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Lathyranoic Acid A: First Secolathyrane Diterpenoid in Nature from *Euphorbia lathyris*

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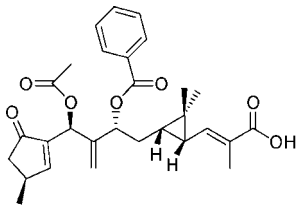
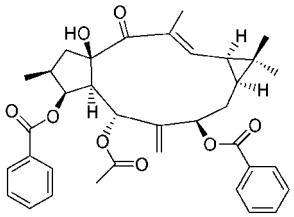
ABSTRACT



Lathyranoic acid (**1**)



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

Lathyranoic acid A (1), the first secolathyrane diterpenoid with an unprecedented skeleton, and a new diterpenoid *Euphorbia* factor L_{11} (2) were isolated from the seeds of *Euphorbia lathyris*. Their structures were elucidated by spectroscopic analysis and chemical methods. A biogenetic route involving an enzymatic Baeyer–Villiger oxidation as the key step was postulated for the transformation of 2 to 1 and mimicked by an unusual chemical Baeyer–Villiger oxidation.

The seeds of *Euphorbia lathyris* (Euphorbiaceae), a common traditional Chinese medicine, have been used to remedy hydropsy, ascites, coprostasis, anuresis, amenorrhea, venous stasis, terminal schistosomiasis, scabies, and snakebite.[1] Chemical and biological research on this plant in past decades[2] has led to the isolation of a series of diterpenoids known as *Euphorbia* factors L_1−L_{10}. Among them, the ingenol-type factors proved to be potent PKC activators[3] as well as anticancer[4] and anti-HIV agents,[5] while the lathyrol-type factors were demonstrated to be powerful P-glycoprotein inhibitors.[2k] The unique structures of these diterpenoids also challenged chemists to convert *Euphorbia* factors into densely functionalized polycyclic diterpenoids via a trans-annular cyclization[6] and to conduct total syntheses.[7] In the current project, lathyranoic acid A (**1**), the first secolathyrane diterpenoid with an unprecedented skeleton, and its biogenetic precursor *Euphorbia* factor L_{11} (**2**) were isolated from the seeds of *E. lathyris*.[8] Their structures were elucidated by spectroscopic analysis (especially two-dimensional NMR techniques) and chemical correlations. A plausible biogenetic

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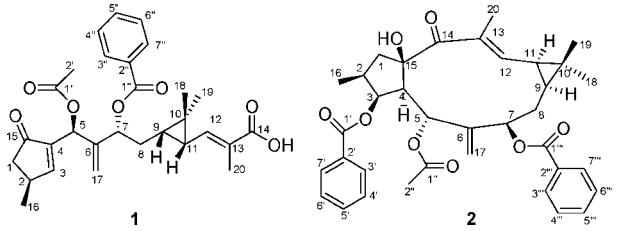
route involving an enzymatic Baeyer−Villiger oxidation as the key step was postulated for the transformation of **2** to **1** and was mimicked by an unusual chemical Baeyer−Villiger oxidation. Herein, we report the structures of lathyranoic acid A (**1**) and *Euphorbia* factor L_{11} (**2**) and their chemical and biogenetic correlations.



Lathyranoic acid A (**1**), a colorless amorphous solid, showed a molecular formula of $C_{29}H_{34}O_7$ as determined by HREIMS at *m*/*z* 494.2316 $[M]^+$ (calcd 494.2305) with 13 degrees of unsaturation. IR absorptions at 3419−2500, 1747, 1716, 1680, and 1635 cm^{-1} implied the existence of carboxyl, acyl, and ketone groups and double bonds, respectively. The ^1H NMR, ^{13}C NMR (Table 1), and DEPT spectra indicated, besides signals attributable to one acetyl group and one benzoyl group, the presence of a ketone group, a carboxyl group, three double bonds (one exocyclic and two trisubstituted), and 12 sp^3 carbons, including four methyls, two methylenes, five methines (two oxygenated), and one quaternary carbon. The above functionalities accounted for 11 degrees of unsaturation, and the remaining two degrees of unsaturation required the presence of two additional rings in lathyranoic acid A (**1**). The aforementioned spectral data, especially the ^1H and ^{13}C NMR patterns of **1**, were different from those of *Euphorbia* factors L_1−L_{10} isolated from the same plant,[2] implying that lathyranoic acid A (**1**) likely possessed a new backbone. The two-dimensional NMR experiments (COSY, HMQC, HMBC, and NOESY measurements) were thus performed to furnish the skeleton of **1**.

