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Jatrophane diterpenoids from *Euphorbia peplus*

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Abstract

From the pro-inflammatory active extract of *Euphorbia peplus*, a new diterpene polyester (**1**) based on the jatrophane skeleton was isolated together with the known compounds **2**–**5**. The irritant activities of some jatrophane diterpenes (**2**, **3** and **6**–**9**) were also investigated: only compound **2** was found to exert a weak pro-inflammatory activity on mouse ear. © 1999 Elsevier Science Ltd. All rights reserved.

Keywords: Euphorbia peplus; Euphorbiaceae; Jatrophane diterpenes; Pro-inflammatory activity

1. Introduction

The widespread genus *Euphorbia* is the source of a large number of biologically active compounds. Besides the well-known skin irritant and tumour-promoting tigliane, ingenane and daphnane diterpenes, considerable attention has recently been paid to the macrocyclic diterpenes because of their high chemical diversity and therapeutically relevant bioactivity (Uemura, Katayama, Uno, Sasaki, & Hirata, 1975; Sahai, Rastogi, Jakupovic, & Bohlmann, 1981; Evans & Taylor, 1983). Moreover, in recent years investigations on *Euphorbia* species have resulted in the discovery of several new classes of diterpenes (Öksüz et al., 1996; Ahmad, Jassbi, & Parvez, 1998; Jakupovic et al., 1998a; Jakupovic, Morgenstern, Bittner, & Silva, 1998b; Jakupovic, Morgenstern, Marco, & Berendsohn, 1998c; Marco, Sanz-Cervera, Yuste, Jakupovic, & Jeske, 1998).

In continuation of our phytochemical studies on biologically active compounds from Hungarian *Euphorbia* species, we now describe the isolation and characterisation of a new jatrophane diterpene (**1**), together with the known jatrophane esters **2**–**5**, which were obtained from the highly irritant extract of whole, fresh plants of *E. peplus* L. (Euphorbiaceae). As only a few experimental data have previously been published on the irritant activities of jatrophane derivatives (Seip & Hecker, 1984), some jatrophane esters (**2**, **3** and **6**–**9**) isolated from *E. peplus* and *E. esula* (Hohmann et al., 1997; Günther et al., 1998) were evaluated in the mouse ear test, in order to establish whether they contribute to the pro-inflammatory activity of the plant extracts.

2. Results and discussion

The mouse ear irritant, dichloromethane extract ($ID_{50}^{4} = ID_{50}^{24} = 25$ μg/ear) of the fresh, whole plant of *E. peplus* was subjected to polyamide CC, silica gel flash chromatography and HPLC to afford a new optically active ($[\alpha]_D^{25}$ −33, *c*, 0.1, $CHCl_3$) constituent **1**, together with the known compounds **2**–**5**.

Compound **1** gave a parent ion in the HREIMS at *m*/*z* 641.2889, appropriate for a molecular formula of

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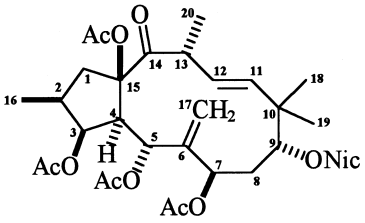

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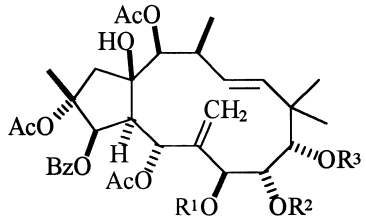
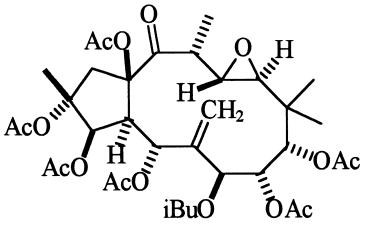


	R^1	R^2	R^3
2	iBu	H	Nic
3	Ac	Ac	Ac
4	iBu	H	Ac
5	Ac	H	Nic



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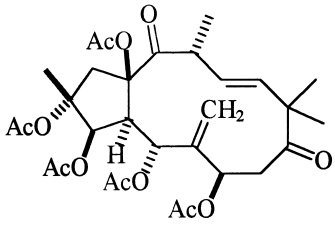

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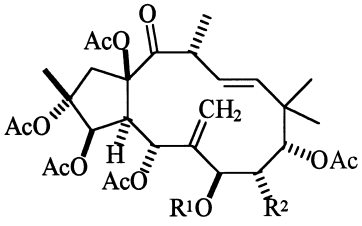

