Isolation and Structure Revision of Pepluane Diterpenoids from *Euphorbia peplus*

Judit Hohmann,*,† Gábor Günther,‡ Andrea Vasas,† Alajos Kálmán,§ and Gyula Argay§

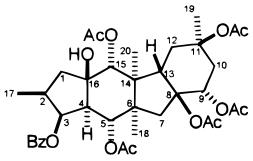
Department of Pharmacognosy, Albert Szent-Györgyi Medical University, P.O. Box 121, H-6701 Szeged, Department of Pharmaceutical Analysis, Albert Szent-Györgyi Medical University, 4 Somogyi u., H-6720 Szeged, Hungary, and Institute of Chemistry, Chemical Research Center, Hungarian Academy of Sciences, P.O. Box 17, H-1525 Budapest, Hungary

Received July 23, 1998

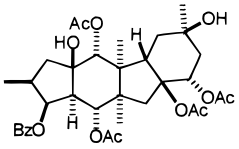
A new pepluane diterpene polyester (**2**) was isolated from a CH_2Cl_2 extract of the whole, undried plant of *Euphorbia peplus*, together with the known compound **1**. The structures were established by high-field spectroscopic methods, including 2D NMR techniques, and by X-ray crystallography, and the stereostructure of the first member of the pepluane diterpenoids (**1**) was revised.

Plants of the genus *Euphorbia* are known to produce a large variety of diterpenoids, some of which are highly irritant and have tumor-promoting activity, while others exhibit antileukemic, cytotoxic, and analgetic activity.[1] *Euphorbia peplus* L. (Euphorbiaceae) is a small, annual, herbaceous plant with milky latex, which occurs all over the world. The plant has been used as an antiasthmatic and anticatarrhal agent and to treat cancerous conditions in traditional medicine in many areas of the world.[2,3] Earlier investigations of this species have led to reports of the isolation of the skin irritant ingenane diterpenes, 2-deoxyingenol 3-*O*-angelate, ingenol 2-*O*-octanoate, and ingenol 2-*O*-acetate-3-*O*-angelate from the acetone extract of the latex and from a diethyl ether-soluble fraction of the plant.[4,5] While the present work was in progress, jatrophane diterpenoids and a tetracyclic diterpene (**1**) based on a new carbon framework, named pepluane, were reported from the dried, whole plant of *E. peplus*.[6]

In the course of our studies on biologically active compounds from Hungarian *Euphorbia* species, we have reinvestigated *E. peplus* for its diterpene constituents and have isolated two pepluane diterpenoids (**1**, **2**) from a dichloromethane extract of the fresh plant. This paper deals with the isolation and structure elucidation of these compounds.



1



2

Results and Discussion

The dichloromethane-soluble fraction obtained from a methanolic extract of the fresh, whole plant of *Euphorbia peplus*, collected in Miskolc, Hungary, in 1996, was subjected to repeated column chromatography on polyamide (H_2O→MeOH) and Si gel ($CHCl_3$−Me_2CO, gradient) followed by HPLC to afford, in crystalline form, compounds **1** (mp 225 °C) and **2** (mp 238−240 °C) in 0.0028% and 0.0012% yields, respectively.

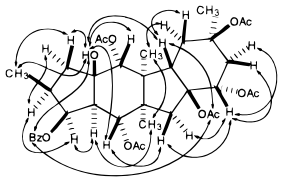


Figure 1. NOESY correlations for **1**.

Mass spectrometry and detailed NMR investigations indicated that compound **1** was identical with the pepluane diterpene **1** isolated earlier from the same plant.[6] We now report the missing physical and spectral data (see Experimental Section) and a stereochemical study of **1** by means of NOESY experiments in solution, and X-ray crystallography.

The NOESY spectrum of **1**, recorded in C_6D_6, demonstrated *trans*-fused A/B rings, as NOE effects were detected between 16-OH and H-1β, 16-OH and H-17, 16-OH and *ortho*-benzoyl protons, H-4 and H-3, and H-3 and H-2 (Figure 1). These NOE interactions revealed a β-oriented methyl group on C-2 and a benzoyl group on C-3. The cross-peaks between H-4 and H-18 and between H-18 and H-20 in the NOESY spectrum proved a cis B/C ring junction in the molecule. The NOESY correlations between 16-OH and H-15 and between 16-OH and H-5 indicated the presence of ester groups in the α position on C-5 and C-15. The 16-OH group also participated in an NOE interaction with H-13, from which the β orientation of H-13 was concluded. In our NOESY experiment, correlative signals were observed between 8-OAc and H-13 and between 8-OAc and *ortho*- and *meta*-benzoyl protons, pointing to cis-fused C/D rings, in contrast with the proposal of Jakupovic and coworkers.[6] We could not establish the stereochemistry of H-9 on the basis of the NOESY spectrum, because this proton gave cross-peaks both with H-7α and H-7β and with H-10α and H-10β. Further, the configuration of C-11 could not be determined because of missing diagnostic NOESY correlations.

