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# Lacitemzine, a novel sesquiterpene acid from the Tunisian plant *Pulicaria laciniata* (*Coss.* et *Kral.*) *Thell.*

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## ABSTRACT

The flowers of *Pulicaria laciniata* (*Coss.* et *Kral.*) *Thell.* (Asteraceae) afforded a new sesquiterpene acid **1**, named lacitemzine together with the three known compounds, 4-hydroxy-3-methoxypyridine **2**, β-sitosterol-3-*O*-β-D-glucoside **3** and 1,3,5-trimethoxybenzene **4**. The structure of compound **1**, 2-(2,6-dimethyl-3,4,5,6,7,8,9,10-octahydro-5,8-oxaazulen-9-yl)acrylic acid, contains a guaiane skeleton and was elucidated by spectroscopic procedures including 2D-NMR and X-ray diffraction.

In continuation of our investigations on the secondary metabolites of Tunisian plants,[1–4] we have studied the plant *Pulicaria laciniata* (*Coss.*et *Kral.*) *Thell.* (Asteraceae). This Mediterranean plant, widely distributed in Tunisia, is also regarded as being an Algero-Tunisian endemic species.[5] The genus *Pulicaria* comprises about 21 species predominately distributed around the Mediterranean. In Tunisia, beside *P. laciniata*, six other species have been found. It is known that the genus *Pulicaria* contains a variety of sesquiterpene lactones[6,7] and various other compounds such as flavonoids.[8–10] Recently, chemical investigations on *P. laciniata* gathered in Algeria led to the isolation of a guaianolide epoxide and a pseudoguaianolide.[11]

The current study describes the isolation and the structure elucidation of a new sesquiterpene acid named lacitemzine **1** in addition to three known compounds, 4-hydroxy-3-methoxypyridine **2**, β-sitosterol-3-*O*-β-D-glucoside **3** and 1,3,5-trimethoxybenzene **4** (Fig. 1). The structure of compound **1** was established principally by two-dimensional NMR spectroscopy, and the relative configurations of the stereogenic carbons were determined through X-ray diffraction analysis.

The dried and powdered flowers (1.1 kg) of *P. laciniata*[12] were extracted with dichloromethane at room temperature for six days. 7.5 g of the crude residue (60 g), obtained after filtration and evaporation of the solvent under reduced pressure, was subjected to silica gel column chromatography eluting with $CH_2Cl_2$/petroleum ether (70:30, 85:15, 90:10), $CH_2Cl_2$, $CH_2Cl_2$/acetone (90:10, 80:20, 70:30, 60:40, 50:50) and acetone successively, to yield 9 fractions.

Fraction 6 (1.3 g) was chromatographed on silica gel eluting with $CHCl_3$/acetone (90:10, 80:20, 70:30, 60:40), and acetone to give eight subfractions, the third of which was purified by column chromatography over silica gel petroleum ether/AcOEt (70:30) to give **4** (7 mg). The seventh fraction was eluted with petroleum ether/AcOEt (60:40) to afford **1** (35 mg; mp = 162–164 °C).

Fraction 5 (2.1 g) was eluted with petroleum ether/AcOEt (70:30) over silica gel to afford seven subfractions, the fourth of which was subjected to preparative silica gel TLC eluting with $CHCl_3$/MeOH (94:6) to give **2** (9 mg).[13] Analysis of the 1 and 2D-NMR spectra of compound **2** confirmed the structure as 4-hydroxy-3-methoxypyridine. Fraction 9 (900 mg) was also purified by column chromatography on silica gel, $CHCl_3$/MeOH (80:20) to give **3** (13 mg), the spectral data of which were in good agreement with those previously reported.[14,15]

Compound **1** was isolated as a white solid from the dichloromethane extract of the *P. laciniata* flowers. Its positive HR-ES-MS showed a pseudo-molecular ion $[M+Na]^+$ at $m/z$ 271.1289 (calcd 271.1310) compatible with the molecular formula $C_{15}H_{20}O_3$ ($M_w = 248$) and six degrees of unsaturation. The $^{13}C$ NMR spectrum showed 15 resonances consistent with the molecular formula. The specific rotation value obtained for **1** was found to be $[\alpha]_D$ +4.37 ($c$ 1.0, $CHCl_3$). The $^1H$ and $^{13}C$ NMR spectra of compound **1** were assigned as shown in Table 1.

