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Isolation and structure determination of pulicazine, a new sesquiterpene lactone from the Tunisian *Pulicaria laciniata* (*Coss.*et *Kral.*) *Thell.*

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ABSTRACT

A new naturally occurring pseudo-guaianolide type sesquiterpene lactone named pulicazine along with *ent*-11β,15β-dihydroxykaur-16-en-19-oic acid is isolated from the Tunisian *Pulicaria laciniata* (*Coss.*et *Kral.*) *Thell.* flowers. Their structures were elucidated by NMR spectroscopy and their stereochemistries were established by X-ray diffraction.

In continuation of our studies of Tunisian fragrant plants,[1–6] primarily in the search for new bioactive principles, we have examined the dichloromethane extract of the flowers of *Pulicaria laciniata* (*Coss.*et *Kral.*) *Thell.* (Asteraceae). We previously isolated a new sesquiterpene acid, lacitemzine, together with the known 4-hydroxy-3-methoxypyridine, β-sitosterol-3-*O*-β-D-glucoside and 1,3,5-trimethoxybenzene from this species.[7]

The genus *Pulicaria* is well documented as a good source of sesquiterpene lactones.[8,9] A review of the literature showed that some sesquiterpene and diterpene derivatives are associated with various biological and pharmacological activities such as anti-fungal, anti-mycobacterial, anti-inflammatory, and anti-cancer.[10–12]

We describe here the isolation and structure determination of a new pseudo-guaianolide type sesquiterpene lactone **1** and the known kaurenoic acid **2** (Fig. 1).[13,14] The structures were established by 2D NMR spectroscopy and their stereochemistries were determined through X-ray diffraction analysis.

The dried and powdered flowers (1.1 kg) of *P. laciniata*[15] were extracted with dichloromethane at room temperature for six days. A total of 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 successively 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 to yield nine fractions.

Fraction 7 (0.66 g) was chromatographed on silica gel eluting with $CHCl_3$–MeOH (95:5, 90:10) to give eight subfractions, the seventh of which was purified by column chromatography over silica gel using $CHCl_3$–MeOH (90:10) as eluent to afford **1** [70 mg; mp = 144–146 °C ($CHCl_3$)].[16]

Fraction 5 (2.1 g) was eluted with petroleum ether–EtOAc (70:30) over silica gel to afford seven subfractions, the second of which was subjected to preparative silica gel TLC eluting with $CHCl_3$–MeOH (95:5) to give **2** (10 mg).[13,14]

Compound **1** was obtained as colourless crystals. Negative and positive ES-MS of this substance gave pseudo-molecular peaks $[M-H]^-$ at *m/z* 265 and $[M+Na]^+$ at *m/z* 289, respectively, which corresponded with the molecular formula $C_{15}H_{22}O_4$ (M_w = 266) and four degrees of unsaturation. The IR spectrum showed absorption bands typical of hydroxy (3450 cm^{-1}) and lactone carbonyl (1750 cm^{-1}) groups. The specific rotation of **1** was found to be $[\alpha]_D^{20}$ -36 (*c* 0.416, $CHCl_3$). The ^1H and ^{13}C NMR spectral data of compound **1** are listed in Table 1.

The ^{13}C NMR spectrum showed 15 carbon resonances, which were identified by CHcorr and HMBC experiments as two methyl, five methylene, four methine and four quaternary carbon atoms.

The presence of an α-methylene-γ-lactone moiety was confirmed by the ^{13}C NMR signals at δ 165.6 (CO), 138.1 and 128.2 ($C{=}CH_2$), and was reinforced by the HMBC spectrum which

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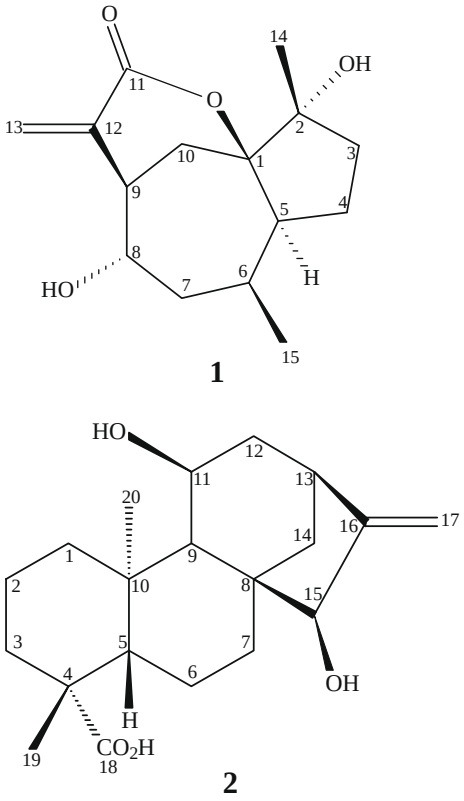


Figure 1. Structures of **1** and **2**.

