A Novel Lathyrane Diterpenoid from the Roots of *Euphorbia lathyris*

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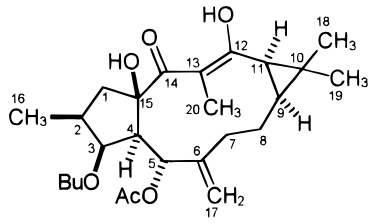
Received July 9, 1998

A new lathyrane diterpene (**1**) has been isolated and characterized from a CH_2Cl_2 extract of the roots of *Euphorbia lathyris*. Detailed spectral analysis revealed that the structure of **1**, including relative stereochemistry, is that of a diester of a hitherto unknown, polyfunctional diterpene parent alcohol.

Euphorbia lathyris L., or caper spurge (Euphorbiaceae), is widely distributed throughout the Mediterranean region of Eastern and Middle Europe.[1] It is sometimes planted in gardens in Hungary to deter moles. Animals nibbling the roots of the plant are killed because of its highly irritant gastric effect. Previous biological studies have demonstrated the proinflammatory property of the roots, seeds, aerial parts, and milky latex, and an antitumor effect of the seeds of *E. lathyris* against sarcoma 180 ascites.[2−4] From the antitumor extract, a diterpenoid, ingenol 3-hexadecanoate was isolated as active principle; this compound displays paradoxical biological action, being also a known tumor-promoting agent.[3] Besides ingenol esters, a series of diterpenes based on the lathyrane skeleton have also been isolated (L_1−L_3, L_7, L_8) from the seeds.[2,5−9] The roots of *E. lathyris* have been less well investigated to date; only the isolation of a lathyrane diterpenoid, jolkinol B, was reported earlier.[4]

As part of our studies on biologically active compounds from *Euphorbia* species found in Hungary, we have examined the roots of *E. lathyris* for its diterpene constituents. The dichloromethane phase of a methanolic extract of the undried roots of *E. lathyris* was fractionated by column chromatography on polyamide, then on Si gel, and further purified by preparative TLC and HPLC to afford compound **1**.



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Compound **1** was shown by HREIMS to have the molecular formula $C_{26}H_{38}O_7$. Its UV spectrum displayed a maximum at 280 nm, indicating an enone chromophore in the molecule. The ^1H and ^{13}C NMR spectra of **1** (Table 1) revealed the presence of one acetate group [δ_H 2.08 s; δ_C 170.4 (*C*O) and 21.0 (*C*H_3)] and one butanoate group [δ_H 2.36 m (H-2′), 1.66 m (H-3′), and 0.81 t (C-4′); δ_C 173.8 (C-1′), 35.9 (C-2′), 18.1 (C-3′), and 13.2 (C-4′)]. Characteristic signals at δ_H 1.10 dd, 1.50 dd, 1.06 s, and 1.05 s and at δ_C 35.9, 28.3, 25.2, 28.3, and 15.7 suggested a *gem*-dimethyl-substituted cyclopropane ring, present in many types of diterpenes from plants in the Euphorbiaceae.[10] Additionally, the ^1H NMR spectrum exhibited 15 signals due to the parent skeleton, which were assigned with the aid of two-dimensional experiments. The ^1H−^1H COSY spectrum revealed the presence of the systems CH_2−CH(CH_3)−CHR−CH−CHR− (A) and −CH_2−CH_2−CH−CH− (B) (R = acyl) in **1**, which represented the structural moieties C-1−C-5 and C-7−C-11 of a lathyrane diterpene, respectively. In position C-6, an exomethylene group was concluded on the basis of the singlet signals at δ_H 5.10 and 5.07, which did not show any correlations to other protons. In the ^1H NMR spectrum, a downfield-shifted tertiary methyl signal (δ_H 1.91 s) and two broad signals at δ_H 8.25 and 7.40 were also observed. The chemical shift assignments of the carbon atoms were established from the HMQC and HMBC spectra. The HMQC correlations