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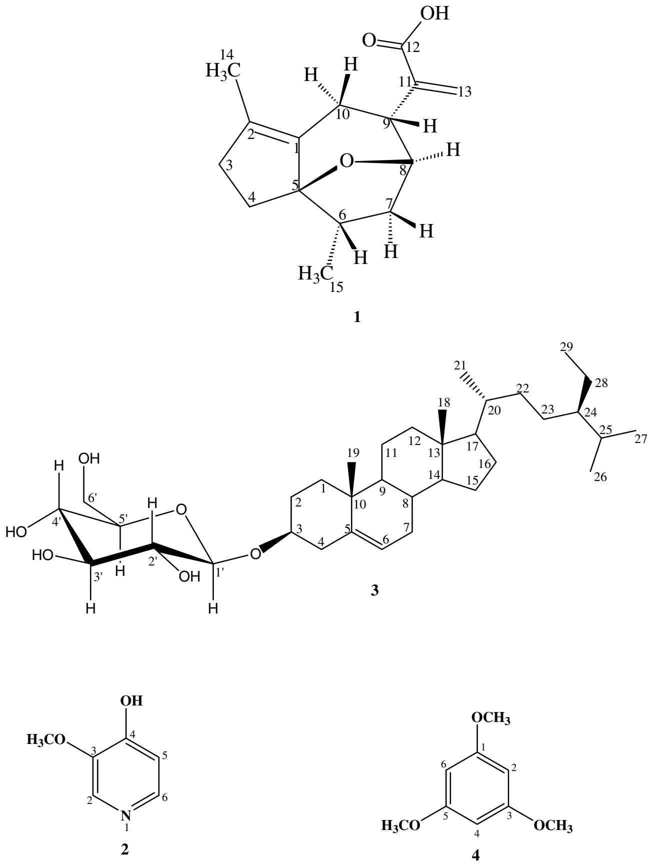


**Figure 1.** Structures **1**–**4**.

The $^{13}C$ NMR spectrum displayed 15 carbon resonances, which were assigned by CHcorr, HMBC and DEPT 135 experiments to five quaternary, three methine, five methylene and two methyl carbon atoms. The presence of an α-methylene-acid moiety was deduced from the $^{13}C$ NMR signals at δ 171.9 (CO), δ 140.1 and δ 126.7 ($C{=}CH_2$), and was reinforced by the HMBC spectrum which showed a correlation between the methylenic protons H-13 and C-12. The position of this acrylic system in the molecule was proved via the heteronuclear correlation H-13/C-9. The presence of a tetrasubstituted double bond was evident from the two signals at 131.4 ppm and 134.2 ppm (both singlets) in the $^{13}C$ NMR spectrum, whereas, the presence of two oxygenated carbon atoms (C-5 and C-8) was proved by the observation of two signals at δ 76.1 (d, C-8) and δ 96.3 (s, C-5). The HMBC spectrum indicated that C-5 and C-8 were separated by an oxygen atom (Fig. 2). The remaining signals observed in the $^{13}C$ NMR spectrum were assigned to non-functionalized $sp^3$ carbons. Thus, compound **1** has three unsaturated functions and possesses three rings in its structure.

The $^1H$–$^1H$ COSY experiment enabled H-9 to be placed at δ 3.03 according to its allylic coupling to H-13a,b.

The complete interpretation of the remaining NMR data was undertaken and established based on the results of conclusive $^1H$–$^1H$ COSY, HMBC and NOESY experiments.