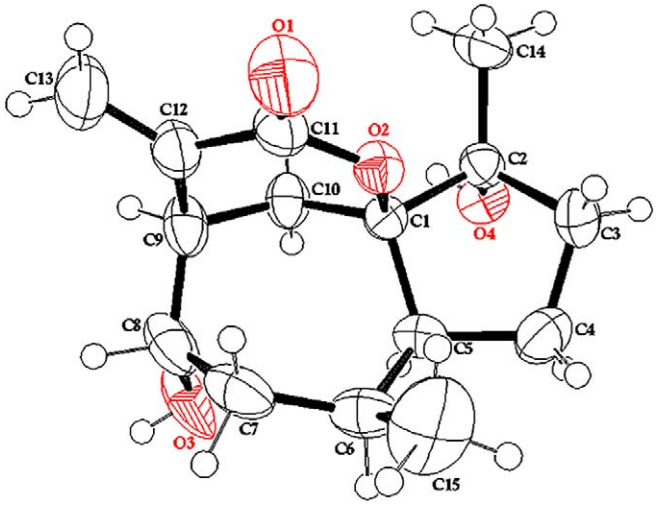


Figure 2. ORTEP drawing of **1** showing the atomic numbering scheme. Displacement ellipsoids are plotted at 50% probability level.

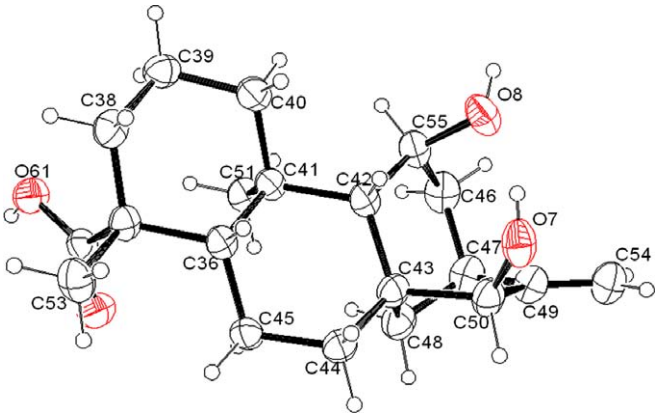


Figure 3. ORTEP drawing of **2** showing the atomic numbering scheme. Displacement ellipsoids are plotted at 50% probability level.

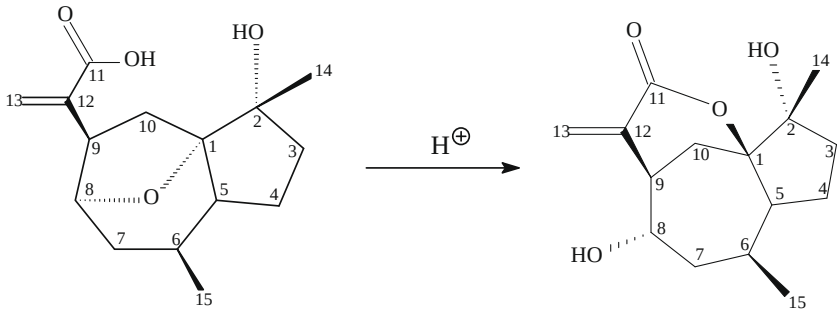
showed correlation of the methylene protons H-13 with C-11 and C-12.

The position of the α-methylene-γ-lactone system on the cycloheptane ring was deduced from the presence of long-range correlations in the HMBC spectrum, which are summarised in Table 1.

The presence of three oxygenated carbon atoms (C-1, C-2 and C-8) was confirmed by the presence of the three signals at δ 96.7 (singlet, C-1), δ 84.0 (singlet, C-2) and δ 74.3 (doublet, C-8). The ^{1}H-^{1}H COSY experiment enabled H-9 to be placed at δ 2.97 according to its allylic coupling to H-13a,b. The complete interpretation of the remaining NMR data was established based on ^{1}H–^{1}H COSY, HMBC and NOESY experiments. The position of the secondary hydroxy-

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

Atom	δ ^{1}H (ppm)	Multiplicity	J (Hz)	δ ^{13}C (ppm)	HMBC (C→H)	COSY
1	–			96.7	H-3a, H-4b, H-10a, H-14	
2	–			84.0	H-14	
3	1.64 (a)	m		37.9	H-14	H-4b
	2.04 (b)	m				H-4a,b
4	1.61 (a)	m		23.7	H-3a,b, H-5, H-6	H-5
	1.79 (b)	m				H-5
5	2.25	m		27.7	H-4a, H-6, H-7b, H-10b, H-15	H-6
6	2.56	m		49.4	H-7a, H-15	H-7a,b, H-15
7	1.69 (a)	ddd	17.4; 8.4; 2.7	39.4	H-15	H-8
	1.89 (b)	m				H-8
8	3.95	dt	8.7; 3	74.3	H-10a	H-9
9	2.97	q	3.5	44.3	H-7a, H-10a, H-13a,b	H-10a,b
10	1.84 (a)	m		25.6		
	2.31 (b)	dd	14.4; 3			
11	–			165.6	H-13a,b	
12	–			138.1	H-10b, H-13b	
13	5.57 (a)	d	1.2	128.3		H-13b
	6.35 (b)	d	1.2			H-13a
14	1.15	s		21.8		
15	0.90	d	7.2	17.2		



