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Lagaspholones A and B: Two New Jatropholane-Type Diterpenes from *Euphorbia lagascae*

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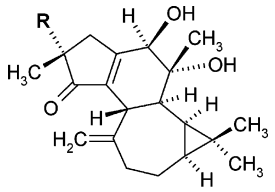
ABSTRACT



1 R = H
2 R = OH

Two new diterpenes, lagaspholones A (1) and B (2), have been isolated from the methanolic extract of *Euphorbia lagascae*, along with the known compounds (+)-dehydrovomifoliol, scopoletin, dehydrodiconiferyl diacetate, 3-indolcarbaldehyde, and 4-hydroxy-3,5-dimethoxybenzaldehyde. Their structures were elucidated by spectroscopic methods. Compounds 1 and 2 contain the rare jatropholane-type skeleton, characterized by a 5:6:7:3 fused ring system. A possible biosynthetic pathway for lagaspholones is proposed.

A great variety of diterpene esters with a unique structure have been isolated from the Euphorbiaceae and Thymeleacea families.[1] Among this class of compounds, jatrophane and lathyrane macrocyclic diterpenes have been extensively investigated and have shown several important biological effects, which include antiproliferative effects,[2] inhibition of cancer cells *P*-glycoprotein,[3] and microtubule interacting activities.[4] Moreover, their exclusive distribution in the Euphorbiaceae family reinforces their importance as biogenetic and chemotaxonomic markers.[5] *Euphorbia lagascae* Spreng (Euphorbiaceae) is a species endemic from the Iberic Peninsula, which was used in traditional medicine for the treatment of cancers and tumors.[6] This species has also a potential industrial value due to the presence in the seeds of high amounts of vernolic acid, a 12,13-epoxyoleic acid that has been used in the oleochemical industry as a plasticizer.[7] In previous reports, we have described the isolation and structural elucidation of several lathyrane-type diterpenes from *E. lagascae*, which have shown to be strong modulators of multidrug resistance, as well as apoptosis inducers.[8,9] Continuing our studies on *E. lagascae*, herein we report the isolation and structure elucidation of two novel jatropholane-type diterpenes (**1** and **2**), together with five known compounds.

The ether-soluble fraction of the methanol extract of the air-dried powdered plant of *Euphorbia lagascae* was submit-

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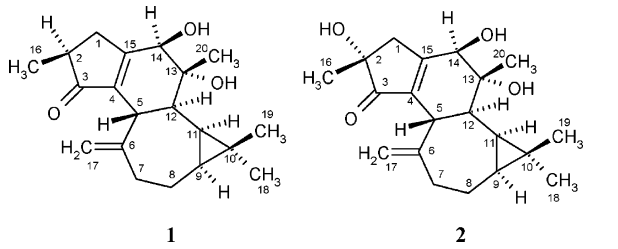
ted to successive chromatographic fractionation to afford the new diterpenes **1** and **2** (Figure 1), as well as the known



1 **2**

Figure 1. Chemical structures of compounds **1** and **2**.

compounds (+)-dehydrovomifoliol (**3**), scopoletin (**4**), dehydrodiconiferyl diacetate (**5**), 3-indolcarbaldehyde (**6**), and 4-hydroxy-3,5-dimethoxybenzaldehyde (**7**). The known compounds were identified by comparison of their physical and spectroscopic data to those described in the literature (see Supporting Information). To the best of our knowledge, compounds **3**, **5**, and **7** were isolated from *Euphorbia* species for the first time.

Compound **1**, named lagaspholone A, was obtained as white crystals of mp 217−219 °C and $[\alpha]_D^{20}$ − 46.7 ($CHCl_3$, *c* 0.10). The molecular formula was determined as $C_{20}H_{28}O_3$ from its HRSIMS that has shown a pseudomolecular ion at 317.2111. The IR spectrum displayed absorption bands for hydroxyl groups (3580 and 3442 cm^{-1}), an α,*β*-unsaturated ketone (1685 cm^{-1}), and an exocyclic double bond (1648 and 887 cm^{-1}).

Inspection of the ^{1}H NMR spectrum suggested a diterpene scaffold for **1** showing signals for three tertiary methyl groups (*δ* 1.02, 1.11, and 1.04), one secondary methyl group, displayed as a doublet at *δ* 1.18 (*J* = 7.32 Hz), and an oxymethine proton (*δ* 4.54, broad singlet). Moreover, the ^{1}H NMR also showed the signals of an exocyclic methylene displayed as two broad singlets at *δ* 4.63 and 4.20. A proton broad signal at *δ* 1.71, without correlation in the HMQC spectrum, is in agreement with the existence of the hydroxyl function in the molecule; this feature was also corroborated by the FABMS spectrum that displayed a fragment peak at *m*/*z* 299, corresponding to the loss of H_2O $[M + 1 - H_2O]^+$.