Two methyl resonances detected at δ 12.7 ($CH_3$-15) and 13.6 ppm ($CH_3$-14) were easily distinguished from the multiplicities of the attached protons in the $^1H$ NMR spectrum. Thus, the doublet at δ 0.90 ($J$ = 6.3 Hz) corresponded to the carbon resonance at 12.7 ppm and the singlet at 1.64 ppm was due to the protons attached to the carbon atom at 13.6 ppm (Table 1).

The position of the above methyl groups was determined with the help of the HMBC spectrum (Fig. 2) which showed correlations between H-14 and C-1, C-2 and C-3 as well as correlations between H-15 and C-5, C-6 and C-7, indicating that the methyl groups $CH_3$-14 and $CH_3$-15 were attached to C-2 and C-6, respectively (Fig. 2).

The position of the quaternary carbon C-5 was deduced from its relatively high $^{13}C$ NMR chemical shift (δ 96.3) and from significant proton–carbon correlations including H-8/C-5, H-4/C-5, H-6/C-5 and H-7α/C-5 in the HMBC spectrum.

On the other hand, the position of the C=C double bond in the cyclopentene moiety was evident from the H-10/C-1 and H-3/C-1 correlations observed in the HMBC spectrum (Fig. 2) and from the NOE between H-10 β/H-14 in the NOESY spectrum (Fig. 3).

**Table 1**
$^1H$ (300 MHz) and $^{13}C$ NMR (75 MHz) spectral data ($CDCl_3$ solution) of compound **1**

| Atom | δ $^1H$ (ppm) | Multiplicity | $J$ (Hz) | δ $^{13}C$ (ppm) |
|---|---|---|---|---|
| 1 | – | | | 134.2 |
| 2 | – | | | 131.4 |
| 3 | 2.33 | m | | 35.7 |
| 4α | 1.90 | ddd | 13.8; 8.4; 3.9 | 23.8 |
| 4β | 2.10 | m | | |
| 5 | – | | | 96.3 |
| 6 | 2.13 | m | | 40.9 |
| 7α | 1.25 | d (br) | 4.8 | 30.1 |
| 7β | 2.17 | m | | |
| 8 | 4.44 | d (br) | 4.8 | 76.1 |
| 9 | 3.03 | d (br) | 11.7 | 41.8 |
| 10α | 2.06 | m | | 23.8 |
| 10β | 2.48 | dd | 14.4; 4.8 | |
| 11 | – | | | 140.1 |
| 12 | – | | | 171.9 |
| 13a | 5.64 | d | 1.5 | 126.7 |
| 13b | 6.42 | d | 1.5 | |
| 14 | 1.64 | s | | 13.6 |
| 15 | 0.90 | d | 6.3 | 12.7 |

