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Triterpenoid saponins from the aerial part of *Gladiolus segetum L.*

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ABSTRACT

A New triterpenoïd was isolated from the aerial parts of *Gladiolus segetum*. Its structure was elucidated on the basis of ESI-MS, NMR, and chemical methods.

KEYWORDS

Saponin;
Gladiolus segetum L.;
Iridaceae;
Triterpenoid.

INTRODUCTION

Gladiolus segetum is a toxic plant[1], lethal for livestock, growing in wheat fields and cultures.

It is well spread in highlands in Algeria, in the Batna region. Former studies (Wasicky and Hoehne, 1951)[2] reported the presence of saponins in different cultivars of gladiolus, 6.38% in leaves and 8.68% in dried bulbs. Saponins were also mentioned in gladiolus in a paper of Chernov and Lytkina[3] who tested their toxic activities on tumors. In 1962, Stanislas and

Galaud[4-5] proved that saponins are well responsible of the toxic effects. In 2000 a medicagenic acid is isolated by M.H.Mohamed and A.M. Nafady[6]. The aim of our study is to elucidate the structure of the toxic compounds responsible of the lethal effect of this plant. From the methanolic extract, we obtained several new triterpenoid saponins with more than four sugar units. Herein, we describe the isolation and structure elucidation of a new saponin, which contains five sugar units. We performed an extractive protocol on 153,22g of dried plant materiel (Seeds and Flowers) of *Gladiolus segetum*. The plant material was subjected to subsequent extraction by hexane, chloroform, ethyl acetate and methanol. The methanol extract, after filtering, was concentrated in vacuum at 35^{0}C to yield 29,50 g of the crud extract. The crud extract was dissolved in 100 ml of chloroform- methanol-water (43:37:20) mixture and filtered through a 0.2µm filter. The mixture solution was subjected to a droplet counter current chromatography (DCCC). One hundred and forty fractions with a volume of 10 ml each were collected and analysed by thin layer chromatography (TLC). All the fractions containing similar compound were combined then concentrated. Five fractions were obtained. The fourth fraction was concentrated and subjected to a low- pressure liquid chromatography (BPLC) to afford 115 fractions with 10ml of volume each. Fraction containing compound **1(17-25)** were pooled and purified using repeated preparative HPLC to give (15mg) of this compound.

Saponin 1 was obtained as a white amorphous powder. The positive and negative ESI-MS showed

$[M+Na]^+$ and $[M+Cl]^-$ at m/z 1125 and 1137, consistent with the molecular formula $C_{54}H_{86}O_{23}$. NMR data (TABLE 1) were characteristic of triterpenoid saponin with four sugar units. After acidic hydrolysis, D-glucuronique, D-xylose, D arabinose and L-rhamnose were identified with authentic samples. The 1H-

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TABLE 1: ^{13}C and 1H NMR data of compound 1 in pyridine-d5 (δppm)

δH	δC	Carbon	δH	δC	Carbon
	Sugar at C-3			Aglycone	
4.81	107,4	Glc-A1		39,9	1
3.99	76.0	2		69,3	2
4.25	79.3	3	3.31	89.9	3
4.48	73.4	4		40.2	4
4.35	76,8	5	0.78	56.1	5
-	170.0	6		18.5	6
		Sugar at C-28		33.6	7
6.48	95,8	Ara-1		39,3	8
4.55	76,0	2	1.40	47.6	9
4.35	69,7	3		37.6	10
4.37	66.2	4		24.9	11
3.99 4,52	63,2	5	5.65	124.0	12
5.65	102,0	Rha-1		144,8	13
4.52	72.6	2		41,1	14
4.48	73,1	3		36.1	15
4.32	84,5	4	5.25	74.5	16
4.35	69.5	5		50.4	17
1.68	19.2	6		49.1	18
5.12	107,7	Xyl-1		47.2	19
4,02	76.8	2		30.8	20
4.08	79.0	3		36.1	21
4.15	71.8	4		32,2	22
δH	**δC**	**Carbon**	**δH**	**δC**	**Carbon**
3.48 4.10	68,2	5	1.23	180.7	23
			1.01	17.8	24
			0.83	15,0	25
			1.07	18.4	26
			1.79	28.3	27
				176.1	28
			0.95	33,8	29
			1.14	24,1	30

NMR spectrum showed the presence of six tertiary methyl groups (H 1.79, 1.04, 1.00, 0.94, 0.88, 086), five anomeric protons δ(6.48 (s1), 5.65 (s), 5.15 (d, j= 7.6 Hz) et 4.81(d, j=7.5Hz) and one trisubstituted olefinic proton (d H 5.65). Additionally, the 13C-NMR spectra displayed six methyl groups, five anomeric carbons, and two olefinic carbons. A detailed comparison between 1 and the reference data of medicagenic acid[7] a major aglycone present in this plant, implied that 1 possessed of the aglycone of medicagenic acid an extra secondary hydroxy function which was located at

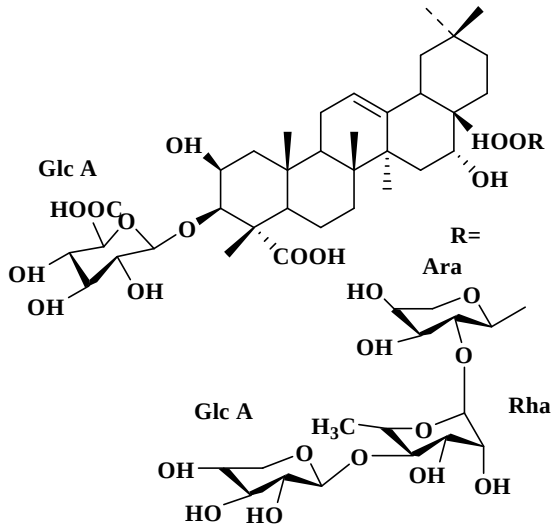

Figure 1: Structure of isolated triterpenoid glycosides

C_{16} based on absence of normal resonance of C_{16} and downfield shift of C_{15} compared with 1. Complete assignments of each glycosidic proton system were achieved by analysis of 2D NMR experiments (TOCSY, HSQC and HMBC). Evaluation of spin-spin couplings and chemical shifts allowed the identification of one β-D-glucopyranosyl unit, one α-L-rhamnopyranosyl unit, one β- Glucuronopyranosyl unit one α -L-arababinofuranosyl unit, and one β-Dxylopyranosyl unit. The sugar sequences of the oligosaccharide chains as well as the glycosidic sites were subsequently determined by a combination of HSQC and HMBC experiments, a δH-1 6.48

(1,2-Ara) and δC-28 176.1 of medicagenic acid, an δH-1 5.65 (1,3,4-Rha) and δC-2 76,0 (1.2- Ara), a δH-1 5.12 (1-Xyl) and δC-4 84.5 (1,4-Rha), a δH-1 4,81 (T-Glc) and δC-3 89,9 of medicagenic acid. In the HMBC spectrum of 1, the correlations could be achieved between

the four anomeric proton: δ6.48 (s1), 5.65 (s), 5.12 (d, j= 7.6 Hz) et 4.81(d, j=7.5Hz)and the four anomeric carbon δ95,8, 102,0, 107.7, and 107.4 respectively.

All the data assigned that saponin 1 is as 16α hydroxy, 3-*O*-β-D-glucopyranosyl medicagenic acid-28-O- β-D-xylopyranosyl(1?4)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside (Figure 1).

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