The diterpenic nature of **1** was more evident by combined inspection of the ^{13}C NMR and DEPT experiments. These spectra showed 20 carbon resonances corresponding to four methyl groups, four methylenes (one sp^2 at δ_C 109.0), six methines (one oxygenated at δ_C 76.1), and six quaternary carbons (a carbonyl group at δ_C 209.3, one oxygenated carbon at δ_C 77.2, and three olefinic carbons at δ_C 171.1, 152.6, and 138.6). The presence of an α,*β*-unsaturated cyclopentenone was supported by the downfield signal corresponding to an olefinic carbon at δ_C 171.1, together with a carbonyl signal, evidencing the mesomeric effects due to the conjugation. The unexpected high value of the corresponding carbonyl resonance (δ_C 209.7) can be explained by its location in the methyl-substituted five-membered ring, which is in agreement with the $^2J_{C-H}$ and $^3J_{C-H}$ correlations of this carbon with the protons H-1 and H-2 and the methyl group at C-16, as well as the cross-peaks of the sp^2 carbons of the conjugated double bond, with H-1 and H-14. Moreover, a quaternary carbon at δ_C 19.6 and two shielded methines at *δ* 0.89 (m) and 0.76 (br t, *J* = 10 Hz) supported the existence of a *gem*-dimethyl-substituted cyclopropane ring.

On the basis of the seven degrees of unsaturation deduced by the molecular formula $C_{20}H_{28}O_3$, a tetracyclic diterpene skeleton was proposed for **1**. The structural features described above were corroborated by two-dimensional NMR experiments (COSY, HMQC, and HMBC), which allowed the assignment of the remaining NMR resonances. The COSY and the HMQC experiments revealed the existence of two sequences of correlated protons: $CH_3CH-CH_2-C(R)-CH(OH)R-$ (A) and $CH_2-CH_2-CH(R)-CH(R)-CH(R)-CH(R)-$ (B) (Table 1 and Figure 2). A long-range W-type

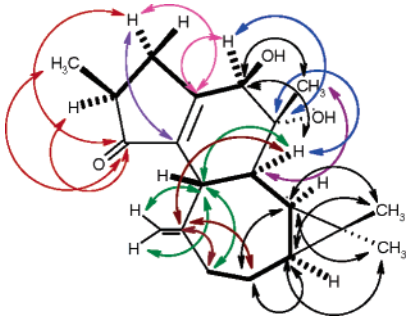


Figure 2. ^{1}H-spin systems (A and B) of compound **1** assigned by the HMQC and COSY experiments (−) and their connection by the principal heteronuclear $^2J_{C-H}$ and $^3J_{C-H}$ correlations displayed in the HMBC spectrum (↔).

coupling between the oxymethine proton of the fragment A, which appeared as a broad singlet (*δ* 4.54, H-14), and one of the diastereotopic methylene protons at C-1 (*δ* 2.34) was also evident in the COSY spectrum of **1**. Furthermore, the ^{1}H−^{1}H COSY spectrum also suggested the linkage of sequences A and B by a ^5J homoallylic coupling between the methine protons at *δ* 4.54 (H-14) and 2.88 (H-5). This connection was supported by the heteronuclear $^2J_{C-H}$ and $^3J_{C-H}$ correlations displayed in the HMBC spectrum of **1**, between the quaternary carbons and the protons of the referred spin systems (Figure 2). In particular, the long-range correlation of both C-5 and C-7 (δ_C 45.5 and 39.7) with the sp^2 methylene protons at δ_C 4.63 and 4.20 (H-17), the cross-peaks between C-6 (δ_C 152.6) and H-7, and the correlations exhibited by C-9, C-11, and the quaternary carbon C-10 with the methyl singlets CH_3-18 and CH_3-19 (*δ* 1.11 and 1.02) allowed the establishment of the exocyclic double bond and of the cyclopropane ring. The analysis of the HMBC spectrum also led to the location of the free hydroxyl groups, at positions 13 and 14, due to the observed cross-peaks between the quaternary C-13 (δ_C 77.2) and H-12, between

Table 1. NMR Data of Lagaspholones A (**1**) and B (**2**) ($CDCl_3$, *J* in Hz)

