–C(7) groups are in β position, as dictated by the NOESY correlations between

Table 1. *NMR Spectral Data of* **1** *in* $CDCl_3$ (δ in ppm, multiplicities, J in Hz)

C-Atom	^{1}H	^{13}C	HMBC (H-atoms)	NOESY
C(1)	2.53 (*m*)	42.4	16	2, 13, 14, 16
C(2)	2.22 (*m*)	37.7	16	1, 3, 13, 16
C(3)	6.36 (*d*, 2.5)	79.1	5, 16	2, 6-OH, 8, 16
C(4)	–	140.4	5	–
C(5)	6.52 (*s*)	137.2	17	12, 17, 8, 7-OAc
C(6)	–	78.0	6-OH, 5, 8, 17	–
C(7)	4.96 (*d*, 6.7)	76.8	6-OH, 5, 8, 17	8, 17
C(8)	5.60 (*d*, 6.7)	70.6	–	3, 5, 7, 9, 18
C(9)	5.52 (*s*)	72.3	18, 19	8, 11, 19
C(10)	–	40.7	9, 18, 19	–
C(11)	5.37 (*d*, 16.7)	139.1	18, 19	9, 13
C(12)	5.47 (*dd*, 16.7, 8.6)	130.5	14, 20	5
C(13)	2.62 (*m*)	38.5	20	1, 2, 11, 14, 20
C(14)	5.97 (br. *s*)	78.7	20	1, 13, 20
C(15)	–	90.2	5, 14	–
C(16)	1.22 (*d*, 6.6)	14.2	–	1, 2, 3
C(17)	1.28 (*s*)	27.8	–	5, 6-OH, 7
C(18)	0.92 (*s*)	21.6	9, 19	8, 11
C(19)	0.88 (*s*)	29.9	18	9
C(20)	1.04 (*d*, 7.0)	23.1	–	13, 14
6-OH	6.07 (*s*)	–	–	3, 17
CO	–	[a])		
C(1′)	–	130.9		
C(2′,6′)	7.93 (*d*, 7.2)	130.0		15-OAc
C(3′,5′)	7.41 (*t*, 7.8)	128.9		
C(4′)	7.56 (*t*, 7.5)	[a])		
7-*C*OMe	–	170.3	7, 7-CO*Me*	
7-CO*Me*	2.12 (*s*)	21.2		
8-*C*OMe	–	171.0	8, 8-CO*Me*	
8-CO*Me*	2.06 (*s*)	22.3		
9-*C*OMe	–	171.6	9, 9-CO*Me*	
9-CO*Me*	2.09 (*s*)	21.5		
14-*C*OMe	–	170.4	14, 14-CO*Me*	
14-CO*Me*	2.20 (*s*)	22.1		
15-*C*OMe	–	171.1	15-CO*Me*	
15-CO*Me*	1.60 (*s*)	22.5		2′,6′

[a]) Not observed.

the *ortho*-Bz H-atoms and AcO–C(15), and between H–C(5) and AcO–C(7). The most-informative *Overhauser* effect was observed between H–C(3) and 6-OH, which unequivocally differentiated compounds **1** and **5** as epimers. All of the above data indicate structure **1** for this compound.

Compound **2** has been isolated as colorless crystals, with $[\alpha]_D^{29} = -85$ ($c = 0.1$, $CHCl_3$), and with the molecular formula $C_{37}H_{48}O_{12}$, as indicated by HR-ESI-MS. In the $CDCl_3$ ^{1}H-NMR spectrum, many broad and overlapping signals were observed; thus, all NMR spectra were also run in (D_6)benzene. The ^{1}H-NMR spectra of **2** exhibited the presence of one Bz, five Ac, and five Me groups (*Table 2*), some of which gave rise to broad *singlets*. The small quantity of **2** isolated did not allow us to record a

