Isolation of a New Steroidal Glycoalkaloid from *Solanum xanthocarpum*

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Abstract: A new steroidal glycoalkaloid was isolated from barries of *Solanum xanthocarpum,* together with two other known steroidal glycoalkaloids. The structure of new steroidal glycoalkaloid was elucidated as *O*-(3){ α-L-rhamnopyranosyl-(1→2_{gal})- β-D- galactopyranosyl}-solasodine on the basis of spectroscopic analysis as well as comparison with reported spectroscopic data of related compounds.

Keywords: *Solanum xanthocarpum,* steroidal glycoalkaloid, *O*-(3) {α-L-rhamnopyranosyl-(1→2_{gal})-β-D-galactopyranosyl}-solasodine.

1. INTRODUCTION

Genus *Solanum* belongs to the family Solanaceae and comprises more than 1700 species, found in the tropical and temprate zones. Some of these plants have long been used as vegetables and medicinal agents. In Pakistan, only 15 species have so far been identified [1]. *Solanum xanthocarpum* Schard & Wendl is a traditional medicine. The plant has been reported to possess antispasmodic, cardiotonic, hypotensive, antitumor, antianaphylactic and cytotoxic activities [2-4]. It is also useful in asthma, cough, fever, enlargement of liver, spleen, controlling stones in bladder and pain in chest [5]. Phytochemical studies of this plant showed that sterols, alkaloids, glycosides have been isolated from the plant. Solasodine, *β*-sitosterol, diosgenin [6, 7], triterpenes [8], steroidal saponins [9] and steroidal glycoalkaloids [10-12] are also reported from the fruit. The pharmacological research showed that plant and its constituents have antifungal [13], anticancer [14], hypoglycemic [15], hepatoprotective [16], antiviral, anti-oxidant and anti-microbial [17, 18] activities.

Keeping into account the pharmacological and phytochemical importance of *Solanum xanthocarpum,* the barries of *Solanum xanthocarpum* from Pakistan have recently been studied and found to contain a new steroidal glycoalkaloid *Solaxanine* (**1**) *O*-(3){α-L-rhamnopyranosyl-(1→2_{gal})- β-D- galactopyranosyl}-solasodine which yielded solasodine as aglycone and galactose and rhamnose as sugar moieties.

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2. EXPERIMENTAL

2.1. Plant Material

*Solanum xanthocarpum*was collected from Karachi, Pakistan and the plant material was identified by herbarium, Department of Botany, University of Karachi, Pakistan.

2.2. Extraction and Isolation

The air dried barries of *Solanum xanthocarpum* (2kg) were crushed and soaked in 95% ethanol at room temperature for 15 days. The ethanolic extract was concentrated under vacuum below 50^0C to give a dark brown residue (74g). After that barries were again air dried and then soaked in 5% acetic acid (pH=3) at room temperature for 3 days. The acidic aqueous fraction was filtered and extracted 3-times (3L each) with chloroform to get a chloroform soluble fraction and concentrated under vacuum below 50^0C to give a brown residue (3.1g).

The $CHCl_3$ extract of barries (3.1g) was subjected to Si-gel column chromatography using hexane, $CHCl_3$ and MeOH mixtures by increasing polarities. The fractions were combined by monitoring with TLC and these collective fractions were individually subjected to further column chromatography to furnish various compounds in pure state. The fraction eluted with $CHCl_3$/MeOH 7.0:3.0, after further CC separation gave compound **1** (40mg) with $CHCl_3$/MeOH 7.5:2.5.

Solaxanine **1** a pale yellow crystalline solid, IR spectrum showed bands (KBr) 3430 cm^{-1} (OH), 1626 cm^{-1} (C=C). ^{1}H NMR and ^{13}C- NMR spectra; see Table **1**. Negative FAB-MS; *m/z* 721 (Calculated 721.4401 for $C_{39}H_{63}NO_{11}$), EIMS *m/z* 413 $(M\text{-}C_{12}H_{20}O_9)^+$, 397 $(M\text{-}C_{12}H_{20}O_{10})^+$, 138 $(M\text{-}C_{30}H_{47}O_{11})^+$, 114$(M\text{-}C_{33}H_{51}O_{10})^+$.

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Table 1: ^{1}H NMR and ^{13}C NMR Data for *Solaxanine* (1)

Position	δ_H (300MHz, C_5D_5N)	δ_C (100.58MHz, $CDCl_3$)
1	0.98m, 1.80m	37.10
2	1.77m, 1.51m	30.7
3	3.85m	70.65
4	1.69d (J,6.0Hz)	39.02
5		140.21
6	5.28brs	121.53
7	1.67m, 1.90m	32.15
8	1.24m	32.17
9	0.91m	49.80
10		36.66
11	1.45m, 1.35m	20.61
12	2.18m, 2.25m	38.50
13		40.78
14	1.06m	56.08
15	1.89m, 1.42m	31.76
16	3.48m	78.87
17	2.03m	61.25
18	0.72s	16.01
19	0.91s	19.20
20	2.14m	41.70
21	1.18s	17.15
22		98.64
23	1.49m	29.50
24	1.82m, 1.50m	29.38
25	1.51m	31.32
26	2.69d (J,10.2Hz)	45.38
27	0.87d (J,6.0Hz)	18.29
1′	4.95d (J,7.8Hz)	101.47
2′	3.45m	75.16
3′	4.59m	83.49
4′	3.59m	70.77
5′	3.22m	74.70
6′	3.72d (J,9.3Hz)	60.64
1″	4.26d (J,1.4Hz)	100.78
2″	3.18m	73.18
3″	3.35m	72.22
4″	3.81m	70.65
5″	3.78m	69.42
6″	1.14d(J,6.0Hz)	14.57

3. RESULTS AND DISCUSSION

Silica gel chromatography of $CHCl_3$ fraction obtained from aqueous acidic extract of barries yielded a new crystalline compound *Solaxanine* 1($C_{39}H_{63}NO_{11}$), was positive to Dragendorff's reagent. The IR spectrum showed the presence of double bond and hydroxyl group. The sugars were identified after acidic hydrolysis as β-D- galactose and α-L- rhamnose by Co-TLC with authentic samples.