HMQC data allowed the assignment of all the protons to their bonding carbons. Two subunits **a** (C-1 to C-3; C-16) and **b** (C-7 to C-9; C-11 to C-12) (Figure 1), drawn with bold bonds, were established on the basis of ^1H−^1H COSY data. The HMBC correlations (Figure 1) enabled assembly of the subunits **a** and **b** with the quaternary carbons and other functionalities. Correlations of C-15 with H_2-1, H-3, and H-5 and correlations of C-4 with H-2 and H-5 established the cyclopentenone ring and the linkage between C-5 and C-4. Similarly, the correlations of H_2-17 with C-5, C-6, and C-7 were critical in locating the olefinic methylene at C-6 and enabled attachment of C-5 and C-7 at C-6. The correlations of H-12 with C-13, C-14, and C-20 established the Δ^{12} double bond and the attachments of C-14 and C-20 to C-13, while the correlations of H_3-18 with C-9, C-10, and C-11 established the cyclopropane ring. The acetoxy and benzoyloxy groups were located at C-5 and C-7, respectively, by the correlations of H-5 and H-7 to the carbon signals corresponding to the ester carbonyls. The planar structure of lathyranoic acid A (**1**) was, therefore, determined as depicted in Figure 1.

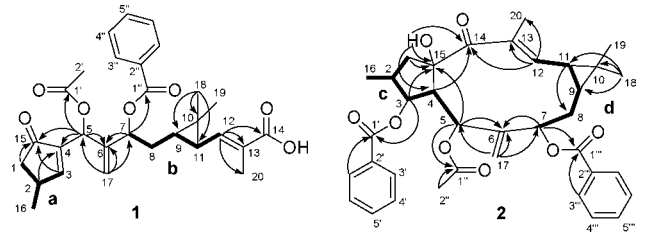


Figure 1. ^1H−^1H COSY (−−) and key HMBC correlations (H⟶C) of **1** and **2**.

The relative stereochemistry of lathyranoic acid A (**1**) was partially assessed by analysis of its ^{13}C NMR and NOESY data (Figure 2). The upfield-shifted carbon signal of C-20 at δ 12.3 revealed an (*E*)-geometry for the Δ^{12} double bond,[9] which was confirmed by a NOESY correlation between H_3-20 and H-11. NOESY correlations of H_3-18 with H-11 and H-9 established a cis orientation for H-9 and H-11. The stereochemistry at C-2, C-5, and C-7 in **1** could not be determined by analysis of the available spectral data since the coupling constants and relevant NOESY correlations did not provide sufficient relevant information.

Euphorbia factor L_{11} (**2**) had a molecular formula of $C_{36}H_{40}O_8$ as deduced from HREIMS data. Spectral analysis suggested that **2** was likely an analogue of the 7-hydroxylathyrol *Euphorbia* factors.[2i,j] Out of 40 proton signals