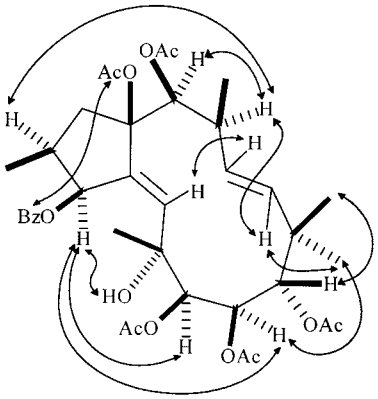


Figure. *NOESY Correlations for* **1**

^{13}C-NMR spectrum; accordingly, the structure was solved by means of 2D-NMR experiments (^{1}H,^{1}H-COSY, NOESY, HMQC, and HMBC). Supported by HMQC measurements, the ^{1}H,^{1}H-COSY spectrum indicated three sequences of correlated H-atoms; $-CH_2-CH(Me)-CH(OR)-CH-CH=$ (C(1)–C(5)), $-CH(OR)-CH(OR)-CH(OR)-$ (C(7)–C(9)), and $-CH=CH-CH(Me)-CH(OR)-$ (C(11)–C(14)). These fragments, the Me groups and the quaternary C-atoms were connected due to the long-range ^{1}H/^{13}C correlations (*cf. Table 2*). Most informative were the two- and three-bond correlations of the H–C(14), H–C(18), and H–C(17) H-atoms, indicating a 3,7,8,9,14,15-hexaacylated jatrophane-5,11-diene. On the basis of the HMBC spectrum, the positions of only three Ac groups could be determined since the Ac groups with broad signals did not show any HMBC correlations. Due to the cross-peaks of H–C(14), H–C(9) and the Ac C=O C-atoms, and based on the weak four-bond correlation between C(15) and the Ac H-atoms (δ(H) 2.30), the three Ac groups were placed at C(9), C(14), and C(15). Regarding the location of the Bz group, compound **6** ((5*E*,11*E*)-8,9,14,15-tetraacetoxy-3-(benzoyloxy)-7-hydroxyjatropha-5,11-diene) isolated from *E. serrulata* [5] was taken as a reference. The nearly identical chemical shifts of C(3) and H–C(3) for both **2** and **6** (**2**: δ(C) 82.3, δ(H) 4.94; **6**: δ(C) 82.4, δ(H) 4.90) clearly showed that the BzO group is situated on C(3).

The relative configuration of **2** was determined on the basis of the NOESY spectrum. Starting from the H–C(4) α configuration as a reference point, NOE-enhanced signals detected between H–C(4)/H–C(7/8), H–C(7/8)/H–C(19), H–C(4)/H–C(3), H–C(3)/H–C(16), H–C(4)/H–C(13), and H–C(13)/H–C(14) provided evidence of the α orientation of these H-atoms. The NOESY cross-peak between H–C(18)/H–C(9) pointed to the β position of H–C(9). The (*E*)-configuration of the C(11)=C(12) bond was derived from the corresponding coupling constant (J = 16.0 Hz). The C(5)=C(6) bond also adopts the (*E*)-configuration since H–C(17) displayed a NOESY correlation with the α-oriented H-atoms H–C(4), H–C(7/8), and H–C(13), as did H–C(5) with the β-oriented H–C(9). In conclusion, the structure of **2** corresponds to (5*E*,11*E*)-7β,8β,9α,11*E*,14β,15β-pentaacetoxy-3β-(benzoyloxy)jatropha-5,11-diene.

