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Lathyranone A: A Diterpenoid Possessing an Unprecedented Skeleton from *Euphorbia lathyris*

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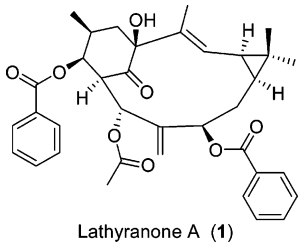
ABSTRACT



Lathyranone A (**1**)

Lathyranone A (1), a novel diterpenoid with a rearrangement skeleton, along with a known diterpenoid, *E*uphorbia factor L_{11} (2), was isolated from the seeds of *Euphorbia lathyris*. The structure and relative stereochemistry of 1 were elucidated by extensive spectroscopic analysis. A possible biosynthetic pathway for lathyranone A (1) was proposed.

Euphorbiaceae species are a prolific source of new diterpenoids with diverse skeletons,[1] of which some have antitumor[2] and antiproliferative effects.[3] The seeds of *Euphorbia lathyris* L. are used in Chinese medicine for the treatment of hydropsy, ascitics, scabies, and snakebites.[4] Recently, lathyranoic acid A, a secolathyrane diterpenoid, has been isolated from *E. lathyris*.[5] Aiming to find novel and potentially bioactive secondary metabolites from this species, we investigated the seeds of *E. lathyris* and isolated a novel diterpenoid, lathyranone A (**1**), with an unprecedented cyclohexanone moiety in the structure, along with a known compound *Euphorbia* factor L_{11} (**2**).[5] In this paper, we describe the isolation and structure elucidation of lathyranone A (**1**).

The seeds of *E. lathyris* (20 kg) were extracted with 95% EtOH under reflux. The residue was suspended in water and

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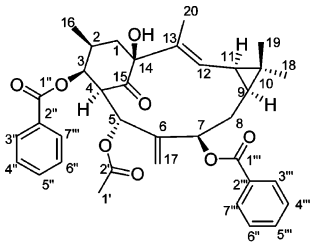
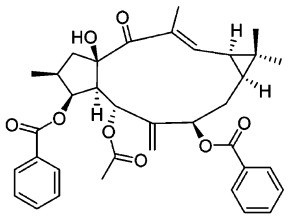
partitioned with petroleum ether. The petroleum ether fraction was roughly separated by silica gel chromatography, using gradient petroleum/acetone (from 0:1 to 1:0) as eluents to give 8 fractions (A−H). Fraction E was purified with RP-18 column chromatography (MeOH/H_2O, 7:3) to afford lathyranone A (**1**) (15 mg) and *Euphorbia* factor L_{11} (**2**) (20 mg), respectively.



Lathyranone (**1**)



Euphorbia factor L_{11} (**2**)

Lathyranone A (**1**) [colorless oil; $[\alpha]^{25}_D$ +189.47 (*c* 0.20, $CHCl_3$)] exhibited a pseudomolecular ion peak at *m*/*z* 623.2615 $[M + Na]^+$ (calcd 623.2620) by HREIMS, consistent with a molecular formula of $C_{36}H_{40}O_8$. The IR spectrum showing absorptions at 3463, 1749, 1722, 1637, and 1602 cm^{-1} implied the existence of hydroxyl, acyl, ketone groups, and double bonds, respectively. The ^1H NMR and ^{13}C NMR and DEPT spectra of 1 (Table 1) displayed signals including one ketone group, one acetyl group, two benzoyl groups, two double bonds (one exocyclic and one trisubstituted), four methyls, two methylenes, seven methines (three oxygenated), and two quaternary carbons (one oxygenated). An extensive comparison of ^1H and ^{13}C NMR data of lathyranone A (**1**) with those of *Euphorbia* factor L_{11} (**2**) suggested they had the same functional groups except that some chemical shifts of **1** were not identical with those of **2**.