	1						**2**	
	^{1}H	^{13}C	DEPT	COSY (H→H)	HMBC (C→H)	NOESY	^{1}H	^{13}C
1α	2.95 ddt (18.9, 6.5, 1.6)	34.5	CH_2	1*β*, 2	CH_3-16	H-1*β*, H-2	2.78 m	41.8
1*β*	2.34 dd (18.9, 2.0)			1α, 14		H-1α, CH_3-16		
2	2.46 m	40.8	CH	1α, CH_3-16	CH_3-16	H−1α, H-14, CH_3-16		76.1
3		209.3	C		1, 2, CH_3-16			208.4
4		138.6	C		1b			135.9
5	2.88 br d (7.6)	45.5	CH	12, 14	7α, 12, 17a, 17b	H-7*β*, CH_3-20	2.88 br d (10)	45.2
6		152.6	C		7α, 7*β*, 8, 12, 17b			152.6
7α	2.51 m	39.7	CH_2	7*β*, 8	17a, 17b	H-7*β*, H-8	2.46 dd (13.2; 5.6)	39.7
7*β*	2.19 br t (12.5)			7α		H-5, H-7α	2.11 br d (12.8)	
8	2.12 m	26.3	CH_2	7α	7α, 7*β*	H-7*β*, H-9	2.08 m	26.3
9	0.89 m	27.0	CH	8, 11	8, CH_3-18, CH_3-19	H-8, H-11, H-12	0.84 m	26.9
10		19.6	C					19.8
11	0.76 br t (10.0)	27.0	CH	9, 12	8, CH_3-18, CH_3-19	CH_3-18	0.67 br t (10.8)	27.0
12	1.38 br t (10.0)	48.5	CH	5, 11	CH_3-20	H-9, H-14, CH_3-18	1.33 dd (1.6; 10.8)	48.4
13		77.2	C		12, 14, CH_3-20			77.2
14	4.54 br s	76.1	CH	1*β*, 5	12, CH_3-20	H-2, H-12	4.48 t (2.0)	76.1
15		171.1	C		1α, 1*β*, 14			171.0
16	1.18 d (7.32)	15.6	CH_3			H-1*β*, H-2	1.31 s	26.1
17a	4.63 s	109.0	CH_2	17b	7α, 7*β*	H-17b, H-7α	4.58 br s	109.0
17b	4.20 s			17a		H-17a	4.11 br s	
18	1.11 s	28.8	CH_3			H-12	1.05 s	28.8
19	1.02 s	16.8	CH_3		11, CH_3-18	−	0.95 s	16.7
20	1.04 s	15.9	CH_3		12	H-5	1.01 s	15.8

H-14 and CH_3-20, and between the secondary C-14 (δ_C 76.1) with H-12 and CH_3-20.

The relative stereochemistry of **1** was deduced from the analysis of the NOESY spectrum, together with the comparison of the coupling constant patterns with those reported in the literature for similar compounds. The *trans*-cyclohexane/cycloheptane ring connection and the *β*-orientation of H-5, which were assumed on the basis of the observation that crotofolane diterpenes, whose stereochemistry was determined by X-ray analysis, exhibited this type of fusion, were taken as a starting point.[10,11] Crotofolane diterpenes are closely related to jatropholones bearing an isopropylidene side chain instead of the cyclopropane ring.[1] The absence of nuclear Overhauser effects (NOE) between H-5 and H-12 is in agreement with the referred *trans*-junction. Therefore, the existence of strong NOE correlations between H-5/CH_3-20 and H-5/H-7*β* indicated the *β* orientation of these protons. Further, cross-peaks between H-12/H-9 and H-12/H-14 established the stereochemistry at C-12 and C-14 and the α configuration of these protons. The *cis* ring C/cyclopropane connection, also observed in lathyrane diterpenes,[1,12,13] was evidenced by the NOE enhancements between H-9 and H-11 and corroborated by the vicinal coupling constant value between these protons ($^3J_{11,9}$ = 10 Hz). The *β* orientation of CH_3-16 was supported by the presence of a NOE correlation between H-2 and H-14. This feature was corroborated by the values of $J_{1\alpha,2}$ (6.5 Hz) and $J_{1\beta,2}$ (2.0 Hz) which are similar to those reported for curcusone A ($J_{1\alpha,2}$ = 6.8, $J_{1\beta,2}$ = 2.3 Hz), with a *β*-methyl group at C-2, and different from that of curcusone B (CH_3-16α, $J_{1\alpha,2}$ = 7.4, $J_{1\beta,2}$ = 3.4 Hz).[14]

Lagaspholone B (**2**) was obtained as a colorless oil with $[\alpha]_D^{20}$ −30.0 ($CHCl_3$, *c* 0.11). Its molecular formula ($C_{20}H_{28}O_4$) was determined by HRSIMS, which showed a pseudomolecular ion at *m*/*z* 333.2068 $[M + 1]^+$ (calcd for $C_{20}H_{29}O_4$: 333.2066). The IR and MS data of **2** clearly resemble those found for lagaspholone A (**1**). The analysis of its ^{1}H NMR spectrum showed signals for four tertiary methyl groups (*δ* 0.95, 1.01, 1.05, and 1.31), one exocyclic methylene displayed as broad singlets at *δ* 4.58 and 4.11, and an oxygenated methine proton at *δ* 4.48 (t, *J* = 2 Hz). The ^{13}C NMR and DEPT spectra showed 20 signal resonances corresponding to four methyl groups, four methylenes (one sp^2 at δ_C 109.0), five methines (one oxymethine at δ_C 76.1), and seven quaternary carbons (one ketone at δ_C 208.4, two oxygenated carbons at δ_C 77.2 and 76.1, and three olefinic carbons at δ_C 171.0, 152.6, and 135.9).