To determine the complete relative and absolute configurations and solid-state conformation of **1**, single-crystal X-ray analysis was performed. Two conformers (I and II) (Figure 2) were detected in the crystal lattice, which form dimers through O−H···O hydrogen bonds. The geometry of these interactions [H...A (Å), D...A (Å), and DHA (°)] for

* To whom correspondence should be addressed. Tel: (36)-62 455 558. Fax: (36)-62 426 146. E-mail: hohmann@pharma.szote.u-szeged.hu.
† Department of Pharmacognosy, Albert Szent-Györgyi Medical University.
‡ Department of Pharmaceutical Analysis, Albert Szent-Györgyi Medical University.
§ Institute of Chemistry, Chemical Research Center, Hungarian Academy of Sciences.

10.1021/np980319l CCC: $18.00
Published on Web 12/05/1998

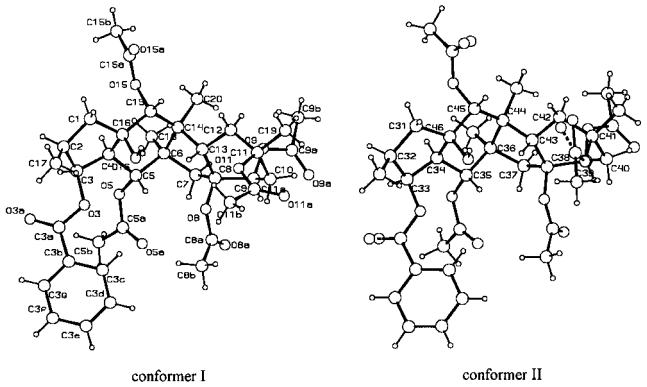


Figure 2. Perspective views of **1** conformers I and II. (In the latter case only the atoms in the molecular skeleton are labeled, using numbers increased by 30 relative to conformer I; bonds to the disordered atoms O33b, O41b, and O45b are drawn with dashed lines.)

O16−H16...O41a and O46−H46...O15a contacts are 2.06, 2.87 (1), 170.4 and 2.27, 3.078 (3), 167.0. The two conformers, which differ only in the internal rotations of the 9-OAc and 11-OAc groups, unambiguously demonstrated an α-oriented acetyl group on C-9 and a *β*-oriented one on C-11. Additionally, it was found that ring B assumed a chair conformation and ring D a boat conformation, differing from the PCMODEL-calculated conformation.[6] The conformations of the symmetry-independent molecules exhibit a few visible differences. The five-membered ring A assumes a distorted envelope shape in molecule I, whereas it has a half-chair form in molecule II, with an almost perfect two-fold symmetry axis bisecting atom C32 and the opposite bond C34−C46. The conformation of the other three rings fused to each other with trans, cis, and cis junctions may be defined as follows: ring B is a slightly flattened (at C14/C44) chair, ring C is a distorted envelope, and ring D assumes a boat (1,4-diplanar) conformation. Assuming the α position, the out-of-plane atoms are C9 (C39) and C12 (C42). This relatively rare conformation of ring D, with numerous substituents, may account for the misinterpretation of the NMR spectra by Jakupovic and co-workers[6] as concerns the C/D junction and the orientation of the acetoxy moieties on C8 and C9. Prior to the X-ray analysis of **1**, this feature of the molecular conformation presumably prevented the correct NMR elucidation of the substituent positions. On the whole, the four acetyl and one benzoyl functions exhibit differences only a few degrees (<10°) in rotation around the ester C−O−C bonds. Only the 11-OAc groups exhibit different rotations ($\Delta\rho \approx 112°$) about the C11−O11 bond. The second largest difference ($\Delta\rho \approx 31°$) in the substituent positions is displayed around the C3−O3 bond, accompanied by a second rotation of $\Delta\varphi \approx 30°$ about the $C_{ph}-C_{acyl}$ bond in the bulky benzoyl moiety.

