ANTI-MICROBIAL ACTIVITY OF THE ALKALOID EXTRACT OF *Genista microcephala*: ISOLATION AND COMPLETE ^{1}H AND ^{13}C CHEMICAL SHIFTS ASSIGNMENTS OF LUPANINE AND S-CALYCOTOMINE

AMAR ZELLAGUI[1], SALAH RHOUATI[1], JOEL CRECHE[2] ,GÀBOR TÓTH[3], AHMED A. AHMED[4] and PAUL W. PARÉ[5]

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ABSTRACT

A chemical investigation of the aerial parts of *Genista microcephala* Coss et Dur, afforded two alkaloids, identified as lupanine (2-oxosparteine) **1** and S-calycotomine **2**. The complete ^{1}H and ^{13}C chemical shifts assignments of Lupanine and S-Calycotomine were determined using 1D and 2D NMR spectroscopy (COSY, DEPT, HMQC, HMBC).The alkaloid extract of *Genista microcephala* was tested against bacteria and fungi strains, which showed a strong sensitivity. The MIC and MBC of the alkaloid extract were determined.

Key words: *Genista microcephala*, Fabaceae, quinolizidine alkaloids, anti-microbial activity.

RESUMEN

La investigación química de las partes aéreas de *Genista microcephala* Coss et Dur, dio como resultado la identificación de dos alcaloides, lupanina (2-oxosparteina) **1** and S-calycotomina **2**. La asignación completa de los desplazamientos químicos de ^{1}H y ^{13}C, se llevo a cabo utilizando espectroscopia de RMN monodimensional (1D) y bidimensional (2D) (COSY, DEPT, HMQC y HMBC).El extracto alcaloideo mostró un fuerte actividad inhibitoria contra hongos y bacterias. Se determinaron los CMI y CMB del extracto alcaloideo.

[1]Laboratory of Natural Product and Organic Synthesis, Department of Chemistry, Faculty of Science, University Mentouri–Constantine, Algeria. [2]EA 2106 Biomolecules et Biotechnologie végétales, Faculté des Sciences Pharmaceutiques, Université de tours. [3]Technical Analytical Research Group of the Hungarian Academy of Sciences, Budapest, University of Technology and Economics, H-111, Budapest, Hungary, [4]Department of Chemistry, Faculty of Science, El-Minia University, El-Minia 61519, Egypt, [5]Department of Chemistry and Biochemistry, Texas Tech University, Lubbock, TX 79409-1061

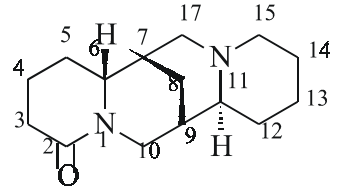
INTRODUCTION

Quinolizidine alkaloids, the largest single group of legume alkaloids, are characteristic secondary metabolites in the family Fabaceae (Kinghorn *et al.* 1984, Pistilli *et al.* 2001). They appear to be restricted in distribution to leguminous plants especially to the tribe Genisteae (Shutte 1969, Southon *et al.* 1994, Wink *et al.* 1994). Quinolizidine alkaloids have proved to have an important role as herbivore repellents, and inhibit the growth of various bacteria and fungi providing protection to the plants from herbivores such as insects and grazing mammals (Waller *et al.* 1978, Tosun *et al.* 1995).

Genista microcephala Coss et Dur. is an endemic plant from north Africa, growing in east Algeria and flourish from May to July (Quezel *et al.* 1962). In the present work we described the isolation and identification of two alkaloids from *G. microcephala*. The total assignments of the signals in the ^{1}H and ^{13}C NMR spectra to 2-oxosparteine and S-calycotomine were determined using 2D NMR (COSY, DEPT, HMQC and HMBC) experiments. Moreover, the alkaloid extract was tested against bacteria and fungi, obtaining significant results. To the best of our knowledge, the species described here has not been previously studied chemically.

RESULTS AND DISCUSSION

Fractionation of the methanol extract of the aerial parts of *G. microcephala* gave two known alkaloids, namely lupanine (2-oxosparteine) **1** and S-calycotomine **2** (Tosun *et al.* 1987a, Tosun *et al.* 1994, Tosun *et al.* 1987b, Kerekes *et al.* 1985, , Pistilli *et al.* 2001)**.** The structure of the isolated compounds were identified using spectroscopic methods and by comparison of their physical and spectroscopic data with literature values. The complete assignments of all protons and carbons were carried out using 2D NMR (COSY, DEPT, HMQC and HMBC) experiments (tables 1 and 2).The total assignment of the signals in the ^{1}H NMR spectra of 2-oxospartein have been published previously, (Golebiewski, 1986, Kolanos *et al* 2003), but there were some differences with our results.



