COMPLETA ASIGNACIÓN DE LOS ESPECTROS DE ^{1}H Y ^{13}C RMN DE DOS GUAYANOLIDAS CONOCIDAS AISLADAS DE *CENTAUREA MUSIMOMUM*

COMPLETE ASSIGNMENT OF THE ^{13}C AND ^{1}H NMR SPECTRA OF TWO KNOWN GUAIANOLIDES ISOLATED FROM CENTAUREA

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RESUMEN

Se complementan los datos espectroscópicos de dos guayanolidas conocidas aisladas de *C. musimomum* por ^{1}H, ^{13}C y 2D RMN (HSQC, HMBC, ^{1}H-^{1}H COSY).

Palabras clave: C*entaurea musimomum*, guayanolidas, ^{1}H RMN, ^{13}C RMN, 2D RMN.

ABSTRACT

Complementary ^{1}H, ^{13}C and 2D NMR (HSQC, HMBC, ^{1}H-^{1}H COSY) spectroscopic data are provided for two known guaianolides.

Key words: C*entaurea musimomum*, guaianolides, ^{1}H RMN, ^{13}C RMN, 2D RMN.

INTRODUCTION

Sesquiterpene lactones are part of a biologically active group of substances found in several plant families, most notably the Compositae which have yielded over 3000 structures. Their biological activity is increasingly appreciated – a recent detailed studies (1,2) have singled out several lactones with useful anthelmintic, antibiotic, allergenic and, above all, cytotoxic properties. One particular series of lactones with marked inhibitory action on the cell growth of many types of tumour (3) has been identified from among the antineoplastic agents obtained from plants (4).

Sesquiterpene lactones irritate the nose, eyes and gastrointestinal tract. Sheep and goats are the main livestock

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species affected (5), primarily because the plants are unpalatable and therefore rarely consumed in toxic quantities by cattle and horses. The toxicity of sesquiterpene lactones is due to the binding of the exocyclic methylene group with tissue constituents, such as sulphydril groups and other nucleophilic components (6).

One and two-dimensional NMR data are given for two known guaianolides from Centaurea *musimomum* (7,8) to supplement the information already available for these two compounds.

EXPERIMENTAL

General Experimental Procedures

^{1}H and ^{13}C NMR spectra were taken on a Bruker AMX-500 spectrometer with standard pulse sequences, operating at 500 MHz for ^{1}H and 125 MHz for ^{13}C. $CDCL_3$

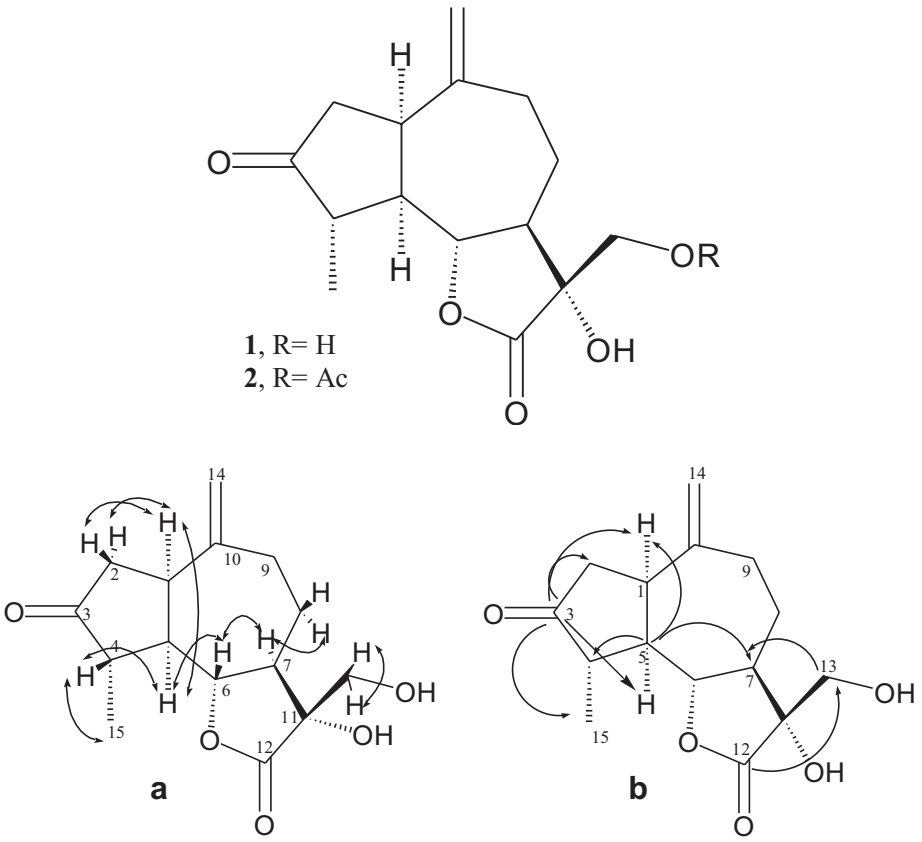

Figure 1. Correlations for compound **1, a)** ^{1}H-^{1}H COSY; **b)** HMBC (arrows ^{13}C-^{1}H).

was used as solvent and TMS as internal standard.