Compound **2** gave a parent ion in the EIMS at *m/z* 658, appropriate for a molecular formula of $C_{35}H_{46}O_{12}$. It exhibited IR absorption bands at 3432, 1738, and 1711 cm^{-1} and UV maxima at 229, 274, and 282 nm, characteristic of hydroxy, ester, and phenyl groups. The ^1H and ^{13}C NMR spectra of **2** revealed four acetate and one benzoate groups (Table 1). Additionally, the spectra exhibited resonances closely related to that of **1**. After the ^1H and ^{13}C NMR data on **2** had been assigned by analysis of its $^1H-^1H$ COSY, HMQC, and HMBC spectra, it was obvious that compounds **1** and **2** were based on the same parent system and differed only in esterification. The absence of one acetate signal and the appearance of one hydroxy signal (δ_H 2.82 s) indicated the replacment of one of the acetyl residues with a hydroxy group. Comparison of the ^1H and ^{13}C signals of **1** and **2** revealed a significant difference in the chemical shift values of C-11 (**1**, δ_{C-11} 79.8; **2**, δ_{C-11} 68.0), from which the position of the hydroxy group on C-11 was concluded. This was substantiated by the occurrence of $^3J_{C-H}$ coupling between 11-OH and C-19 in the HMBC spectrum. A careful comparison of the NOESY spectra of compounds **1** and **2** (Table 1) enabled us to assume the same stereochemistry for **2** as that of **1**. Thus, the structure of this compound was elucidated as shown in formula **2**.

Table 1. NMR Data on Compound 2 [$CDCl_3$, δ (ppm) J = Hz)]

atom	$^1H^a$	$^{13}C^b$	NOESY H no.	HMBC C no.
1α	2.17 dd (14.2, 11.6)	44.7	2	2, 3, 15, 16, 17
1*β*	1.51 dd (14.2, 5.1)		16-OH	2, 3, 4, 16, 17
2	2.54 m	35.9	1α, 3, 17	17
3	5.80 m	76.4	2, 4	1, 4, 16
4	2.40 dd (12.0, 4.3)	48.3	3, 18	5, 6, 16
5	5.83 d (12.0)	69.7	7*β*, 13, 16-OH	3, 4, 7, 16, 18
6		49.0		
7*β*	2.48 d (16.0)	39.8	5	5, 6, 8, 13, 14
7α	1.58 d (16.0)		18	5, 6, 8, 9, 18
8		88.6		
9	5.78 d (4.9)	68.3	8-OAc, 10α,*β*	7, 8, 11, 13
10*β*	1.97 dd (16.9, 5.7)	41.6	11-OH	11, 12
10α	1.85 d (16.9)		19	8, 9, 19
11		68.0		
12*β*	1.75 m	33.9		8, 10, 11, 13, 19
12α	1.69 t (12.9)			13, 14
13	4.30 dd (12.8, 6.6)	47.0	5, 11-OH, 15, 16-OH	9, 11, 12, 14, 15, 20
14		52.0		
15	5.07 s	73.3	13, 16-OH	1, 4, 6, 13, 14, 16
16		84.2		
17	1.05 d (7.3)	16.6	16-OH, 2	1, 2, 3
18	1.08 s	16.7	4, 7α, 20	5, 6, 7, 14
19	1.29 s	31.5	10α	10, 11, 12
20	0.92 s	16.3	18	6, 13, 14, 15
11-OH	2.82 s		13, 10*β*	19
16-OH	3.17 s		1*β*, 5, 13, 15, 17	4, 15, 16

[a] ^1H NMR signals of the acyl groups: 5-OAc: 1.72 s, 8-OAc: 1.96 s, 9-OAc: 2.03 s, 15-OAc: 2.13 s, 3-OBz: 7.92 d (7.4) (H-2′, 6′), 7.54 't′ (7.4) (H-4′), 7.41 't′ (7.7) (H-3′, 5′). [b] ^{13}C NMR signals: 5-OAc: 22.0, 170.4, 8-OAc: 20.9, 170.2, 9-OAc: 21.4, 169.3, 15-OAc: 20.9, 170.0, 3-OBz: 165.8 (CO), 133.2 (C-4′), 129.3 (C-2′, 6′), 128.5 (C-3′, 5′), 130.0 (C-1′).