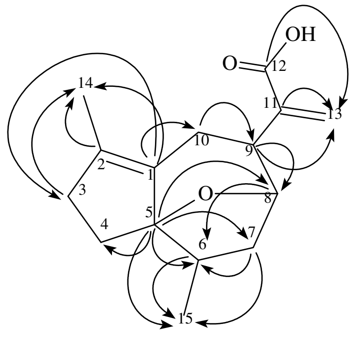


**Figure 2.** Representative HMBC correlations of **1** arrows point from carbon to proton.

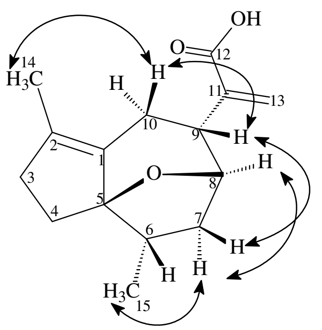


**Figure 3.** Dipolar couplings observed in the NOESY experiment of **1**.

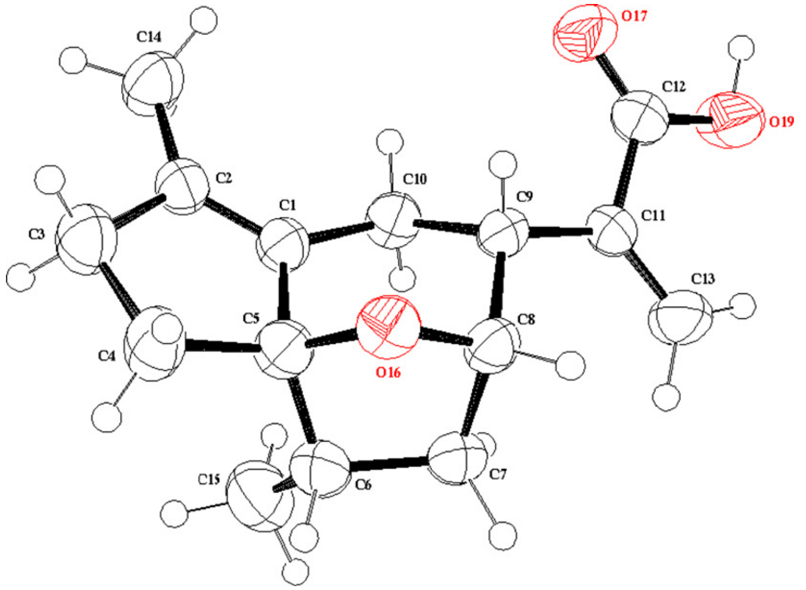
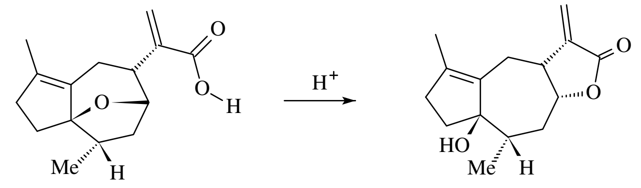


**Figure 4.** ORTEP drawing of the molecule **1** showing the atomic numbering scheme. Displacement ellipsoids are plotted at the 50% probability level.



**Scheme 1.** A possible biosynthetic pathway to a sesquiterpene lactone.

A careful examination of the $^1H$–$^1H$ COSY, CHcorr and HMBC spectra indicated additional correlations permitting the establishment of the connectivities as depicted in Figure 2.

Suitable white X-ray quality crystals of compound **1** were obtained by crystallization from hexane/$CHCl_3$ (7:3). The X-ray diffraction analysis of **1** was carried out on a single crystal,[16] Figure 4. This study confirmed the structure of **1** and clearly establishes that the relative configurations of the four stereogenic carbons are, in fact, 5*R*, 6*S*, 8*S* and 9*R* (or 5*S*, 6*R*, 8*R* and 9*S*). The tetrasubstituted double bond is between C-1 and C-2 as the C-1–C-2 bond length is 1.315 Å, and the oxygen atom O-16 connects carbon C-5 with C-8 as the O-16–C-5 and O-16–C-8 bond lengths are 1.468 Å and 1.447 Å, respectively. These results were in good agreement with all the dipolar couplings observed in the NOESY spectrum, and are illustrated in Figure 3.

In summary, besides three known compounds, a new sesquiterpene acid named lacitemzine **1** was isolated from the aerial parts of *P. laciniata* (*Coss.* et *Kral.*) *Thell.* The main novelty of this compound is the presence of a guaiane skeleton with a tetrahydrofuran ring and an acrylic acid moiety in its structure. We believe that this compound may be implicated in the biosynthesis of a guaianolide type sesquiterpene lactone through acid-induced cyclization of **1** as depicted in Scheme 1.