	R^1	R^2
8	Ac	H
9	iBu	OAc

$C_{34}H_{43}NO_{11}$ (calcd 641.2836). It exhibited UV maxima at 217 and 264 nm, characteristic of the nicotinoyl group. The ^1H and ^{13}C NMR spectra of **1** revealed the presence of one nicotinoyl and four acetyl groups (Table 1). Additionally, the ^1H and ^{13}C NMR spectra contained resonances for skeletal carbons and protons, which were assigned on the basis of the interpretation of the ^1H–^1H-COSY and HMQC spectra. The proton and carbon connectivities detected here demonstrated that a diterpene core containing 20 carbons is present, consisting of two tertiary and two secondary methyls, one *trans* disubstituted olefin, one exocyclic and two simple methylenes, seven methine groups, one ketone and three quaternary carbons. The ^1H–^1H COSY spectrum revealed three structural fragments with correlated protons: δ_H 3.01 d, 1.65 d, 2.22 m, 0.90 d, 5.42 t, 2.71 d and 5.81 s [–C*H*$_2$–C*H*(C*H*$_3$)–C*H*(OR)–C*H*–C*H*(OR)–] (A); δ_H 4.84 dd, 2.18 m and 5.09 dd, [–C*H*(OR)–C*H*$_2$–C*H*(OR)–] (B); δ_H 5.94 d, 5.65 dd, 3.53 dq and 1.20 d [*trans*–C*H*=C*H*–C*H*(C*H*$_3$)–] (C) (R = acyl). Further, in the ^1H–^1H COSY spectrum, ^4J couplings were also detected between the exomethylene (δ_H 5.07 s (2H) (H-17)) and two acyloxymethines (δ_H 5.81 s (H-5) and 4.84 dd (H-7)), indicating the linkage of sequences A and B. The remaining connectivities were established by inspection of long-range C–H cor-

Table 1
NMR spectral data on **1** ($CDCl_3$, TMS, δ (ppm), (J = Hz))

Atom	^1H	^{13}C	HMBC (H → C) C No.	NOESY
1α	3.01 dd (14.0, 7.7)	46.3	2, 3, 4, 14, 15	1β, 2
1β	1.65 dd (14.0, 12.7)		2, 14, 15, 16	1α, 16
2	2.22 m	38.3	–	1α, 3, 4, 16
3	5.42 t (3.0)	76.7	15, 3-COMe	2, 4, 16
4	2.71 d (3.0)	52.9	3, 14, 15	2, 3, 5, 7
5	5.81 s	68.5	3, 4, 6, 7, 15, 17, 5-COMe	4, 8, 11, 13, 17
6	–	146.9	–	–
7	4.84 dd (7.1, 2.3)	69.1	5, 8, 7-COMe	4, 8, 9, 11, 17, 18/19
8	2.18 m (2H)	34.0	7, 9, 10	5, 7, 9, 17
9	5.09 dd (2.7, 7.9)	75.9	7, NicCO	7, 8, 18/19
10	–	40.7	–	–
11	5.94 d (16.0)	137.9	10, 13, 18, 19	5, 7, 13, 18/19
12	5.65 dd (16.0, 9.0)	130.3	10, 13, 14, 20	13, 17, 18/19, 20
13	3.53 dq (9.0, 6.7)	43.3	11, 12, 14, 20	5, 11, 12, 20
14	–	212.6	–	–
15	–	92.9	–	–
16	0.90 d (6.6)	13.3	1, 2, 3	1β, 2, 3
17	5.07 s (2H)	110.2	5	5, 7, 8, 12
18	1.13 s	26.5	9, 10, 11, 19	7, 8, 9, 11, 12,
19	1.11 s	23.3	9, 10, 11, 18	7, 8, 9, 11, 12,
20	1.20 d (6.7)	19.4	12, 13, 14	12, 13
Nic CO	–	164.1	–	
2′	9.23 d (1.5)	151.1	3′, 4′, 6′	
3′	–	125.7	–	
4′	8.21 dt (7.9, 1.5)	136.8	2′, 6′, NicCO	
5′	7.43 dd (7.9, 4.8)	123.5	3′	
6′	8.80 dd (4.8, 1.5)	153.8	4′, 5′	
Acetyls				
3-CO		169.9	–	
3-COMe	2.13 s	21.2	3-COMe	
5-CO		169.6	–	
5-COMe	2.15 s	21.2	5-COMe	
7-CO		169.7	–	
7-COMe	1.52 s	20.5	7-COMe	
15-CO		169.9	–	
15-COMe	2.11 s	21.2	15-COMe	