When comparing the NMR spectra of compounds **1** and **2** and taking into account their molecular formula, the presence of another free hydroxyl group, located at C-2, was evidenced for the latter. In this way, CH_3-16, which appears as a doublet (*J* = 7.3 Hz) in compound **1**, is displayed as a lower field singlet (*δ* 1.31) and located at an oxygenated

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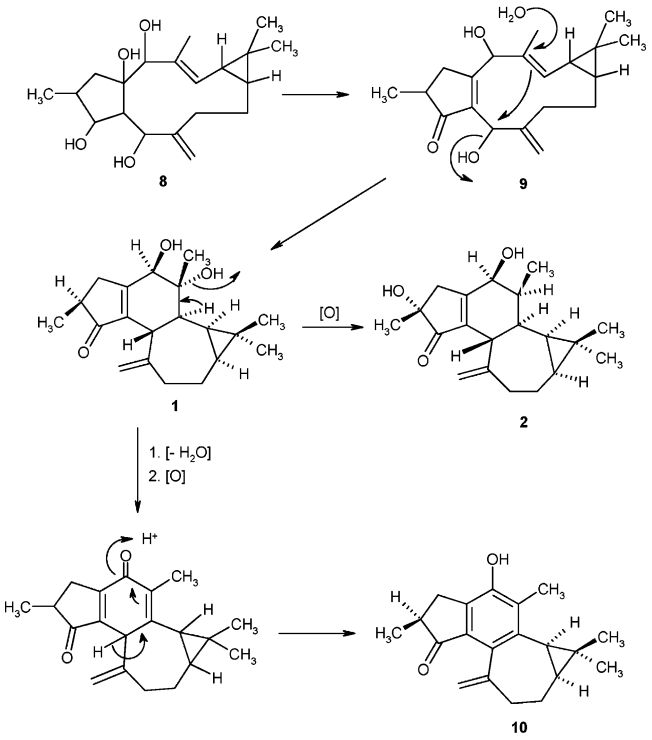
Scheme 1. Proposed Biogenetic Pathway for Lagaspholones A and B as Biosynthetic Intermediates of Jatropholones



quaternary carbon (δ_C 76.1) in the spectrum of **2**. Besides, the H-2 signal in the spectrum of **1**, at δ 2.46, is absent in the ^{1}H NMR spectrum of **2**. The structure of **2** was confirmed by 2D NMR experiments, and its relative stereochemistry was deduced from the NOESY spectrum and J_{H-H} values.

As far as we know, this is the first reported occurrence of jatropholane diterpenes in *Euphorbia* species.

Jatropholane diterpenes, characterized by the unusual 5:6:7:3 fused ring system, have been rarely isolated. In fact, jatropholones A (CH_3-16β, **10**) and B (CH_3-16α) were, until now, the only isolated compounds with this type of skeleton. They were obtained for the first time from the roots of *Jatropha gossypiifolia* (Euphorbiaceae)[15] and later on were also found in the roots of *Jatropha elliptica*[16] and *Jatropha curcas*.[17] Furthermore, because of its unusual scaffold, the synthesis of jatropholones was also described by several authors.[18,19]

A possible biosynthetic pathway for lagaspholones is proposed in Scheme 1. As can be observed, compounds **1** and **2** might be considered intermediates in the biosynthetic process leading to jatropholones. In this way, the oxidation of carbon C-13 in a lathyrane derivative such as **9**, followed by transannular closure of the macrocycle at position C-5/C-12, would lead to lagaspholone A (**1**) which would be further oxidized at C-16 giving rise to lagaspholone B (**2**). Dehydration of the tertiary hydroxyl group at C-13 of lagaspholone A (**1**), followed by the aromatization of ring B, would form jatropholone A (**10**).

The isolation of lagaspholone A and B from *Euphorbia* species reinforces the importance of casbene-derived diterpenes as useful chemotaxonomic biomarkers for the diverse plant family of Euphorbiaceae.[1]

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Supporting Information Available: Full experimental details on the isolation and identification of compounds **1**−**7** and 1D and 2D spectra of lagaspholones A and B (**1** and **2**). This material is available free of charge via the Internet at http://pubs.acs.org.

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