Table 2. *NMR Spectral Data of* **2** *and* **3** *in* $CDCl_3$ (δ in ppm, multiplicities, J in Hz)

C-Atom	**2**				**3**
	^1H[a])	^{13}C[b])	HMBC (H-atoms)	NOESY	^1H[c])
C(1a)	3.52 (br. *d*, 12.1)	41.9	16, 14	1b	2.74 (*dd*, 14.4, 9.7)
C(1b)	1.61 (*t*, 12.1)			1a, 14	1.55 (*m*)
C(2)	2.35 (*m*)	37.6	16	16	2.56 (*m*)
C(3)	4.94 (*t*, 7.1)	82.3	16	4, 16	5.80 (br. *s*)
C(4)	3.29 (br. *t*)	47.0		3, 7/8, 13, 17	–
C(5)	5.69 (br. *s*)	122.1	17	9	5.60 (*d*, 2.0)
C(6)	–	131.9	17, 7/8	–	–
C(7)	5.02 (br. *s*)	77.0	17	4, 17, 19	5.89 (*d*, 4.0)
C(8)	5.02 (br. *s*)	68.9			5.69 (*d*, 4.0)
C(9)	5.13 (br. *s*)	72.8	18	5, 12, 18	–
C(10)	–	39.6	18	–	–
C(11)	5.15 (*d*, 16.0)	136.3	18.13	12, 13, 19, 7/8/14	5.48 (*d*, 16.2)
C(12)	5.78 (*dd*, 16.0, 7.6)	131.9	20	9, 11, Ac δ 2.30, 18	5.39 (*dd*, 16.2, 8.9)
C(13)	2.58 (*m*)	38.6	20, 11, 14	4, 11, 17, 14, 20	3.20 (*m*)
C(14)	5.01 (*d*, 2.7)	80.8	20	1b, 13, 20	4.94 (*d*, 9.2)
C(15)	–	93.5	14	–	–
C(16)	1.10 (*d*, 6.9)	17.4		2, 3	1.25 (*d*, 7.0)
C(17)	1.82 (br. *s*)	16.0		4, 7/8, 13	1.37 (*s*)
C(18)	0.99 (*s*)	23.1	11, 19	9, 12, Ac δ 2.30	1.21 (*s*)
C(19)	0.88 (br. *s*)	20.7	18, 11	7/8, 11	0.91 (*s*)
C(20)	0.96 (*d*, 7.0)	20.0	12, 14	13, 14	1.00 (*d*, 6.9)
9-OH	–	–	–	–	3.26 (*s*)
CO	–	165.8			–
C(1′)	–	130.4			–
C(2′,6′)	7.93 (*d*, 7.5)	129.8			7.78 (*d*, 7.4)
C(3′,5′)	7.40 (*t*, 7.7)	128.6			7.34 (*t*, 7.5)
C(4′)	7.52 (*t*, 7.4)	133.1			7.51 (*t*, 7.4)

[a]) ^1H-NMR Signals of Ac groups: 2.30 (*s*), 2.13 (*s*), 2.05 (*s*), 2.00 (br. *s*), and 1.42 (br. *s*). [b]) ^{13}C-NMR Signals of Ac groups: 171.0, 169.8, and 169.2 (C=O); 23.1, 3 × 21.0, and 20.0 (Me). [c]) ^1H-NMR Signals of Ac groups: 2.20 (*s*), 2.16 (*s*), and 2.00 (*s*).

Compound **3**, an amorphous solid, with $[\alpha]_D^{29} = +20$ ($c = 0.01$, $CHCl_3$), exhibited an $[M+Na]^+$ peak at m/z 741.2876 in its HR-ESI mass spectrum, corresponding to the molecular formula $C_{40}H_{46}O_{12}$. The ^1H-NMR spectrum contained signals due to two Bz, three Ac, and five Me groups. Additionally, methine and methylene H-atoms were detected in the ^1H-NMR spectrum. On the basis of the $^1H,^1H$-COSY spectrum, these were assigned as $-CH_2-CH(Me)-CH(OR)-C=CH-$ $(-C(1)-C(2)(C(16))-C(3)-C(4)=C(5)-)$, $-CH=CH-CH(Me)-CH(OR)-$ $(-C(11)=C(12)-C(13)-(C(20))-C(14)-)$, and $-CH(OR)-CH(OR)-$ $(-C(7)-C(8)-)$. When the ^1H-NMR data of **3** were compared with those on other jatrophane polyesters, the *E. serrulata* metabolite **7** was found to be very similar [7]. The close chemical shifts and coupling constants revealed that the two compounds possess the same diterpene core, differing only in one ester group: the tigloyloxy substituent of **7** is replaced by a BzO group in **3**. The paramagnetically shifted H–C(7) signal (**3**: δ(H) 5.89 (*d*); **7**: δ(H) 5.70