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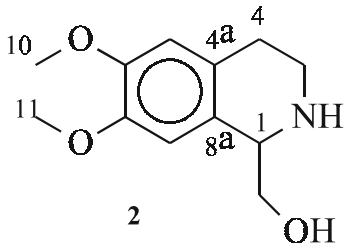

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Chemical structures of the isolated compounds.

Table 1: NMR data of compound **1** ($CDCl_3$, TMS, as int. standard)

position	^{13}C (ppm)	^{1}H (ppm)	m,*J* (Hz)	COSY	HMBC	NOESY
2	171.9	-				
3α	32.9	2.335	td; 13.5, 5.4		171.9	
β		2.54	d; 13.5		171.9, 19.2	
4α	19.2	1.89	d; 13.2			3.38, 2.55, 2.36
β		1.67	qdd: 13.2 , 4.2 , 3.0	2.55	60.6, 32.9, 27.4	3.38, 2.35
5α	27.4	1.38	qd; 13.2; 2.6	3.38		
β		1.90	d; 11.5			
6β	60.6	3.38	dd; 11.5, 4.8	1.90, 1.38	171.9, 51.4, 30.8, 27.4, 25.9	2.55, 2.37, 1.90, 1.67, 1.55, 1.38
7α	30.8	2.37	d; 4.5			
8a	25.9	1.55	d;14.2	1.97	65.2, 60.6, 51.4, 46.1, 33.5, 30.8	
b		2.96	d;13.0			
9α	33.5	1.97	s; broad	4.58, 2.56, 1.55		4.58, 2.96, 2.55, 2.42, 1.82, 1.55
10α	46.1	4.58	dt; 13.5, 2.2	1.97	171.9, 65.2, 60.6, 33.5, 25.9	2.43, 1.97, 1.82 (w)
β		2.55	d; 13.5		171.9	
11α	65.2	2.43	d; 5.0			
12α	31.3	1.82	d; 13.5	1.46		4.58 (w), 2.42 , 1.97, 1.46
β		2.07	dd; 13.8, 2.6	-	-	-
13α	22.9	1.46	qt; 13.3, 3.8	1.82, 1.76	31.3	2.42, 1.82, 1.76
β		1.90	d; 13.3			
14α	22.7	1.76	d;14.5	1.46	-	3.44, 2.49, 1.46
β		2.40	d; 14.5			
15α	55.7	2.48	m			
β		3.44	d; 10.6		22.9	1.76
17α	51.4	2.36	d; 11.6			
β		3.49	t; 11.6		65.2, 60.6, 30.8	-

The antibacterial and anti-fungal activity of the alkaloid extract (Tables 3 and 4) showed a strong sensitivity for various bacteria and fungi, and the MIC (minimum inhibitory concentration) and MBC (minimum bactericide concentration) were determined and the results are shown in Table 5.

CHEMOTAXONOMIC SIGNIFICANCE

The isolation of lupanine and S-calycotomine from *Genista microcephala*, previously reported from *G. burdurensis* (Tosun *et al.* 1987a), *G. albida* and *G. involucrata* (Tosun *et al.* 1987b), supports a close relationship among these species. Moreover our results confirm the chemotaxonomic significance of these alkaloids in the *Genista* genus.

Table 2: NMR data of compound **2** ($CDCl_3$, TMS, as int. standard)

position	^{13}C (ppm)	^{1}H (ppm)	m, *J* (Hz)	COSY	HMBC
1	56.5	4.46	m	4.04, 3.83	-
3α	38.9	3.25	m	2.93	125.0, 56.5, 25.9
3β		3.39	m	2.93	125.0, 56.5, 25.9
4 α	25.9	2.93	m	3.39, 3.25	125.0, 121.8, 111.5-6, 38.9
4 β		2.93	m	3.39, 3.25	125.0, 121.8, 111.5, 38.9
4a	125.0	-			
5	111.5	6.57	s	-	148.1, 121.8, 25.9
6	148.6	-			-
7	148.1	-			
8	109.2	6.63		2.93	148.6, 125.0, 56.5
8a	121.8	-	s		
9a	62.9	3.83	dd, 11.5,3.8	4.46	148.1
9b		4.04	d; 11.5	4.46	-
10	55.8/56.1	3.81		-	148.6
11	55.8/56.1	3.88		-	148.1

