NOVEL SESQUITERPENE LACTONE AND SESQUITERPENE LACTONE GLUCOSIDE FROM THE FLOWERS OF THE TUNISIAN *PULICARIA LACINIATA* (COSS. ET KRAL.) THELL.

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Abstract: A sesquiterpene lactone **1** Lacinin and a sesquiterpene lactone glucoside **2** Laciniside, were isolated from the flowers of *Pulicaria laciniata*. Their structures were elucidated by extensive spectroscopic methods including 1D-(^{1}H and ^{13}C) and 2D-NMR experiments, (CHcorr, HMBC, ^{1}H-^{1}H COSY and NOESY) as well as high resolution ES-MS.

Keywords: *Pulicaria laciniata*, Asteraceae, Sesquiterpene lactone glucoside, 2D-NMR.

INTRODUCTION

The large Asteraceae family contains 25.000-30.000 species, belonging to over 1000 genera. The chemistry of numbers of this family has been studied intensively and more than 28.000 substances have been identified so far [1]. The genus *Pulicaria* is one of the largest and most widely distributed genera of the Asteraceae. Different types of sesquiterpene lactones have been reported from this genus, eudesmanolides, guaianolides and pseudo-guaianolides being the most common [2].

As sesquiterpenoids exhibit a wide range of bioactivities which include toxicity of certain cancer cell lines and induction of detoxifying enzymes, the sesquiterpene content of salads and vegetables might contribute to the health promoting properties of these groceries [3,4]. Some other sesquiterpene and diterpene derivatives are also associated with various other biological and pharmacological activities such as anti–fungal, anti-mycobacterial and anti-inflammatory [5-7]. An earlier phytochemical investigation performed in our laboratory on *P. laciniata*, led to the isolation of two new sesquiterpenes, lacitemzine and pulicazine, together with four known compounds, 4-hydroxy-3-methoxypyridine, β-sitostrol-3-*O*-β-D-glucoside, 1,3,5-trimethoxybenzene and kauranoic acid [8,9].

In the present study, we describe the isolation and structure determination of a new sesquiterpene lactone **1** Lacinin and a new sesquiterpene lactone glucoside **2** Laciniside. Their structures were established by 1D (^{1}H and ^{13}C) and 2D-NMR spectroscopy.

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RESULTS AND DISCUSSION

The dichloromethane and butanolic extracts of *P. laciniata* flowers were fractionned by silica gel chromatography, leading to the isolation of two compounds **1** and **2**.

Compound 1:

Was obtained as colorless oil. Its negative and positive ES-MS showed pseudo-molecular peaks $[M-H]^-$ and $[M+Na]^+$ at *m/z* 265 and at *m/z* 289, respectively, in concordance with the molecular formula $C_{15}H_{22}O_4$ and five degrees of unsaturation.

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

The presence of an α-metylene-γ-lactone moiety was revealed by the ^{13} C-NMR signals at δ171 .4 (CO), 140.4 and 120.1 ($C=CH_2$) [10,11].

The position of this α-methylene-γ-lactone system in the molecule was deduced from the presence of some significant long range correlations in the HMBC spectrum such as H_{13}-C_{11} and H_{13}-C_{12} (Table 1).

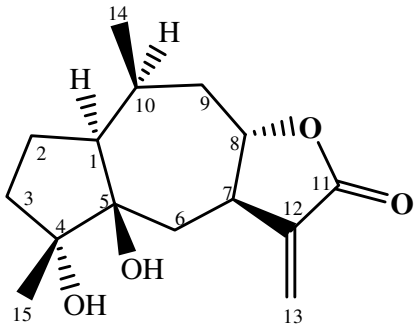
The position of the methyl groups (CH_3)-14 and (CH_3)-15 was determined from the HMBC experiment (table 1) which showed correlations between H_{14} (δ 0.95, d, J= 6.6 *Hz*) and C_1 (δ 51.8) and C_9 (δ 36.0) as well as correlation between H_{15} (δ 1.2, s) and the two quaternary hydroxylated carbons C_4 (δ 81.9) and C_5 (δ 82.5), indicating that the methyl groups (CH_3)-14 and (CH_3)-15 were attached to C_{10} and C_4, respectively. The position of (CH_3)-14 was also reinforced by the peak cross H_{14}-H_{10} revealed from the ^{1}H-^{1}H COSY spectrum.

Table 1: ^{1}H, ^{13}C, HMBC and NOESY spectroscopic data of compound **1**.