The main differences were as follows: the chemical shifts of C-1 (δ_C 42.2, δ_H 2.55, δ_H 1.87), C-2 (δ_C 34.1, δ_H 2.37), C-7 (δ_C 70.7, δ_H 5.52), C-12 (δ_C 133.5, δ_H 4.65), C-13 (δ_C 135.6), and C-14 (δ_C 81.2) were shifted upfield while C-17 (δ_C 124.8) shifted downfield as compared to those of **2**. So, the two-dimensional NMR experiments (COSY, HMBC, HSQC, and ROSEY) were required to determine the scaffold. The $^1H-^1H$ COSY spectra revealed connectivity of two partial structures **a** (C-1 to C-5; C-16) and **b** (C-7 to C-9; C-11 to C-12) drawn with a bold bond (Figure 1). The HMBC correlations between H_2-1, H-3, and H-5 with C-15 and correlations between H-1 with C-14 revealed the presence of an unprecedented hexacyclic moiety in lathyrane-type diterpenoids. The linkage of two quaternary carbons C-13 and C-14 could be determined by the HMBC correlations between H-1 with C-13, H-12 and C-14, H-12 and C-13′ and correlation between H_3-20 and C-14. The connectivities of fragments **a** and **b** through C-6 and the olefinic methylene was suggested by the critical HMBC correlations between H_2-17 and C-6, C-5, and C-7, and was further confirmed by correlations H-4 and H-8 with C-6 and correlations H-5 and H-7 with C-17. Similarly, the correlations of H_3-18 with C-10, C-9, and C-11 established the cyclopropane ring. A proton signal at δ_H 3.75 suggested the existence of a hydroxyl function in the molecule. The assignment of the hydroxyl was located at C-14 by the HMBC correlations between OH with C-14, C-15′, and C-13. The HMBC correlations of H-3/C-1″ and H-7/C-1‴ sug-

Table 1. NMR Data and HMBC Correlation of Lathyranone A (**1**) in $CDCl_3$[a]

no.	^1H (δ_H, mult, *J* in Hz)	^{13}C (δ_C)	HMBC (C→H)
1α	2.55 (dd, 14.0, 3.0)	42.2 (t)	2,3,14,16
1β	1.87 (t, 14.0)		2,3,13,14,16
2	2.37 (m)	34.1 (d)	1α,1β,4,16
3	5.76 (br s)	74.7 (d)	1α,1β,2,4,5,15,16,1″
4	4.02 (dd, 11.0, 1.1)	52.3 (d)	3,5,6,15
5	5.55 (d, 11.0)	71.8 (d)	3,4,6,7,15,17b,2′
6		143.3 (s)	
7	5.52 (d, 5.6)	70.7 (d)	5,6,8α,17b,1‴
8α	2.35 (m)		7,9,11
8β	1.58 (m)	32.5 (t)	6,7,9,10,11
9	1.48 (m)	31.6 (d)	6,8,10,12,18,19
10		24.0 (s)	
11	1.55 (m)	26.9 (d)	8,10,12,18,19
12	4.65 (d,10.0)	133.5 (d)	9,10,14,20
13		135.6 (s)	
14		81.2 (s)	
15		207.1 (s)	
16	0.99 (d, 7.0)	16.9 (q)	1β,2,3,14
17a	5.79 (s)	124.8 (t)	4,5,6,7
17b	5.70 (s)		
18	1.09 (s)	28.7 (q)	7,9,10,11,19
19	0.97 (s)	15.6 (q)	9,10,11,18
20	2.10 (s)	12.7 (q)	1α,10,11,13,14,15
14-OH	3.75 (s)		
1″		165.4 (s)	
2″		129.9 (s)	
3″,7″	7.92 (2H, d, 7.5)	129.8 (d)	
4″,6″	7.40 (2H, d, 7.5)	128.4 (d)	
5″	7.55 (1H, m)	133.1 (d)	
1′	1.62 (3H, s)	20.6 (q)	
2′		169.3 (s)	
1‴		166.1 (s)	
2‴		121.1 (s)	
3‴,7‴	7.99 (2H, d, 7.5)	129.4 (d)	
4‴,6‴	7.40 (2H, d, 7.5)	129.4 (d)	
5‴	7.55 (1H, m)	133.3 (d)	

[a] ^1H NMR recorded at 400 MHz. ^{13}C NMR recorded at 100 MHz.

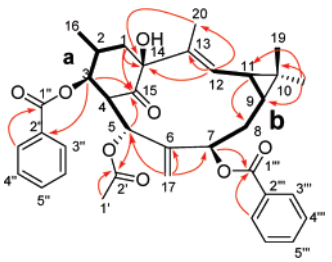


Figure 1. $^1H-^1H$ COSY (bold) and key HMBC correlations (arrows) of **1**.

gested that two benzoyloxy groups were linked to C-3 and C-7, respectively. The acetoxy attachment to C-5 could be determined by the HMBC correlations of H-5/C-2′. Therefore the planar structure of **1** was assigned as shown in Figure 1.