Table 1. NMR Spectral Data of Compound **1**[a]

position	^1H[b]	^{13}C	HMBC (H→C)
1α	3.47 m	50.3	
1β	2.04 t (12.3)		2, 15, 16
2	2.4 m	37.9	
3	5. 83 t (3.6)	79.9	1′, 15
4	3.01 dd (3.6, 10.5)	53.4	6, 14
5	6.56 d (10.5)	67.5	6, AcCO
6		149.8	
7a	2.54 dd (15.0, 5.4)	35.3	
7b	2.13 m		
8	1.94 m (2H)	21.6	
9	1.10 dd (15.3, 8.3)	35.9	
10		25.2	
11	1.50 dd (11.8, 8.3)	28.3	13, 18
12		152.4	
13		134.2	
14		202.2	
15		88.1	
16	1.03 d (6.8)	14.1	1, 2, 3
17a	5.10 s	114.7	5
17b	5.07 s		5
18	1.06 s	28.3	9, 10, 11, 19
19	1.05 s	15.7	9, 10, 11, 18
20	1.91 s	12.2	12, 13, 14
12-OH	8.25 br s		11
15-OH	7.40 br s		

[a] Pyridine-d_5, TMS, δ (ppm), J = Hz. [b] Additional assignments: 3-*O*-butanoyl: δ_H 2.36 m (H-2′), 1.66 m (H-3′), 0.81 t (7.4) (H-4′); δ_C 173.8 (C-1′), 35.9 (C-2′), 18.1 (C-3′), 13.2 (C-4′); 5-*O*-acetyl: δ_H 2.08 s; δ_C 170.4 (*C*O), 21.0 (*C*H_3).

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10.1021/np980294q CCC: $18.00
Published on Web 11/19/1998

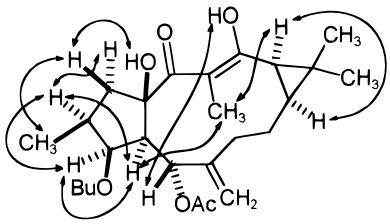

Figure 1. Major NOESY correlations of **1**.

indicated, besides ester groups, the presence of 14 protonated carbons: six methines, four methylenes, and four methyls. Moreover, it was considered that the two broad singlets at δ_H 8.25 and 7.40 corresponded to hydroxyl groups because of their missing HMQC cross peaks.

The HMBC spectrum of **1** revealed the presence of six quaternary carbons, whose correlative signals confirmed the connection of the partial structures proposed. Long-range correlations between the signals at δ_C 88.1 (C-15) and at δ_H 2.04 (H-1β) and 5.83 (H-3) proved that structural fragment A comprised the five-membered ring of **1**. The $^2J_{CH}$ and $^3J_{CH}$ correlations of protons H-4, H-5 and H-17a,b (Table 1) confirmed the presence of an exomethylene group at C-6. The cross peak between the signals at δ_C 202.2 and δ_H 3.01 (H-4) indicated that a keto group must be sited at C-14. This keto group showed a correlation in the HMBC spectrum with the singlet methyl signal at δ_H 1.91, indicating that C-20 is attached to a quaternary carbon. Further, long-range $^1H-^{13}C$ couplings from H-20 to the quaternary carbons at δ_C 152.4 (C-12) and 134.2 (C-13) and from H-11 to δ_C 134.2 (C-13) were observed, and thus the assignments of C-12 and C-13 could be determined. A hydroxy group (δ 8.25) was sited at C-12, from the observation of its correlative signals with C-11 in the HMBC spectrum. The coupling of OH-12 and H-11 in the ^1H NMR spectrum was detected in a proton decoupling experiment. On irradiation of the OH-12 signal (δ_H 8.25), simplification of the H-11 signal (δ_H 1.50 dd) to a doublet was observed.

The positions of ester groups in **1** were also established via the HMBC experiment. The correlation of the carbonyl signals at δ_C 173.8 (butanoyl *C*O) and 170.4 (acetyl *C*O) with the proton signals at δ_H 5.83 (H-3), 2.36 (H-2′), and 6.56 (H-5), and at δ_H 2.08 (acetyl methyl), respectively, demonstrated the presence of the butanoyl group at C-3 and the acetyl group at C-5. All of the above data are compatible with the structure of **1** being 5-*O*-acetyl-3-*O*-butanoyl-12-hydroxylathyrol.