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(8) (a) Dried, powdered seed material (1 kg) of *E. lathyris* was percolated with 95% EtOH to give 500 g of crude extract, which was defatted on a silica gel column eluted with petroleum ether to afford 285 g of refined extract. After fractionation on a MCI gel column eluted with 80% MeOH in H_2O, six major fractions (fractions 1−6) were obtained. Fractions 3 and 4 were chromatographed on silica gel columns (petroleum ether/EtOAc = 10:1) to obtain two major compounds, each of which was then purified on a RP-18 column (75% MeOH in H_2O) to give lathyranoic acid A (**1**) (0.0005%) and *Euphorbia* factor L_{11} (**2**) (0.011%), respectively. Lathyranoic acid A (**1**): colorless amorphous solid; mp 57−58 °C; $[\alpha]^{20}_D$ −27° (*c* 0.67, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 230 (4.50); IR (KBr) ν_{max} 3419−2500, 2958, 2926, 1747, 1716, 1680, 1635, 1452, 1375, 1271, 1230, 1111, 1026, 714 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; positive ESIMS *m*/*z* 517 $[M + Na]^+$, 1011 $[2M + Na]^+$; negative ESIMS *m*/*z* 1009 $[2M - 2H + Na]^-$; EIMS *m*/*z* 434 $([M - HOAc]^+, 9)$, 312 (12), 294 (7), 105 (100); HREIMS at *m*/*z* 494.2316 $[M]^+$ (calcd for $C_{29}H_{34}O_7$, 494.2305). *Euphorbia* factor L_{11} (**2**): white amorphous powder; mp 117−118 °C; $[\alpha]^{20}_D$ +26° (*c* 0.94, $CHCl_3$); CD (MeOH) $\Delta\epsilon$ (λ_{max}) +10.7 (274), +4.3 (238), −8.5 (222), −9.8 (202); UV (MeOH) λ_{max} (log ϵ) 230 (3.51), 274 (3.13) nm; IR (KBr) ν_{max} 3435, 2922, 2852, 1720, 1620, 1385, 1279, 1113, 714 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; positive ESIMS *m*/*z* 623 $[M + Na]^+$, 1224 $[2M + Na]^+$; negative ESIMS *m*/*z* 645 $[M + HCOOH - H]^-$; EIMS *m*/*z* 418 (8), 296 (12), 136 (27), 105 (100); HREIMS at *m*/*z* 600.2718 $[M]^+$ (calcd for 600.2723 $C_{36}H_{40}O_8$). (b) Biological evaluation of compounds **1** and **2** has not been conducted.

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Table 1. ^{1}H and ^{13}C NMR Data of Lathyranoic Acid A (**1**) and *Euphorbia* Factor L_{11} (**2**) in $CDCl_3$

	1			**2**		
	δ_H[a]	(mult J_{HH})	δ_C[b]	δ_H[a]	(mult J_{HH})	δ_C[b]
1α	2.69	dd (19.0, 6.3)	43.7	2.82	dd (14.6, 9.6)	47.6
1*β*	2.02	br dd (19.0, 1.7)		1.86	dd (14.6, 10.3)	
2	2.96	m	33.7	2.53	m	37.4
3	7.47	br s	166.0	5.84	dd (3.6, 3.6)	76.7
4			142.1	2.89	dd (4.5, 3.6)	52.0
5	6.05	s	67.7	5.48	d (4.5)	69.1
6			144.2			143.9
7	5.42	dd (5.5, 4.9)	73.8	5.27	br d (5.1)	74.1
8α	1.97	m	30.2	2.04	ddd (15.3, 6.8, 4.1)	30.1
8*β*				1.70	ddd (15.3, 12.4, 2.5)	
9	1.23	dd (9.6, 9.6)	29.7	1.40	m	31.8
10			24.9			24.1
11	1.45	dd (10.3, 9.6)	27.6	1.48	dd (10.1, 9.1)	26.3
12	6.68	d (10.3)	142.4	6.00	br d (10.1)	139.7
13			127.8			138.4
14			172.1			207.1
15			206.5			85.8
16	1.17	d (7.2)	19.8	1.07	d (6.7)	14.5
17a	5.41	s	115.2	5.25	br s	113.5
17b	5.34	s		5.20	br s	
18	1.09	s	28.8	1.14	s	28.4
19	1.10	s	16.0	1.22	s	15.6
20	1.89	s	12.3	2.13	d (1.0)	14.0
15-OH				4.24	br s	
3-*O*-benzoyl				δ_H 8.15 (2H, H-3′, H-7′, dd, J = 7.3, 1.4 Hz), 7.44 (2H, H-4′, H-6′, br dd, J = 8.0, 7.3 Hz), 7.56 (H-5′, m); δ_C 165.9 (C-1′), 130.1 (C-2′), 130.0 (C-3′, C-7′), 128.4 (C-4′, C-6′), 132.9 (C-5′)		
5-*O*-acetyl	δ_H 1.91 (H_3-2′, s); δ_C 169.4 (C-1′), 20.8 (C-2′)			δ_H 1.32 (H_3-2″, s); δ_C 169.7(C-1″), 20.6 (C-2″)		
7-*O*-benzoyl	δ_H 8.05 (2H, H-3″, H-7″, d, J = 7.3 Hz), 7.45 (2H, H-4″, H-6″, br t, J = 7.6 Hz), 7.57 (H-5″, t, J = 7.4 Hz); δ_C 165.5 (C-1″), 130.2 (C-2″), 129.7 (C-3″, C-7″), 128.5 (C-4″, C-6″), 133.1 (C-5″)			δ_H 8.07 (2H, H-3‴, H-7‴, br dd, J = 8.3, 1.4 Hz), 7.44 (2H, H-4‴, H-6‴, br dd, J = 8.0, 7.3 Hz), 7.56 (H-5‴, m); δ_C 166.2 (C-1‴), 130.7 (C-2‴), 129.7 (C-3‴, C-7‴), 128.5 (C-4‴, C-6‴), 133.3 (C-5‴)		