(*d*)) demonstrated that an aromatic ester group is present at C(7) in **3**, instead of an aliphatic group. Therefore, **3** was identified as *rel*-(2*R*,3*S*,4*E*,6*S*,7*R*,8*S*,9*R*,11*E*,-13*R*,14*S*,15*R*)-8,14,15-triacetoxy-3,7-bis(benzoyloxy)-6,9-epoxy-9-hydroxyjatropha-4,11-diene.

Compound **4** was found to be identical in all of its characteristics, including melting point, specific rotation, and NMR spectral data, with *rel*-(2*R*,3*S*,4*E*,6*S*,7*R*,8*R*,-9*S*,11*E*,13*S*,15*R*)-7,8,9,15-tetraacetoxy-3-(benzoyloxy)-6-hydroxy-14-oxojatropha-4,11-diene. This compound had previously been isolated from *E. serrulata*, and was found to be active in reversing the multidrug resistance of tumor cells [5].

Conclusions. – *Euphorbia platyphyllos* accumulates highly functionalized jatrophane diterpenes containing jatrophane diene polyols esterified by benzoic and acetic acids. Compounds **1**–**4** do not represent a stereochemically uniform series because they differ in the orientations of the Me groups at C(2), C(6), and C(13). Structurally, the compounds obtained from *E. platyphyllos* are very similar to the diterpenes isolated from *E. serrulata*, *e.g.*, compounds **1**, **3**–**5**, **7**, and serrulatin A and B are uniquely unsaturated at C(4)/C(5) [12]. Moreover, **3** contains the same heterocyclic ring system previously found only in the *E. serrulata* metabolites serrulatin A and its C(2) epimer (**7**). This chemical relationship supports the botanical similarity of the two species. Taxonomically, *E. platyphyllos* and *E. serrulata* are closely related and can be distinguished only *via* the morphological details of the fruits [13].

Experimental Part

General. For column chromatography, polyamide (*ICN*), and, for vacuum-liquid chromatography (VLC), silica gel (*Kieselgel GF$_{254}$*, 15 μm, *Merck*) were used. Chromatographic fractions were monitored by TLC on silica-gel plates (*Merck 5715*), and visualized by spraying with conc. H_2SO_4, followed by heating. HPLC was carried out on *LiChrospher-Si-100* and *LiChrospher-RP-18* (5 μm, 200 × 4 mm) columns on a *Waters Millipore* instrument at a flow rate of 0.4 ml/min, with detection at 254 nm. Melting points are uncorrected. Optical rotations were determined with a *Perkin-Elmer 341* polarimeter. NMR spectra were recorded in $CDCl_3$ on a *Bruker Avance DRX-500* spectrometer at 500 MHz (^{1}H) and 125 MHz (^{13}C), with $SiMe_4$ as internal standard. Two-dimensional data were acquired and processed with standard *Bruker* software. HR-ESI-MS measurements were carried out on a *Perkin-Elmer Q-STAR Pulsar Q-TOF* mass spectrometer equipped with an electrospray ion source.

Plant Material. E. platyphyllos was collected from wild stock growing in the region of Csáfordjánosfa, Nagyacsád, and Vitnyéd (Hungary) in August and September 1999, and identified by Dr. *Gyula Pinke* (Department of Botany, Institute of Biology and Environmental Science, University of West Hungary, Mosonmagyaróvár, Hungary). A voucher specimen (No. 549) was deposited in the Herbarium of the Department of Pharmacognosy, University of Szeged, Szeged, Hungary.