Table3. Medium zone of inhibition fromalkaloid extract at 100 mg/L concentrationin gelose nutritive medium

Strain bacteria	medium diameter
E . coli	1,95
Staphylococcus blanc	1,45
Pseudomonas aeruginosa	1,60
Enterobacter Sp.	1,05
Proteus merabilis	1,15
Serratia Sp	1,05
Proteus vulgaris	1,15

Table 4. Strain fungi sensitivity from alkaloid extract at 100-mg/L in sabouraud medium

Strain fungi	
Aspergilus fumigatus	-
Gliocladium Sp	-
Rhizoctomnia solani	+
Alternaria alternata	+
Aspergilus remosus	+
Rhizopus Sp	+

+ important growth ,- no growth.

EXPERIMENTAL

General

The following adsorbents were used for separation and purification: silica gel 60 (0.040-0.063mm) (1230-400 mesh ASTM) Merk, Sephadex LH-20; analytical TLC (silica gel GF254). The TLC plates were visualized under UV light at 254 and 365 nm and sprayed with dragendroff reagent. The NMR spectra were recorded on Brucker Avance DRX-500 spectrometer at 500 MHz for ^{1}H NMR and 125 MHz for ^{13}C NMR. The CIMS mass spectra were recorded on TSQ-70-Triple Stage Quadrupole mass spectrometer (70 eV).

Plant Material. The aerial parts of *G. microcephala* Coss et Dur. were collected in June 2000, at Chillia, Khenchla, Algeria, and were identified by Prof. Dr. M. Kaabache, Department of Biology, Faculty of Science, University of Ferhat Abbass, Setif, Algeria. A voucher specimen (RS/71) was deposited at the Chemistry Department, University of Mentouri-Constantine, Algeria .

Extraction. Air dried aerial parts of *G. microcephala* Coss et Dur. (1.35 Kg) were extracted in a soxhlet apparatus with MeOH (80%). The concentrated MeOH extract was dissolved in HCl 2% aqueous solution, and extracted with CH_2Cl_2 (three times). The acidic solution was brought to PH 9 with NH_4OH 25%, and extracted again with CH_2Cl_2 (four times) according to Sagen et al. (2002). The CH_2Cl_2 extracts were evaporated to give the crude alkaloids (10.85 g).

Isolation of lupanine (2-oxosparteine) 1 and S-calycotomine 2.

The crude alkaloid extract was chromatographed on a silica gel open column (CC) using CH_2Cl_2-MeOH-NH_4OH (85:14:1) as mobile phase to obtain five primary fractions. Fraction H (2.4 g) was re-chromatographed on a silica gel column eluted with ethyl acetate-acetone-NH_4OH (2:2:1) and followed by Sephadex LH-20 column to obtain compound **1**. Fraction (O) (2.84 g) was also re-chromatographed on a silica gel column and eluted with ethyl acetate-acetone-NH_4OH (2:2:1) to give three fractions. The first one (O1) fraction was separated by TLC using CH_2Cl_2-MeOH-NH_4OH 25 % (85:14:1) to obtain compound **2**.

The anti-microbial activity tests were carried out on the alkaloid extract of *Genista microcephala* using the disk diffusion method (Carbonnelle *et al*. 1987).

Lupanine (2-oxoparteine**) 1.** reddish oil. $[\alpha]^{25}_{D}$ +95.0° (*c* 0.3, $CHCl_3$); CIMS : 249. (100).

S-Calycotomine **2**, yellowish oil. $[\alpha]^{25}_{D}$ +25.0° (*c* 0.3, $CHCl_3$); CIMS: 224 (100).

Table 5, MIC and MBC from alkaloid extract.

strain bacteria	MBC mg/mL	MIC mg/mL
Escherichia Coli	8	4
Staphylococcus blanc	16	4
Pseudomonas aeruginosa	16	8
Enterobacter Sp.	8	2
Proteus merabilis	8	2
Proteus vulgaris	16	8
Serratia Sp.	8	8
Sanetta Sp.	8	2

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