[a] Recorded at 400 MHz. [b] Recorded at 100 MHz.

observed in the ^{1}H NMR (Table 1), 39 were assigned to the bonding carbons by analysis of the HMQC spectrum. The only remaining signal at δ 4.24 was assumed to be the proton signal of a hydroxyl group (IR absorption at 3435 cm^{-1}). The 7-hydroxylathyrol-type *Euphorbia* factor structure proposed for **2** was confirmed by $^{1}H-^{1}H$ COSY and HMBC (Figure 1). The proton signal at δ 4.24 (OH) correlated with the carbon signal at δ 85.8 (C-15), locating the only hydroxyl at C-15. Two benzoyloxy groups were linked to C-3 and C-7 by HMBC correlations of H-3/C-1′ and H-7/C-1‴, and the acetoxy was assigned attachment to C-5 on the basis of the HMBC correlation of H-5/C-1″. Thus, the planar structure of **2** was established as shown in Figure 1.

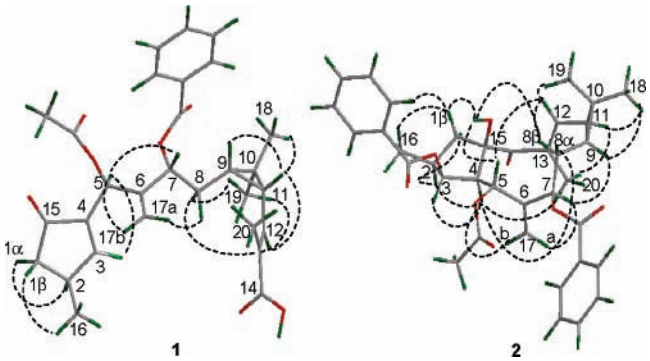


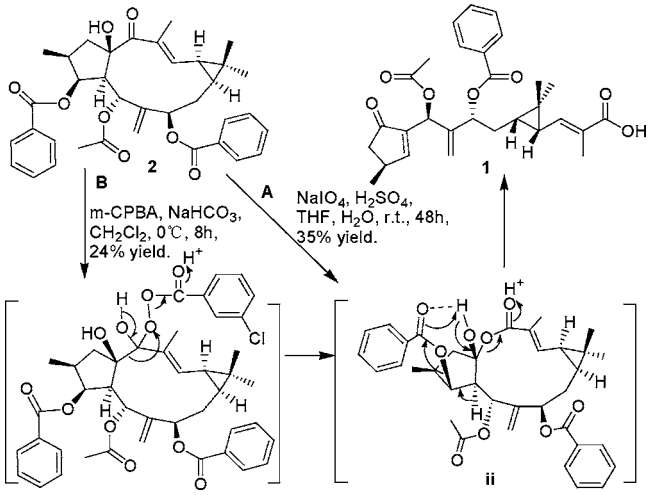
Figure 2. Key NOESY correlations of **1** and **2**.