Scheme 1. A possible biosynthetic pathway to compound **1**.

bearing C-8 at 74.3 ppm was deduced from H-7/H-8 and H-8/H-9 correlations in the ^{1}H-^{1}H COSY spectrum. The two methyl resonances at 21.8 (CH_3-14) and 17.2 (CH_3-15) were easily distinguished from the multiplicities of the attached protons in the ^{1}H NMR spectrum. Thus, the doublet at δ 0.90 (J = 7.2 Hz) corresponded to the carbon resonance at 17.2 ppm and the singlet at 1.15 ppm was due to the protons attached to the carbon atom at 21.8 ppm (Table 1). The position of the methyl groups was determined from the HMBC experiment (Table 1) which showed correlations between H-15 and C-5, C-6 and C-7 as well as correlations between H-14 and C-1, C-2 and C-3, indicating that the methyl groups CH_3-14 and CH_3-15 were attached to C-2 and C-6, respectively (Table 1).

A careful examination of the ^{1}H–^{1}H COSY, CHcorr and HMBC spectra indicated additional correlations, permitting the establishment of the connectivities as listed in Table 1.

White X-ray quality crystals of compound **1** were obtained by crystallisation from hexane/$CHCl_3$ (75:25). The X-ray diffraction analysis of **1**[17] (Fig. 2) confirmed the structure and clearly established the relative configurations of the stereogenic carbons as 1*R*, 2*R*, 5*S*, 6*S*, 8*S* and 9*R* (or 1*S*, 2*S*, 5*R*, 6*R*, 8*R* and 9*S*).

Analysis of the 1D and 2D NMR spectra of compound **2** confirmed the structure as *ent*-11β,15β-dihydroxykaur-16-en-19-oic acid.[18] X-ray analysis of a crystal of compound **2** obtained from $CHCl_3$–MeOH confirmed the proposed stereochemistry (Fig. 3).

We believe that compound **1** could be obtained by a possible biosynthetic pathway involving acid-induced cyclisation of a sesquiterpene acid, possibly present in the same plant,[7] as depicted in Scheme 1.

In summary, we have isolated and elucidated the structure of a new sesquiterpene lactone **1**, pulicazine, together with a known diterpene acid **2** from the flowers of the Tunisian *Pulicaria laciniata* (*Coss.*et *Kral.*) *Thell.* Of note is the unusual position of the α-methylene-γ-lactone moiety on the cycloheptane ring in **1**.

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15. *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.
16. Compound **1**: White solid; mp = 144–146 °C ($CHCl_3$); $[\alpha]_D^{20}$ −36 (*c* 0.416, $CHCl_3$); ES-MS: *m/z* = 289.1 ($[M+Na]^+$), *m/z* = 265.1 ($[M-H]^-$); IR (KBr): ν (cm^{-1}) 3450, 1750; ^{1}H and ^{13}C NMR data (see Table 1).
17. Crystal data for compound **1**, $C_{15}H_{22}O_4$, were collected at room temperature using a Bruker-APEX II Kappa CCD diffractometer, M = 266.33, orthorhombic, $P2_12_12_1$, a = 9.6144(2) Å, b = 10.5735(2) Å, c = 14.1280(3) Å, V = 1436.22(5) Å^3, Z = 4, D_c = 1.232 g/cm^{-3}, X-ray source MoKα (radiation), λ = 0.71073 Å, $F(000)$ = 576, T = 293(2) K, colourless prism 0.44 × 0.34 × 0.25 mm. The structure solution was obtained by direct methods and was refined with anisotropic thermal parameters using full-matrix least squares procedures on F^2 to give R = 0.0494, wR = 0.1231 for 3710 independent observed reflections and 173 parameters. Crystallographic data (excluding structure factors) for the given structure in this Letter have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication number CCDC 705510. Copies of these 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).
18. Crystal data for compound **2**, $C_{20}H_{30}O_4$, were collected at room temperature using a Bruker-APEX II Kappa CCD diffractometer, M = 334.44, monoclinic, $P2_1$, a = 14.8703(3) Å, b = 8.7397(2) Å, c = 17.7146(4) Å, β = 100.6460(10) Å, V = 2262.60(9) Å^3, Z = 4, D_c = 0.982 g/cm^{-3}, X-ray source MoKα (radiation), λ = 0.71073 Å, $F(000)$ = 728, T = 293(2) K, colourless prism 0.49 × 0.38 × 0.20 mm. The structure solution was obtained by direct methods and was refined with anisotropic thermal parameters using full-matrix least squares procedures on F^2 to give R = 0.0493, wR = 0.1209 for 6578 independent observed reflections and 439 parameters. Crystallographic data (excluding structure factors) for the structure given in this Letter have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication number CCDC 705509. Copies of these 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).