Plant Material

Centaurea musimomum was collected in the Souk-Ahras area in the East of Algeria in June 1992. It was identified by Dr Nadra Khalfallah and deposited in the herbarium of Research Unit of Chemistry, University of Constantine.

Extraction and Isolation

Air-dried powdered aerial parts (150 g) were soaked in MeOH (2 L). The MeOH extract was evaporated and the residue dissolved in H_2O (100 mL). The solvent was treated with Pb(OAc). After 3 min centrifugation, the mixture was filtered and the yellow-coloured filtrate extracted with $CHCl_3$ (3 × 50 ml). The extract was concentred in vacuo at room temp, the residue weighing 6 g. A part of this residue (3 g) was separated by CC (silica ge1 230-400 mesh) using solvent mixtures ($CHCl_3$- Me_2CO) with increasing polarities. The fraction was divided into nine crude ones. Fractions 3 (210 mg) and 8 (180 mg) were studied. Fraction 3 on rechromatography on silica gel using solvent mixtures (hexane- Et_2O-Me_2CO) with increasing polarities gave **2** (70.2 mg) and fraction 8 on rechromatography on a silica gel column with $CHCl_3$-hexane Et OAc, 1:1:1 gave **1** (16.1 mg).

Spectral data for compounds 1 and 2:

4β,15-Dihydro-3-dehydrosolstitialin A **(1).** Crystals, mp (154-156°); $[\alpha]_D^{25}$ +145 ($CHCL_3$, C 0.04); IR ν_{max} 3400, 1770, 1730,and 1640 cm^{-1}; EI-MS: 280 $[M]^+$ ($C_{15}H_{20}O_5$) ^1H and ^{13}C NMR data, see Table 1.

4β,15-Dihydro-3-dehydrosolstitialin A monoacetate **(2)[9].** Crystals, mp (143-144°); $[\alpha]_D^{25}$ +125 ($CHCL_3$, c 0.1); IR ν_{max} 3410, 1770, 1720, and 1640 cm^{-1}; EI-MS: 322 $[M]^+$ ($C_{17}H_{22}O_6$) ^1H and ^{13}C NMR data, see Table 1.

RESULTS AND DISCUSSION

The ^{13}C NMR, DEPT and mass spectra of **1** and **2** showed 15 and 17 carbon signals, respectively, indicating molecular formula $C_{15}H_{20}O_5$ and $C_{17}H_{22}O_6$.

Complete ^1H and ^{13}C connectivity was established by extensive use and interpretation of 2D (^1H-^1H) COSY, HSQC (one bond ^{13}C-^1H correlation) and HMBC (long-range ^{13}C-^1H correlations) NMR spectra (Table 1)

The arrangement of the functional groups and the full structures of **1** and **2** were attributed from the ^1H-^1H COSY spectrum of **1** in which the following $\delta_{H/H}$ (ppm) correlations were observed (Figure 1): H-1α (3.09) with H-2β (2.52), H-2α (2.56) and H-5α (2.28); H-4β (2.32) with CH_3-15α (1.26) and H-5α; H-5α with H-1α and H-6β (4.15); H-6β with H-5α, H-7α (2.63); H-7α with H-8α (1.56); H-13a (3.77) with H-13b (3.83).

The functional groups in **1** were confirmed by HMBC data (Figure 1), the important correlations being between C-3 (δ 219.74) and H-1α, H-5α, H_2-2; between C-5 (δ 51.40) and H-1α, H-4β, H-7α,

Table 1. ^{1}H and ^{13}C NMR chemical shifts for **1** and **2** including ^{13}C-^{1}H Long-Range correlations[a,b]