Position	^{1}H	^{13}C	HMBC	NOESY
1	2.02 m	51.8	H_{14}, $H_{2\alpha,\beta}$, $H_{3\alpha,\beta}$	$H_{9\alpha}$, $H_{6\alpha}$, $H_{2\alpha}$
2	1.31 (α) m	18.8		
	1.68 (β) m			
3	1.64 (α) m	35.0	H_{15}	
	1.88 (β) m		$H_{2\beta}$	
4	-	81.9	H_{15}, $H_{3\alpha,\beta}$	
5	-	82.5	H_{15}, $H_{6\alpha,\beta}$, H_1	
6	1.64 (α) m	35.0		H_7
	1.88 (β) m			H_8, H_{13a}
7	3.07 m	39.5	$H_{13a,b}$, $H_{6\beta}$	H_{10}
8	4.18 dd (9;8.4)	83.6	$H_{9\alpha,\beta}$	$H_{9\beta}$, H_{15}, H_{14}
9	1.84 (α) m	36.0	-	
	2.04 (β) m			
10	2.28 m	27.2	-	H_{14}
11	-	171.4	$H_{13a,b}$	
12	-	140.4	$H_{13a,b}$	
13	5.53 (a) d (3)	120.1		
	6.13 (b) d (3)			
14	0.95 d (6.6)	21.5		
15	1.2 (s)	24.6		

The relative stereochemistry of the **1** was determined by the NOESY spectrum. The absence of the *nOe* correlation between H_7 and H_8 indicated that these two hydrogens have a *trans* orientation. Clear *nOe* correlations between H_8, $H_{9\beta}$ and H_{14} confirmed that the methyl group (CH_3)-

14 at C_{10} and the proton $H_{9\beta}$ have the same orientation than H_8. The *nOe* observed between H_{10} and H_7 confirmed their α orientation. A *nOe* interaction between H_{14}, H_{15} and $H_{6\beta}$ allowed the assignment of H_{15} as being α orientation. Measurement of through-space interatomic distances based on molecular models reinforced the proposed relative stereochemistry.



Compound **1**

Compound 2:

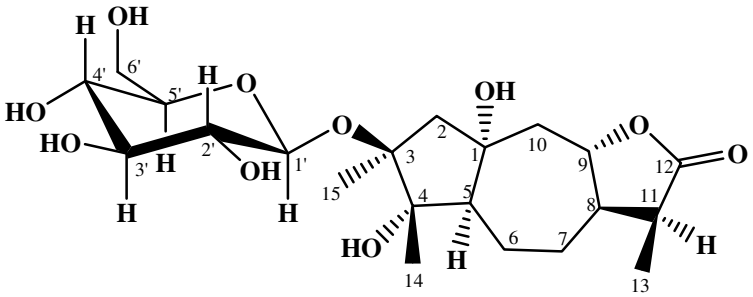
Was isolated as a white solid, and its molecular formula, $C_{21}H_{34}O_{10}$, $[M+Na]^+$ at *m/z* 469 and $[M-H]^-$ at *m/z* 445 was established by ES-MS. The structure of **2** was elucidated via its acetylated derivative **2a**. The ^{13}C and ^{1}H-NMR spectra (table 2) showed the typical signals for a β-*D*-glucopyranose with the doublet for the anomeric proton $H_{1'}$ at δ 4.66 ppm and the resonance for the corresponding carbon $C_{1'}$ at δ 94.9 ppm.

In the ^{13}C-NMR spectrum, the signal for C_{12} at δ 180.0 ppm, which is typical for a saturated lactone ring, indicating the absence of an exo-methylene group at C_{11} [12]. The position of the lactone ring was deduced from the presence of long range correlations in the HMBC experiment which are assigned in table 2. The position of the methyl (CH_3)-13 at δ 10.4 ppm was determined from the HMBC spectrum showing the ^2J (H_{13}-C_{11}) and ^3J (H_{13}-C_8) correlations (Table 2). The two methyl resonances at δ 23.1 (C_{14}) and δ 23.3 (C_{15}) corresponding to the singlets at δ1.27 and 1.21 ppm, respectively, were determined from the CHcorr experiment. Their positions were proved from the HMBC spectrum (table 2) which showed correlations between H_{14}-C_4 and H_{15}-C_3, indicating that the methyl groups (CH_3)-14 and (CH_3)-15 were attached to C_4 and C_3, respectively.

The presence of four oxygenated carbon atoms (C_1, C_3, C_4 and C_9) in the aglycone moiety was confirmed by the presence of the four signals at δ 83.5 (C_1), δ 82.3 (C_3), δ 82.6 (C_4) and δ 80.7 (C_9).

The HMBC spectrum, which displayed correlation between the anomeric proton of the β-glucopyranose at δ4.66 ppm (1H, d, J= 7.8 *Hz*, $H_{1'}$) and C_3 at 82.3 ppm, reinforced the deduction of the glycosilation site as C_3. Assignement of the sugar resonances was achieved using the anomeric proton resonance at δ 4.66 ppm as a starting point for the interpretation of the ^1H-^1H COSY spectrum.