Experimental Section

General Experimental Methods. NMR spectra were recorded on a Bruker Avance DRX 400 spectrometer at 400 MHz (^1H) and 100 MHz (^{13}C). The signals of the deuterated solvents were taken as the reference. Two-dimensional experiments were performed with standard Bruker software. MS measurements were carried out on a Finnigan MAT 8430 spectrometer operating at 70 eV ionizing energy. IR spectra of KBr disks were run on a Perkin−Elmer Paragon 1000 PC FTIR instrument. Optical rotations were determined in $CHCl_3$ at ambient temperature, using a Perkin−Elmer 341 polarimeter. Melting points were not corrected. 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 (LiChrospher Si 100, 5 μm, 200 × 4 mm, Merck) and on a reversed-phase (LiChrospher RP-18, 5 μm, 200 × 4 mm, Merck) column.

Plant Material. *E. peplus* was collected in June 1996, in Miskolc, Hungary. A voucher sample has been deposited at the Department of Pharmacognosy, Albert Szent-Györgyi Medical University, Szeged, Hungary.

Extraction and Isolation. The fresh plant material (200 g) was percolated with MeOH (1800 mL) at room temperature. The crude extract was concentrated in vacuo to 100 mL, and

exhaustively extracted with CH_2Cl_2. Evaporation of the organic phase gave a greenish-brown residue (4.7 g), which was chromatographed on a polyamide column (28 g) with mixtures of H_2O−MeOH (3:2 and 1:4) as eluents. Fractions obtained with H_2O−MeOH (2:3) were combined and subjected to Si gel flash chromatography, using a gradient system of $CHCl_3$ and Me_2CO. Fractions 6−10 eluted with $CHCl_3$ were further fractionated by reversed-phase HPLC with MeOH−H_2O (7:3) as eluent at a flow rate of 0.7 mL/min. Finally, compounds observed at retention times of 13.5 and 20.4 min were transferred to a normal-phase HPLC column eluted with cyclohexane−EtOAc−EtOH (30:10:1) to afford compounds **1** (5.5 mg) and **2** (2.3 mg).

Compound 1: white needles, mp 225 °C (from diethyl ether−*n*-hexane); $[\alpha]^{25}_D$ +5° (*c* 0.06, $CHCl_3$); UV (MeOH) λ_{max} 230 (ϵ 13 040), 272 (ϵ 1210), 280 (ϵ 1070) nm; IR (KBr) 3430, 1741, 1712, 1370, 1246, 1032, 717 cm^{-1}; ^1H NMR (C_6D_6, 400 MHz) δ 1.86 (1H, dd, J = 13.7, 11.4 Hz, H-1α), 1.12 (1H, dd, J = 13.7, 4.6 Hz, H-1β), 2.0 (1H, m, H-2), 5.76 (1H, dd, J = 5.7, 4.7 Hz, H-3), 2.18 (1H, dd, J = 11.9, 4.7 Hz, H-4), 6.09 (1H, d, J = 11.9 Hz, H-5), 1.66 (1H, d, J = 15.7 Hz, H-7α), 2.77 (1H, d, J = 15.7 Hz, H-7β), 6.29 (1H, d, J = 5.0 Hz, H-9), 1.80 (1H, d, J = 17.1 Hz, H-10α), 2.52 (1H, ddd, J = 17.1, 5.0, 1.1 Hz, H-10β), 1.59 (1H, t, J = 13.3 Hz, H-12α), 2.59 (1H, ddd, J = 13.3, 5.8, 1.1 Hz, H-12β), 4.32 (1H, dd, J = 13.3, 5.8 Hz, H-13), 5.26 (1H, s, H-15), 0.84 (3H, d, J = 7.2 Hz, H-17), 1.07 (3H, s, H-18), 1.48 (3H, s, H-19), 0.89 (3H, s, H-20), 2.91 (1H, s, 16-OH), 1.76 (3H, s, 5-OAc), 1.80 (3H, s, 11-OAc), 1.98 (3H, s, 8-OAc), 1.58 (3H, s, 9-OAc), 1.71 (3H, s, 15-OAc), 8.15 (2H, d, J = 6.9 Hz, 3-OBz 2′, 6′), 7.03−7.13 (3H, m, 3-OBz 3′, 4′, 5′); ^{13}C NMR (C_6D_6, 100 MHz) δ 45.4 (C-1), 36.7 (C-2), 76.7 (C-3), 49.5 (C-4), 70.7 (C-5), 49.8 (C-6), 41.2 (C-7), 87.9 (C-8), 68.3 (C-9), 39.5 (C-10), 80.4 (C-11), 31.9 (C-12), 47.7 (C-13), 52.1 (C-14), 74.7 (C-15), 84.8 (C-16), 16.9 (C-17), 17.3 (C-18), 29.2 (C-19), 16.9 (C-20), 21.2, 170.4 (5-OAc), 23.1, 171.2 (11-OAc), 21.2, 170.4 (5-OAc), 21.4, 168.9 (9-OAc), 21.0, 169.6 (15-OAc), 166.4, 133.6, 131.8, 130.5, 129.1 (3-OBz); HREIMS obsd *m*/*z* 538.2619 [M − 2 × AcOH − CH_2CO]$^+$, calcd for $C_{31}H_{38}O_8$ 538.2567, and obsd *m*/*z* 460.2392 [M − 4 × AcOH], calcd for $C_{29}H_{32}O_5$ 460.2250.