The relative stereochemistry of **1** was studied by means of NOESY measurements. The trans-linked cyclopentane ring and the β-oriented OBu-3 and CH_3-16 groups followed from the NOE interactions between H-1β, H-16, and OH-15 and between H-1α, H-2, and H-4, as shown in Figure 1. The cross peak between H-4 and H-20 in the NOESY spectrum required that the methyl group on C-13 be oriented below the plane of the molecule. The NOE effect observed between H-20 and H-11 revealed a trans configuration for the C-12/C-13 double bond. Correlative signals between H-11 and H-9 dictated a cis-fused cyclopropane ring with α stereochemistry of H-9 and H-11, which is usual in lathyrane diterpenoids.[10] The stereochemistry of H-5 was concluded to be β from its NOESY cross peak with OH-12, both oriented above the plane of the molecule. On the above basis, the structure of this compound was elucidated as shown in formula **1**. The identical configuration of C-5 was found in lathyrane ester L_9, jolkinol B, and 15β-*O*-benzoyl-5α-hydroxyisolathyrol,[8,11,12] and the opposite configuration in esters L_1, L_2, and L_{7b}.[5−7] Our findings support the suggestion of Manners and co-workers that lathyrane and related jatrophane diterpenes should be stereochemically similar and that a stereochemical reexamination of some lathyrane diterpenes is necessary.[13]

The demonstrated enolic form of compound **1** is presumably stable because of hydrogen bonding, which can exist between the 14-keto group and OH-12 and OH-15. A similar enol structure was presented in the presumed biogenetic route of the antitumor-active jatrophatrione by Torrance et al.[14]

Experimental Section

General Experimental Procedures. The optical rotation was determined in $CHCl_3$ at ambient temperature, using a Perkin−Elmer 341 polarimeter. The UV spectrum in MeOH was obtained on a Shimadzu UV-2101 spectrophotometer. The NMR spectra were recorded on a Bruker DRX 400 Avance spectrometer at 400 MHz (^1H) and 100 MHz (^{13}C), using pyridine-d_5 as solvent and TMS as internal standard. MS measurements were carried out on a Finnigan MAT 8430 spectrometer operating at 70 eV ionizing energy. 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).

Plant Material. The roots of *E. lathyris* were collected from a two-year-old plant in October 1996, in Székesfehérvár, Hungary. A voucher specimen has been deposited at the Department of Pharmacognosy, Albert Szent-Györgyi Medical University, Szeged, Hungary.

Extraction and Isolation. The fresh roots of *E. lathyris* (840 g) were extracted with MeOH (8 L) at room temperature. The crude extract was concentrated in vacuo and partitioned between CH_2Cl_2 (3 × 0.2 L) and H_2O. On evaporation, the organic phase residue (4.27 g) was obtained, which was chromatographed on a polyamide column (30 g) with mixtures of H_2O−MeOH (4:1, 3:2, 2:3, and 1:4) as eluents. The fractions obtained with H_2O−MeOH (3:2 and 2:3) were combined and subjected to Si gel vacuum−liquid chromatography, using a gradient system of cyclohexane and cyclohexane−EtOAc (9:1, 4:1, 7:3, and 1:1). Fractions eluted with cyclohexane−EtOAc (9:1) were further purified by preparative TLC on Si gel, using $CHCl_3$−Me_2CO (19:1) as solvent, and by HPLC, using a normal-phase column and *n*-hexane−EtOAc (19:1) as eluent, to yield 2.3 mg of compound **1**.

Compound 1: amorphous solid; $[\alpha]^{25}_D$ +76° (*c* 0.1, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 218 (3.63), 280 (3.92) nm; ^1H and ^{13}C NMR, see Table 1; EIMS *m/z* $[M - CH_3]^+$ 447 (16), $[M - CH_3 - HOAc]^+$ 387 (36), $[M - CH_3 - HOAc - C_3H_7COOH]^+$ 299 (23), $[M - CH_3 - HOAc - C_3H_7COOH - H]^+$ 298 (100), $[C_3H_7CO]^+$ 71 (43), $[CH_3CO]^+$ 43 (100); HREIMS *m/z* 447.2299 (calcd for 447.2383 $C_{25}H_{35}O_7$) $[M - CH_3]^+$, 387.2165 (calcd for 387.2172 $C_{23}H_{31}O_5$) $[M - CH_3 - HOAc]^+$.

Acknowledgment. This investigation was funded by the Hungarian Ministry of Culture and Education, project no. MKM 495, and by the National Scientific Research Fund, project no. OTKA T022587.

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NP980294Q