Identification of the sugar as β-glucopyranose was supported by the $^3J_{H1'-H2'}$ coupling constant of 7.8 Hz as well as by the trans di-axial couplings between $H_{2'}$-$H_{3'}$, $H_{3'}$-$H_{4'}$ and $H_{4'}$-$H_{5'}$ (table 2) [13]. The stereochemistry of the molecule was proposed by the NOESY spectrum. The *nOe* correlations observed between H_8 and H_{11} as well as between H_8 and H_{13}, confirming the β-position of the methyl group at C_{11}. The absence of a *nOe* correlation between H_8 and H_9 is in concordance with the 8α (12), 9α *trans*-fusion of the lactone ring. Clear *nOe* correlation between H_5 and H_{15} confirmed that the methyl group at C_4 is β-oriented and the methyl group at C_3 is α-oriented.



Compound **2**

Table 2: ^{1}H, ^{13}C, HMBC and NOESY spectroscopic data of compound **2a**.

Position	^{1}H	^{13}C	HMBC	NOESY
1	-	83.5	H_2, H_5	
2	1.67 m	36.5	H_{15}	
3	-	82.3	H_2, $H_{1'}$, H_{15}	
4	-	82.6	H_5, H_{14}	
5	2.26 t (9.3)	58.7	H_2, H_7, $H_{10\beta}$, H_{14}	$H_{6\beta}$, H_2, H_{15}, H_{14}
6	1.56 (α) m	30.1		H_{15}
	1.81 (β) m			
7	1.58 m	23.2	H_5, $H_{6\beta}$	$H_{10\beta}$, H_{14}
8	2.70 dbr	39.8	$H_{6\alpha}$, H_{13}, $H_{10\alpha}$	H_{13}
9	4.20 m	80.7	$H_{10\beta}$, H_{11}, H_8	
10	1.73 (α) m	44.5		
	2.48 (β) dd (13.8, 5.4)			H_{14},H_7
11	2.60 q (7.8)	40.7	H_{13}	
12	-	180.0	H_{11}, H_{13}	
13	1.03 d (8.1)	10.4		
14	1.27 s	23.1		
15	1.21 s	23.3		$H_{1'}$
1’	4.66 d (7.8)	94.9	$H_{2'}$	
2’	4.90 t (9.6)	71.7		
3’	5.14 t (9.6)	73.2		
4’	4.95 t (9.6)	69.0	$H_{3'}$	
5’	3.61 ddd (9.6, 4.8, 2.4)	72.0	$H_{4'}$, $H_{3'}$	
6’a, b	4.07 (a)	62.5		
	4.13 (b)			

EXPERIMENT SECTION

1- General methods: ^{1}H (300 *MHz*), ^{13}C (75 *MHz*) and 2D NMR spectra of **1** were obtained in a mixture of $CDCl_3$ and CD_3OD with a Bruker NMR-300 spectrometer. ^{1}H (300 *MHz*), ^{13}C (75 *MHz*) and 2D NMR spectra of **2a** were recorded in $CDCl_3$ with a Bruker NMR-300 spectrometer. The residual solvent resonances were used as internal references. Coupling constants are given in Hertz. Mass spectra were obtained with an Automass Multi Thermo Finingam ES-MS spectrometer.

2- Plant material: *Pulicaria laciniata* (coss. et kral.) Tell. [14] was collected in the region of EL Hwareb (Kairouan, Tunisia) in april 2006. The plant was identified by Prof. Fethia HARZALLAH-SKHIRI in the Laboratoire de Biologie Végétale et Botanique, Institut Supérieur Agronomique de Chott merriem, Université de Sousse, Tunisia and a voucher specimen (PL-06) was deposited in the same laboratory.

3- Extraction and isolation: The dried and powdered flowers (1.1kg) of *P. laciniata* were extracted successively with dichloromethane and methanol at room temperature for six days. The corresponding extracts were obtained after filtration and evaporation of the solvents under reduced pressure.

The methanolic extract (80g) was dissolved in water then extracted successively with ethyl acetate and butanol to yield the corresponding extracts.

A total of 7.5g of the CH_2Cl_2 extract (60g) was subjected to column chromatography over silica gel eluted with (EP/CH_2Cl_2/Acetone) in the increasing order of polarities to afford 9 fractions.

Fraction 7 (0.66g) was chromatographed on silica gel eluting with ($CHCl_3$/MeOH) gradients to give eight subfractions, the fiveth of which was purified by column chromatography over silica gel using ethyl acetate as eluent to afford **1** (20mg).

The butanolic extract (14g) was subjected to silica gel column chromatography eluting with ($CHCl_3$/MeOH gradients). Ten main fractions were collected. Fraction 8 (103mg) was chromatographed on silica gel eluting with $CHCl_3$/MeOH (8:2) to give nine subfractions, the seventh of which was purified by preparative TLC $CHCl_3$/MeOH (75:25) to afford **2** (10mg).

Acetylation of 2: The crude polar compound **2** (9mg) was acetylated (Ac_2O/pyridine, room temperature, 12h) then chromatographed on silica gel eluting with $CHCl_3$/MeOH (9:1) to give 12 mg of the derivative **2a** as a white solid.

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