The relative configuration of **1** was established by analysis of key correlations observed in the ROESY spectrum based on the computer-generated lower energy conformation (Figure 2), together with the comparison of the coupling

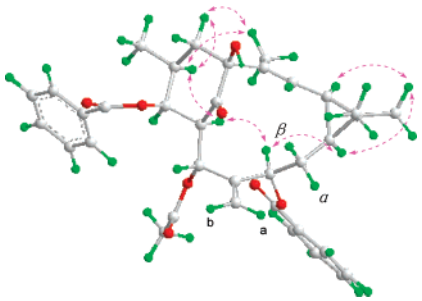


Figure 2. Key ROSEY correlations of lathyranone A (**1**).

constant patterns with the literature of similar compounds, specially with *Euphorbia* factor L_{11} (**2**). The ROESY cross-peak between H_3-20 and H-11 indicated an (*E*)-geometry for the Δ^{12} double bond.[7] The crucial ROESY correlation pairs of H_3-20/H-1α and H-2/H_3-20 revealed that the hydroxyl group at C-14 and the methyl at C-2 were *β*-oriented. In addition, the correlations H-2/H-4, H-4/H-7 suggested that H-4 and H-7 were α-oriented. The H-3 was assigned as being α-originated, evident from correlations between H-3 and H-4, and by the coupling constant value ($J_{3,4}$ = 1.1 Hz). Similarly H-5 was determined to be *β*-oriented by the coupling value ($J_{4,5}$ = 11.0 Hz). The correlations of H-4/H-7, H-7/H-9, H-9/H-18, and H-18/H-11 established a cis orientation for H-9 and H-11.

The configuration of lathyranone A (**1**) could also be deduced by the inspection of the proposed biosynthesis

(6) **Lathyranone A (1):** a colorless oil, $[\alpha]^{25}_{D}$ +189.47 (*c* 0.198, $CHCl_3$); IR (KBr) ν_{max} 3463, 1949, 1722, 1637, and 1602 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; ESI-MS *m*/*z* 623.2615 $[M + Na]^+$.

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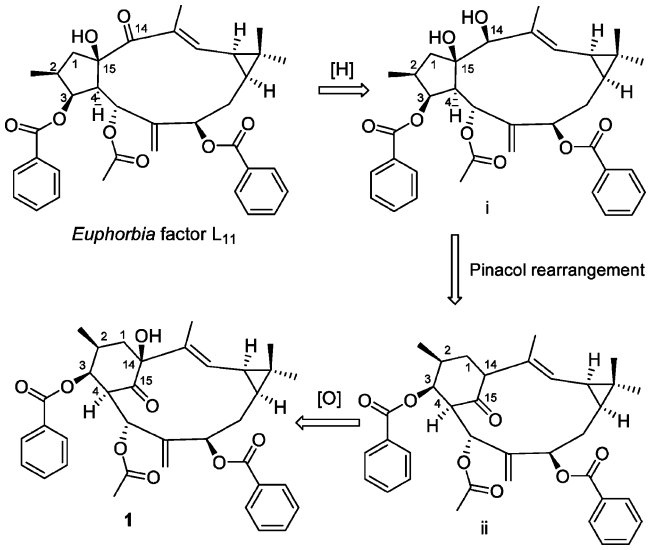
Scheme 1. Biogenetic Pathway Proposed for Lathyranone A (**1**)



(Scheme 1). From a biogenetic point of view, lathyrane-type diterpenoids represented by *Euphorbia* factor L_{11} may be produced from a key intermediate **ii** via a pinacol rearrangement, then oxidation at C-14 giving rise to lathyranone A (**1**).

The cytotoxicity activities of lathyranone A (**1**) against the growth of tumor cell lines [BEL-7402 (human liver carcinoma), MDA-MB-231 (human breast cancer), SGC-7901 (human gastric cancer), HT-29 (human colon adenocarcinoma), and MCF-7 (human breast cancer) cell lines] were evaluated. The results indicated that **1** was inactive against the above cancer cells (50% effective dose of clonal inhibition (ED_{50}) > 10 μg/mL).

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Supporting Information Available: Experimental section, EIMS, ESIMS, and 1D and 2D spectra for compound **1**. This material is available free of charge via the Internet at http://pubs.acs.org.

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