relations observed in an HMBC spectrum (Table 1). The correlation of the quaternary carbon signal at δ_C 92.9 (C-15) with the proton signals at δ_H 3.01, 1.65 (H-1α, β), 2.22 (H-2), 2.71 (H-4) and 5.81 (H-5) showed that structural fragment A construct a methyl-substituted five-membered ring, present in many types of Euphorbiaceae diterpenes (Evans & Taylor, 1983). Cross-peaks between the carbon signal at δ_C 40.7 (C-10) and the proton signals at δ_H 2.18 (H-8), 5.94 (H-11), 5.65 (H-12), 1.13 (H-18) and 1.11 (H-19) indicated that structural parts B and C comprised the twelve-membered ring of a jatrophane diterpene. The $^2J_{C-H}$ and $^3J_{C-H}$ correlations between the carbon signal at δ_C 212.6 (C-14) and the proton signals at δ_H 3.01, 1.65 (H-1α, β), 2.71 (H-4), 5.65 (H-12), 3.53 (H-13) and 1.20 (H-20) fixed the location of the keto group at C-14. The positions of ester groups were also determined via the HMBC experiment, from an evaluation of the J_{C-H} couplings between the oxymethine protons and the carbonyl carbons. The acetyl group at δ_H 2.11, which did not exhibit any long-range correlations, was of necessity situated on a quaternary carbon (C-15). All of the above data are compatible with the structure of **1** being 3,5,7,15-tetraacetoxy-9-nicotinoyloxy-14-oxojatropha-6(17),11-diene.

The stereochemistry of **1** was assessed by analysing the coupling constants and the results of a NOESY experiment. Starting from the α position of the proton at the ring junction (H-4), it was found that a β-oriented methyl group is present on C-2 and a β-oriented ester group on C-3, with regard to the diagnostic NOE effects between H-1α, H-2, H-3 and H-4 and between H-1β and H-16 (Table 1). The position of H-5 was concluded to be β on the basis of the zero coupling constant between H-4 and H-5, similarly as in case of co-occurring jatrophanes and esulatins (Hohmann et

al., 1997; Günther et al., 1998; Jakupovic et al., 1998b). The NOE interaction between H-4 and H-7 required a β-oriented acyl group on C-7. The NOESY cross-peak between H-5 and H-13 dictated the β position of H-13. The α orientation of the nicotinoyl group could be concluded from the coupling constants of H-9, which were found to be similar to those of esulatin D (Günther et al., 1998) and 2α,3β,5α,7β,15β-pentaacetoxy-9α-nicotinoyloxy-14-oxojatropha-6(17),11-diene (Jakupovic et al., 1998b). Such a configuration of C-7 and C-9 signified a parallel position of the ester groups, as described by Jakupovic et al. (1998b), which is manifested in an upfield-shifted acetyl group on C-7 (δ_H 1.52), influenced by the aromatic ring effect of the nicotinoate on C-9. The stereochemistry of the 15-OAc group could not be determined on the basis of NOESY correlations. However, all known jatrophane diterpenes exhibit a *trans* ring junction (Hohmann et al., 1997; Jakupovic et al., 1998a, 1998b, 1998c; Günther et al., 1998; Marco et al., 1998) and, thus, the β orientation of the 15-OAc could be presumed. With regard to the above data, the structure of this compound is formulated as **1**.

From the dichloromethane extract of *E. peplus*, compounds **2**–**5** were also isolated and fully characterised by means of EIMS, HREIMS and NMR spectroscopy, including 2D NMR techniques, since these compounds had not been described while this work was in progress. All the spectral data were identical with those reported previously (Jakupovic et al., 1998b). The stereochemistry of **4** was analysed by X-ray crystallography and the absolute configuration was established as (2*R*,3*R*,4*S*,5*R*,7*S*,8*S*,9*S*,13*S*,14*S*,15*R*)-2,5,9,14-tetraacetoxy-3-benzoyloxy-8,15-dihydroxy-7-isobutanoyloxyjatropha-6(17),11*E*-diene (**4**) (Kálmán & Argay, in preparation). The solid-state conformation, determined here, agreed well with the calculated conformation published by Jakupovic et al. (1998b).