The relative configuration of **2** was determined by analysis of its ^{13}C NMR and NOESY spectra (Figure 2) and by comparison with the literature data of this compound class. The upfield-shifted carbon signal of C-20 at δ 14.0 revealed an (*E*)-geometry for the Δ^{12} double bond,[9] and this was confirmed by a NOESY correlation between H_3-20 and H-11. The NOESY correlation pairs of H_3-16/H-1*β*, H-1*β*/15-OH, and 15-OH/H-5 indicated that CH_3-16, 15-OH, and C-5 were on the same face of the five-membered ring and were

arbitrarily assigned β-configuration. Meanwhile, the NOESY correlations of H-1α/H-2, H-2/H-4, H-3/H-4, H-4/H-7, H-7/H-9, and H-9/H_3-18 were also observed. The crucial NOESY correlations of H-5/H-12, H-12/H-19, H-17b/H-4, and H-17a/H-7 indicated the presence of a favorable conformation of **2** in $CDCl_3$ solution, which is consistent with the perspective view of X-ray structure of 7-hydroxylathyrol triacetate[2d] and the major conformation of *Euphorbia* factor L_9 in $CDCl_3$ solution.[2i] The NOESY correlation of H-5/H-12 also indicated a β-configured H-5. A computer-modeled structure of **2** (CS Chem 3D Pro Version 8.0 using MM2 force field calculation for energy minimization), on which the NOESY correlations were depicted (Figure 2), further supported the stereochemistry assignments. *Euphorbia* factor L_{11} (**2**) was thus identified as (2*S**,3*S**,4*R**,5*R**,7*R**,9*S**,11*S**,15*R**)-5-acetoxy-3,7-dibenzoyloxy-14-oxolathyra-6(17),12*E*-diene.

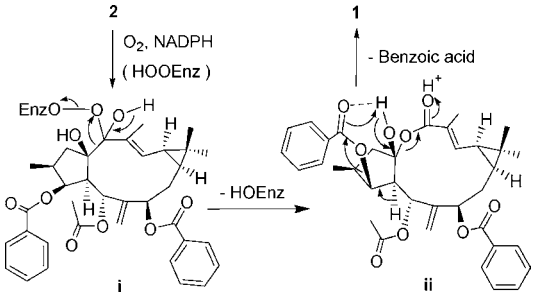
On the basis of the above results, the secolathyrane skeleton and esterification pattern of **1** seemed biogenetically related to **2**. To verify such a correlation and to deduce the stereochemistry of lathyranoic acid A (**1**), *Euphorbia* factor L_{11} (**2**) was subjected to oxidation via two classical routes **A**[10] and **B**[11] (Scheme 1). The product was identified to be **1**

Scheme 1. Chemical Transformations from **2** to **1**



in both reactions. The stereochemistry of lathyranoic acid A (**1**) was thus established as (2*S**,5*S**,7*R**,9*S**,11*S**)-14,-15-seco-5-acetoxy-7-benzoyloxy-3,6(17),12*E*-trien-15-oxo-14-lathyranoic acid by chemical correlation with *Euphorbia* factor L_{11} (**2**). On the basis of these aspects, a route for the biosynthetic transformation of **2** to **1** was postulated (Scheme 2). By way of NADPH- and O_2-involved

Scheme 2. Hypothetical Biogenetic Route from **2** to **1**



cytochrome P450- or FAD-dependent enzymatic Baeyer−Villiger oxidation of **2**,[12] a peroxy-enzyme activated complex (**i**) would first be formed. The complex **i** could be further transformed to a key intermediate (**ii**) by a mechanism similar to that of the chemical Baeyer−Villiger oxidation,[11] and the orientation of C-15-OH must be retained during the migration. The insertion of oxygen could occur between C-14 and C-15 because the fully substituted C-15 carbon has a migratory aptitude comparable to that of the vinyl group.[13] The intermediate **ii** would then undergo rearrangement to produce **1**. This hypothetical biogenetic route proposed for the origin of **1** was successfully mimicked by the chemical Baeyer−Villiger oxidation (Scheme 1, route B), which also shared the same key intermediate **ii** as depicted in Scheme 2.

Acknowledgment. Financial support from the National Natural Science Foundation (30025044) and the Foundation from the Ministry of Science and Technology (2002CB-512807) is gratefully acknowledged.

Supporting Information Available: Experimental section, EIMS, ESIMS, one- and two-dimensional NMR, and IR spectra of lathyranoic acid A (**1**) and *Euphorbia* factor L_{11} (**2**), UV and CD spectra of **2**, and the ^{1}H NMR spectrum of transformed lathyranoic acid A (**1**) (from **2**). This material is available free of charge via the Internet at http://pubs.acs.org.

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