Extraction and Isolation. Dried, ground whole plants of *E. platyphyllos* (131.6 g) were extracted with $CHCl_3$ and evaporated to a crude residue (6.61 g), which was fractionated on a polyamide column with mixtures of MeOH/H_2O 3:2 (600 ml) and 4:1 (900 ml), respectively. The fraction eluted with MeOH/H_2O 3:2 was subjected to silica-gel vacuum liquid chromatography (VLC) using a gradient of cyclohexane/AcOEt/EtOH 19:1:0, 9:1:0, 4:1:0, 70:30:1, 60:40:1.5, 50:50:2, and 50:50:10. In total, 70 fractions of 20 ml were collected and successively combined after TLC monitoring. Fractions 17 and 18 were further chromatographed by prep. TLC (SiO_2, cyclohexane/AcOEt/EtOH 60:13:0.7), yielding four subfractions (I–IV). From the CH_2Cl_2 soln. of subfraction I, compound **2** (3.8 mg) spontaneously crystallized. Subfraction II was purified by RP-HPLC (MeCN/H_2O 4:1). The fraction eluting at t_R 15.38 min was subjected to NP-HPLC (cyclohexane/AcOEt/EtOH 85:7:0.7), affording **1** (4.0 mg). Subfraction III, which was a complex mixture, was purified by means of prep. TLC (PhH/AcOEt 17:1), and then by RP-HPLC (MeCN/H_2O 7:3) to yield **3** (0.8 mg). The

same chromatographic purification, including prep. TLC and NP-HPLC, also furnished **2** (10.4 mg) from VLC fractions 19–21. In VLC fractions 25–29, crystallization of **4** (2 mg) was observed on standing.

rel-*(2S,3S,4E,6S,7R,8R,9S,11E,13S,14S,15R)-7,8,9,14,15-Pentaacetoxy-3-(benzoyloxy)-6-hydroxyjatropha-4,11-diene* (**1**). Amorphous solid. $[\alpha]_D^{29} = +10$ ($c = 0.025$, $CHCl_3$). UV (MeOH): 234 (3.73), 273 (2.64), 286 (2.54). ^{1}H- and ^{13}C-NMR: *cf. Table 1*. HR-ESI-MS: 723.2958 ($[M+Na]^+$, $C_{37}H_{48}O_{13}Na^+$; calc. 723.2992).

rel-*(2S,3S,4S,5E,7S,8S,9S,11E,13S,14S,15R)-7,8,9,14,15-Pentaacetocy-3-(benzoyloxy)jatropha-5,11-diene* (**2**): Colorless crystals. M.p. 213–215°. $[\alpha]_D^{29} = -85$ ($c = 0.1$, $CHCl_3$). UV (MeOH): 229 (3.67), 273 (2.64), 279 (2.58). ^{1}H- and ^{13}C-NMR in $CDCl_3$: *cf. Table 2*. ^{1}H-NMR (500 MHz, (D_6)benzene[1]): δ 3.87 (br. *d*, $J = 12.0$, $H_a-C(1)$); 1.38 (*m*, $H_b-C(1)$); 2.35 (*m*, H–C(2)); 4.92 (*t*, $J = 7.1$, H–C(3)); 2.94 (br. *t*, $J = 7.0$, H–C(4)); 5.90 (br. *s*, H–C(5)); 5.26 (br. *s*, H–C(7)); 5.38 (br. *s*, H–C(8)); 5.43 (*s*, H–C(9)); 5.16 (*d*, $J = 16.0$, H–C(11)); 6.04 (*dd*, $J = 16.0$, 7.4, H–C(12)); 2.43 (*m*, H–C(13)); 4.92 (*s*, H–C(14)); 1.01 (*d*, $J = 6.8$, 3 H–C(16)); 1.79 (br. *s*, 3 H–C(17)); 1.03 (*s*, 3 H–C(18)); 1.00 (br. *s*, 3 H–C(19)); 0.97 (*d*, $J = 6.5$, 3 H–C(20)); 8.09 (*d*, $J = 7.1$, 2 H, H–C(2′,6′)); 7.08 (*m*, 3 H, H–C(3′–5′)); 2.39, 1.95, 2 × 1.73, 1.45 (5*s*, 5 Ac). HR-ESI-MS: 707.3016 ($[M+Na]^+$, $C_{37}H_{48}NO_{12}Na^+$; calc. 707.3043).