Crystal Data on 1: $C_{37}H_{48}O_{13}$, M = 700.75, orthorhombic, space group $P2_12_12_1$, a = 10.178(5), b = 18.387(2), c = 39.621-(5) Å, V = 7415(4) Å^3, Z = 8, D_c = 1.255 gcm^{-3}, F(000) = 2992, μ(Cu Kα = 1.5418 Å) = 0.789 mm^{-1}. Data were collected on an Enraf−Nonius CAD4 diffractometer in the range 2.23 < θ < 74.51°. The structure was determined by direct methods and refined by full-matrix least-squares analysis. All nonhydrogen atoms were refined anisotropically. There is disorder of some oxygen atoms, which have been modeled using two atomic sites and occupation factors of 0.5. The hydrogen atoms were introduced in idealized positions and added to the structure factor calculations. The final R values were R_1 = 0.062, wR_2 = 0.1617 for 10 738 reflections taken with Fo > 4σFo and R_1 = 0.083, wR_2 = 0.1749 for all 14 974 data. Crystallographic data on **1**, including atomic coordinates, have been deposited with the Cambridge Crystallographic Data Centre. Copies of the data can be obtained free of charge, on application to the Director, CCDC, 12 Union Road, Cambridge CB2 1EZ UK [Fax: +44-(0)1223-336033 or e-mail: deposit@ccdc.cam.ac.uk].

Compound 2: white needles, mp 238−240 °C (from diethyl ether); $[\alpha]^{25}_D$ +40° (*c* 0.2, $CHCl_3$); UV (MeOH) λ_{max} 229 (ϵ 13 200), 274 (ϵ 975), 282 (ϵ 766) nm; IR (KBr) 3432, 1738, 1711, 1369, 1241, 1027, 715 cm^{-1}; ^1H and ^{13}C NMR, see Table 1; EIMS (70 eV) *m*/*z* (% rel int) 658 (0.1) [M]$^+$, 598 (1) [M − AcOH]$^+$, 538 (55) [M − 2 × AcOH]$^+$, 523 (53) [M − 2 × AcOH − CH_3]$^+$, 478 (9) [M − 3 × AcOH]$^+$, 460 (12) [M − 3 × AcOH − H_2O]$^+$, 320 (49) [M − 3 × AcOH − BzOH − 2 × H_2O]$^+$; HREIMS obsd *m*/*z* 538.2593, calcd for $C_{31}H_{38}O_8$ 538.2567 and obsd *m*/*z* 320.1786, calcd for $C_{22}H_{24}O_2$, 320.1776.

Acknowledgment. We are grateful to Dr. Gyula Jerkovich (Spectroscopic Department, Institute for Drug Research Ltd., Budapest, Hungary) for the mass spectroscopic measurements. This investigation was funded by the National Scientific Research Fund, project no. OTKA T022587.

References and Notes

(1) Evans, F. J.; Taylor, S. E. *Prog. Chem. Org. Nat. Prod.* **1983**, *44*, 1−99.
(2) Khafagy, S. M.; Gharbo, S. A.; Salam, N. A. A. *Planta Med.* **1975**, *27*, 387−394.
(3) Hartwell, J. L. *J. Nat. Prod.* **1969**, *32*, 153−205.
(4) Gotta, H.; Adolf, W.; Opferkuch, H. J.; Hecker, E. *Z. Naturforsch.* **1984**, *39b*, 683−694.
(5) Rizk, A. M.; Hammouda, F. M.; El-Missiry, M. M.; Radwan, H. M.; Evans, F. J. *Phytochemistry*, **1985**, *24*, 1605−1606.
(6) Jakupovic, J.; Morgenstern, T.; Bittner, M.; Silva, M. *Phytochemistry* **1998**, *47*, 1601−1609.

NP980319L