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## References and notes

1. Bergaoui, A.; Hammami, S.; Ben Jannet, H.; Mighri, Z. *Nat. Prod. Res.* **2007**, 686–691.
2. Ben Jannet, H.; Sakka-Rouis, L.; Neffeti, M.; Mighri, Z. *Jeobp* **2007**, *10*, 151–156.
3. Tekaya-Karoui, A.; Ben Jannet, H.; Mighri, Z. *Pakistan J. Biol. Sci.* **2007**, *10*, 2495–2499.
4. Oueslati, M. H.; Ben Jannet, H.; Mighri, Z.; Chriaa, J.; Abreu, P. M. *J. Nat. Prod.* **2006**, *69*, 1366–1369.
5. Pottier-Alapetite, G. *Flore de la Tunisie.* Angiospermes-Dicotylédones. Publications Scientifiques Tunisiennes: Imprimerie Officielle de la République Tunisienne, Tunis, **1981**.
6. Abdel-Mogib, M.; Jakupovic, J.; Dawidar, A. M. M.; Metwally, A.; Abou-Elzahab, M. *Phytochemistry* **1990**, *29*, 2581–2584.
7. Rustaiyan, A.; Habibi, Z.; Saberi, M.; Jakupovic, J. *Phytochemistry* **1991**, *30*, 2405–2406.
8. Williams, C. A.; Harborne, J. B.; Greenham, J. R.; Grayer, R. J.; Kite, G. C.; Eagles, J. *Phytochemistry* **2003**, *64*, 275–283.
9. El-Negoumy, S.; Ragaa, M. A.; Saleh, A. M. *Phytochemistry* **1982**, *21*, 953–954.
10. Pares, J. O.; Oksuz, S.; Ulubelen, A.; Mabry, T. J. *Phytochemistry* **1981**, *20*, 2057.
11. Aberkane, M. C.; Dibi, A.; Haba, H.; Benkhaled, M.; Benkouider, A.; Mokhtari, M.; Mosset, P.; Pale, P. *Asian J. Chem.* **2007**, *19*, 4954–4956.
12. *Pulicaria laciniata* (*Coss.*et *Kral.*) *Thell.* was collected in the region of El Hwareb (Kairouan, Tunisia) in April 2006. The plant was identified in the Laboratoire de Biologie Végétale et Botanique, Institut Supérieur Agronomique de Chott meriem, Université de Sousse, Tunisia and a voucher specimen (PL-06) was deposited in the same laboratory.
13. Compound **2**: White solid; 9 mg; $R_f$ 0.3 ($CHCl_3$/MeOH 95:5); $^1H$ NMR (300 MHz, $CD_3OD$): $\delta$ (ppm) 3.88 (3H, s, $CH_3$-7), 6.79 (1H, d, $J = 8.1$ Hz, H-5), 7.51 (1H, d, $J = 8.1$ Hz, H-6), 7.57 (1H, s, H-2); $^{13}C$ NMR (75 MHz, $CD_3OD$): $\delta$ (ppm) 54.9 (C-7), 112.5 (C-2), 114.1 (C-5), 123.3 (C-6), 146.9 (C-3), 149.8 (C-4).
14. Hichri, F.; Hamouda, O.; Ben Jannet, H.; Mighri, Z.; Abreu, P. J. M. *J. Soc. Chim. Tun.* **2002**, *4*, 1565–1569.
15. Hammami, S.; Ciavatta, M. L.; Ben Jannet, H.; Cimino, G.; Mighri, Z. *Croatia Chim. Acta* **2006**, *79*, 215–218.
16. Crystal data for compound **1,** $C_{15}H_{20}O_3$ were collected at room temperature using a Bruker-APEX II Kappa CCD diffractometer, $M = 248.31$, Orthorhombic, $P2_12_12_1$, $a = 7.109$ (5) Å, $b = 12.772$ (5) Å, $c = 14.596$ (5) Å, $V = 1325.3$ (12) Å$^3$, $Z = 4$, $D_c = 1.245$ g/cm$^{-3}$, X-ray source Mo–K$\alpha$ (radiation), $\lambda = 0.71073$ Å, $F$ (000) = 134, $T = 293(2)$ K, colourless prism 0.48 × 0.31 × 0.15 mm. The structure solution was obtained by direct methods and refined with anisotropic thermal parameters using full-matrix least-squares procedures on $F^2$ to give $R = 0.051$, $wR = 0.0863$ for 1235 independent observed reflections and 166 parameters. Crystallographic data (excluding structure factors) for the structure in this Letter have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication number CCDC 686108. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: +44(0) 1223 336033 or e-mail: deposit@ccdc.cam.ac.uk).