rel-*(2R,3S,4E,6S,7R,8S,9R,11E,13R,14S,15R)-8,14,15-Triacetoxy-3,7-bis(benzoyloxy)-6,9-epoxy-9-hydroxyjatropha-4,11-diene* (**3**). Amorphous solid. $[\alpha]_D^{29} = +20$ ($c = 0.01$, $CHCl_3$). ^{1}H-NMR: *cf. Table 2*. HR-ESI-MS: 741.2876 ($[M+Na]^+$, $C_{40}H_{46}O_{12}Na^+$; calc. 741.2886).

rel-*(2R,3S,4E,6S,7R,8R,9S,11E,13S,15R)-7,8,9,15-Tetraacetoxy-3-(benzoyloxy)-6-hydroxy-14-oxojatropha-4,11-diene* (**4**). Colorless crystals. M.p. 219–221°. The ^{1}H- and ^{13}C-NMR data agreed with the literature values [5].

This work was supported by grants OTKA T035200 and FKFP 0024/2001. The authors thank Dr. *Gyula Pinke* (Department of Botany, Institute of Biology and Environmental Science, University of West Hungary, Mosonmagyaróvár) for the collection and identification of the plant material.

REFERENCES

[1] R. Sahai, R. P. Rastogi, J. Jakupovic, F. Bohlmann, *Phytochemistry* **1981**, *20*, 1665.
[2] A. Miglietta, L. Gabriel, G. Appendino, C. Bocca, *Cancer Chemother. Pharmacol*. **2003**, *51*, 67.
[3] I. Mucsi, J. Molnár, J. Hohmann, D. Rédei, *Planta Med*. **2001**, *67*, 672.
[4] L. Betancur-Galvis, J. Checa, J. A. Marco, E. Estornell, *Planta Med*. **2003**, *69*, 177.
[5] J. Hohmann, J. Molnár, D. Rédei, F. Evanics, P. Forgo, A. Kálmán, G. Argay, P. Szabó, *J. Med. Chem*. **2002**, *45*, 2425.
[6] J. L. Hartwell, *J. Nat. Prod*. **1969**, *32*, 153.
[7] D. Rédei, J. Hohmann, F. Evanics, P. Forgo, P. Szabó, I. Máthé, *Helv. Chim. Acta* **2003**, *86*, 280.
[8] G. Appendino, S. Jakupovic, G. C. Tron, J. Jakupovic, V. Milon, M. Ballero, *J. Nat. Prod*. **1998**, *61*, 749.
[9] L. G. Liu, R. X. Tan, *J. Nat. Prod*. **2001**, *64*, 1064.
[10] C. Valente, M. J. U. Ferreira, P. M. Abreu, M. Pedro, F. Cerqueira, M. S. J. Nascimento, *Planta Med*. **2003**, *69*, 361.
[11] G. Appendino, P. Spagliardi, M. Ballero, G. Seu, *Fitoterapia* **2002**, *73*, 576.
[12] J. Hohmann, D. Rédei, F. Evanics, A. Kálmán, G. Argay, T. Bartók, *Tetrahedron* **2000**, *56*, 3619.
[13] 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.

Received July 10, 2003

[1]) For better comparison, the data are ordered according to atomic sequence rather than chemical shifts.