Compounds **2** and **3**, together with jatrophanes **6--9**, obtained from *E. esula* earlier (Hohmann et al., 1997; Günther et al., 1998), were tested for their skin irritant activity on mouse ear, using the standard assay (Hecker, Immich, Bresch, & Schairer, 1966). Compounds **3** and **6**–**9** were shown to be inactive up to a dose of 200 μg/ear, while **2** exhibited a pro-inflammatory activity of $ID_{50}^{4} = ID_{50}^{24} = 29$ μg/ear. These data, together with those published on jatrophanes of *E. characias* (Seip & Hecker, 1984), indicated that this type of diterpenes do not play a significant role in the skin irritant activity of *Euphorbia* species. For the highly irritant effect of *E. peplus*, which causes many toxicological problems, primarily ingenane diterpenes are responsible (Rizk, Hammouda, El-Missiry, Radwan, & Evans, 1985; Zayed, Farghaly, Taha, Gotta, & Hecker, 1998).

3. Experimental

3.1. General

M.p.'s are uncorrected. UV: MeOH. EIMS: 70 eV, direct inlet, Finnigan MAT 8430 spectrometer. ^1H, ^{13}C and 2D NMR (^1H–^1H COSY, NOESY, HMQC, HMBC): 400 MHz (^1H) and 100 MHz (^{13}C), $CDCl_3$, with TMS as internal standard, Bruker Avance DRX 400 spectrometer. Optical rotations were determined in $CHCl_3$ at ambient temp.; CC: polyamide (ICN). VLC: silica gel (Kieselgel GF_{254} 15 μm, Merck). HPLC: Li Chrospher RP-18 (200x4 mm, 5 μm) and LiChrospher Si 100 (200 × 4 mm, 5 μm) with RI detection.

3.2. Assay for irritant activity

Irritant doses 50 (ID_{50}) were determined on the ears of mice according to a standard procedure (Hecker et al., 1966). The redness of the mouse ear was estimated 4 and 24 h after the application of solutions in Me_2CO. As a reference, 12-*O*-tetradecanoylphorbol 13-acetate (TPA) was used: ID_{50}^{4} 0.00938 μg/ear, ID_{50}^{24} 0.00645 μg/ear.

3.3. Plant material

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

3.4. Extraction and isolation

Fresh and entire plants of *E. peplus* (200 g) were extracted with MeOH (1.8 l) at room temperature. The crude extract was concentrated *in vacuo* and partitioned between CH_2Cl_2 and H_2O. Evaporation of the organic phase gave a residue (4.7 g), which was chromatographed over a polyamide column with mixtures of H_2O/MeOH (3:2 and 1:4) as eluents. Fractions obtained with H_2O/MeOH (2:3) were chromatographed by silica gel VLC, using $CHCl_3$ and $CHCl_3$/Me_2CO mixtures of increasing polarity as eluents. Fractions 6–10 eluted with $CHCl_3$ were transferred to a reverse-phase HPLC column and eluted with MeOH/H_2O as mobile phase at a flow rate of 0.7 ml/min. Compounds observed at RT 20.0, 29.2, 16.2 and 23.4 min were further purified by HPLC on a normal-phase column with cyclohexane/EtOAc/EtOH (30:10:1) to yield compounds **1** (2.7 mg), **2** (10.2 mg), m.p. 182–185°C, $[\alpha]_D^{25}$ +53 (*c*, 0.51, $CHCl_3$), **3** (4.7 mg), $[\alpha]_D^{25}$ −10 (*c*, 0.19, $CHCl_3$) and **4** (3.1 mg), m.p. 220–223°C, $[\alpha]_D^{25}$ +8 (*c*, 0.3, $CHCl_3$), respectively. Fractions 11–18 obtained from VLC with $CHCl_3$/Me_2CO (49:1) after HPLC purification on a normal-phase column with

cyclohexane/EtOAc/EtOH (30:10:1) as eluent afforded compound **5** (3.8 mg), $[\alpha]_D^{25}$ +27 (*c*, 0.04, $CHCl_3$).

3.5. Compound *1*

Amorphous solid. $[\alpha]_D^{25}$ −33 (*c*, 0.1, $CHCl_3$). UV λ_{max} nm (log ε): 217 (3.96), 264 (3.27). EIMS, *m*/*z* (rel. int.): 641 $[M]^+$ (13), 571 [M? CH_2CO – CO]$^+$ (7), 391 [571-3 × HOAc]$^+$ (3), 328 [571–2 × HOAc–C_5H_4NCOOH]$^+$ (7), 124 $[C_6H_4NCOOH + H]^+$ (100), 123 $[C_8H_{11}O]^+$ (7), 96 $[C_7H_{12}]^+$ (27). HRMS: *m*/*z* 641.2889 $[M]^+$ $C_{34}H_{43}NO_{11}$ required 641.2836. ^1H and ^{13}C NMR: see Table 1.

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