1				**2**		
Position	**δC**	**δH (mult.)**	**HMBC (C→H)**	**δC**	**δH (mult.)**	**HMBC (C→H)**
1α	39.62	3.09ddd (8.4, 7.8, 3.6)	H_2-2, H-5, H_2-14	39.52	3.04 ddd (8.3, 7.8, 3.4)	H_2-2, H-5, H_2-14
2α	43.86	2.56 dd (19, 7.8)	H-1, H-4	43.72	2.54 dd (19, 7.8)	H-1, H-4, H-5
2β		2.52 dd (19, 3.6)			2.49 dd (19, 3.4)	
3	219.74		H_2-2, H-4, H-5, Me-15	219.09		H-1, H_2-2, H-4, H-5, Me-15
4β	47.08	2.32 m	H_2-2, H-6, Me-15	47.06	2.30 m	H-6, H_2-8, Me-15
5α	51.40	2.28 t, br (8.5)	H-1, H-4, H-7, Me-15	51.18	2.20 br t (8.3)	H-1, H-4, H-7, Me-15
6β	87.20	4.15dd (9.7, 8.9)	H-7, H_2-8	86.77	4.05 dd (10, 8.3)	H-5, H-7, H_2-8
7α	49.40	2.63 m	H-6, H_2-8, H_2-13	49.46	2.61 m	H-6, H_2-8, H_2-13
8 ax	26.85	1.56 dq (12.7, 5.1)	H-6, H_2-9, H_2-14	27.08	1.40 m	H-6, H_2-9, H_2-14
8 eq		2.22 m			2.19 m	
9 ax	38.56	2.11 m	H-1, H-7, H_2-8, H_2-14	38.31	2.09 m	H-1, H-7, H_2-8, H_2-14
9 eq		2.65 m			2.57 m	
10	148.90		H-1, H-5, H_2-8, H_2-9, H_2-14	148.74		H-1, H_2-8, H_2-9, H_2-14
11	77.20		H-7, H_2-13	75.49		H-7, H_2-13
12	178.80		H_2-13	176.23		
13a	63.12	3.77 d (11.2)	H-7	63.99	4.29 d (11.5)	H-7
13b		3.83 d (11.2)			4.11 d (11.5)	
14a	112.48	4.68 s	H-1, H_2-9	112.68	4.93 d (3.7)	H-1, H_2-9
14b		4.99 s			4.62 d (3.7)	
15	13.89	1.26 d (7.1)	H-4, H-5	13.97	1.20 d (6.8)	H-4, H-5
C=O				169.19		H_2-13, Me-17,
Actyl-Me				20.52	2.05 s	

a Assignments confirmed by decoupling, ^{1}H-^{1}H COSY, HSQC and HMBC spectra.
b J values are given in Hz.

H_3-15; between C-11 (δ 77.20) and H_2-13, H-7α; between C-12 and H_2-13; and between C-13 (63.12) and H-7α. We definitively determined the structure of **1** by X-ray analysis (7).

These signals show certain similarities to those of the monoacetyl derivative **2** (Table 1), the structure of which was obtained on the basis of its 2D NMR spectrum. Compound **2** was previously described by Herz *et al*. (8,9) on the basis of ^{1}H and ^{13}C NMR spectra and X ray analysis. We observed that the assignments of C-1 and C-4 given by those authors were interchanged with respect to those found by us for compound **2.** HMBC spectrum (Figure 2) shown important correlations between C-1 (δ 39,52) and H_2-14, between C-4 (δ 47,06) and H_3-15. (Figure 2).

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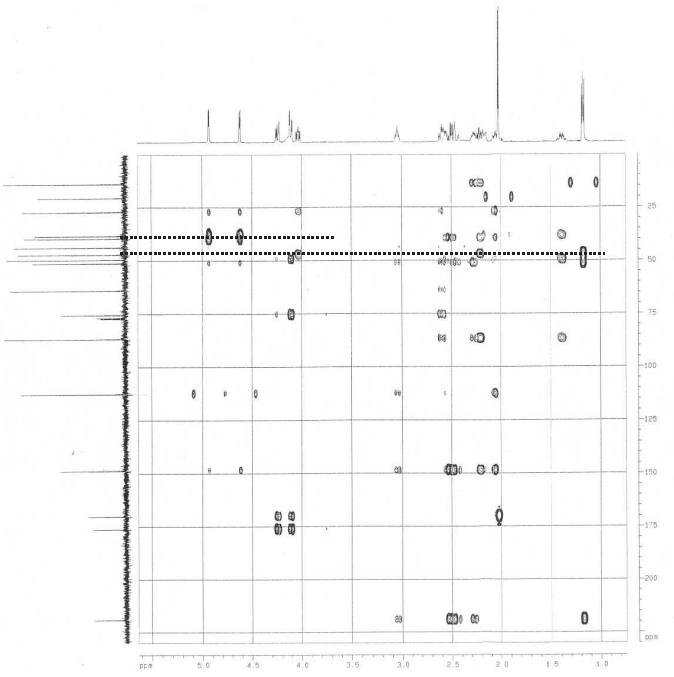

Figure 2. HMBC (long-range ^{13}C-^{1}H correlations) spectrum of compound **2**.

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