In the FAB Mass spectrum (Negative mode) the peak of highest mass number was observed at *m/z* 720 (M-H) (30), corresponds to the molecular formula $C_{39}H_{63}NO_{11}$ (Calculated 721.4401 for $C_{39}H_{63}NO_{11}$). Other fragments appeared at *m/z* 574(M-H-$C_6H_{10}O_4$) (10), m/z 412(M-$C_{12}H_{20}O_9$-H) (18). The EIMS of **1** showed no molecular ion but a peak at *m/z* 413 due to the loss of two sugar units ($C_{12}H_{20}O_9$) Figure **1**, 397 due to the loss of $C_{12}H_{20}O_{10}$, with typical ions at *m/z* 138 and 114 were due to the losses of $C_{30}H_{47}O_{11}$ and $C_{33}H_{51}O_{10}$ respectively which are characteristic of a solasodine skeleton [19]. The peaks appeared in EIMS are explained in Figure **2** which indicate the sequential loss of two sugars from solaxanine.

The ^{13}C-NMR (Broad band and DEPT) spectrum indicated the presence of thirty nine carbons including nineteen methine (CH), eleven methylene (CH_2), five methyl (CH_3) and four quaternary carbons. Full assignments of the proton and carbon signals of new compound **1** are based on the analysis of 1D NMR (^{1}H NMR, BB and DEPT) and 2D NMR (COSY, HSQC, HMBC) spectral data and showed in Table **1**.

In ^{13}C-NMR two kinds of anomeric protons appeared at δ 100.78 and δ101.47.In this latter spectrum six carbon signals at δ101.47, 75.16, 83.49, 70.77, 74.70, 60.64 were reasonably assigned to galactosyl carbons, attached at the 3β-hydroxyl group of solasodine, by comparison with the chemical shift values of galactosyl carbons of solasonineand solalyratine A and solalyratine B. Six carbon signals at δ 100.78, 73.18, 72.22, 70.65, 69.42, 14.57 were assigned to the rhamnosyl carbons by comparison with the chemical shift values of rhamnosyl carbons of salasonine [20, 21] and indiosides A-E [22]

The ^{1}H NMR (In pyridine Table **1**) spectrum showed the presence of two anomeric protons at δ 4.95 (d, J=7.8Hz) and 4.26 (d, J=1.4Hz). The ^{1}H NMR of compound **1** also showed two singlets of H-18, H-19 resonated at δ 0.72 and δ 0.91 respectively. Three doublets at δ 1.69 (J=6.0Hz), δ 2.69 (J=10.2 Hz) and at δ 0.87 (J=6.0 Hz) were due to H-4, H-26 and H-27. Other doublets appeared at δ 3.72(J=9.3Hz) due to H-6′ of galactose and at δ 1.14 (J=6.0Hz) due to H-6″ of

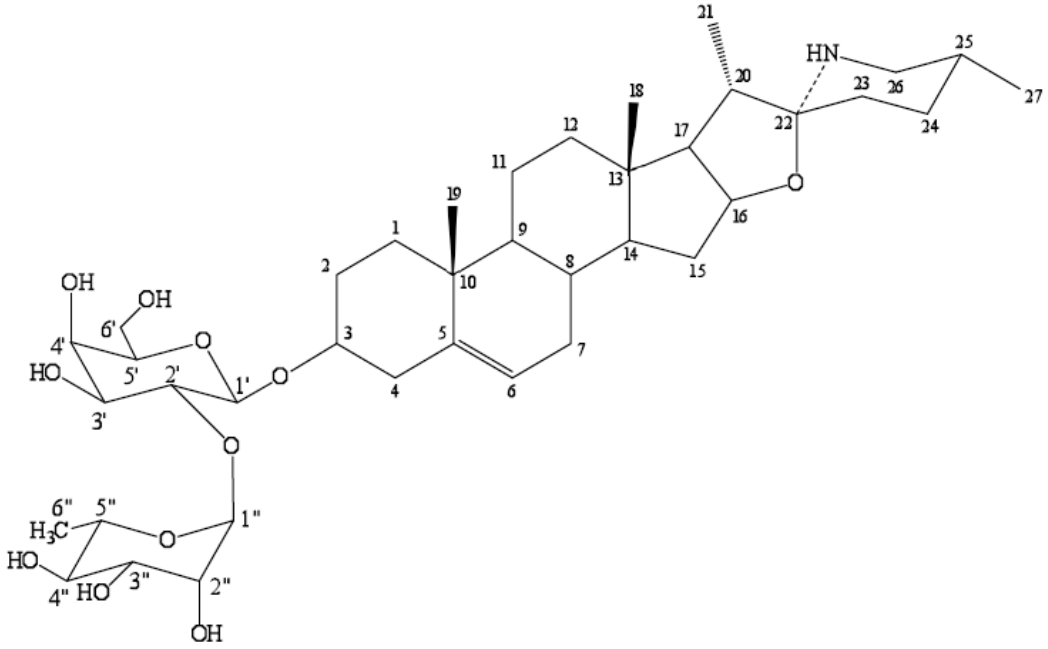

Figure 1: Structure of *Solaxanine* **1**.

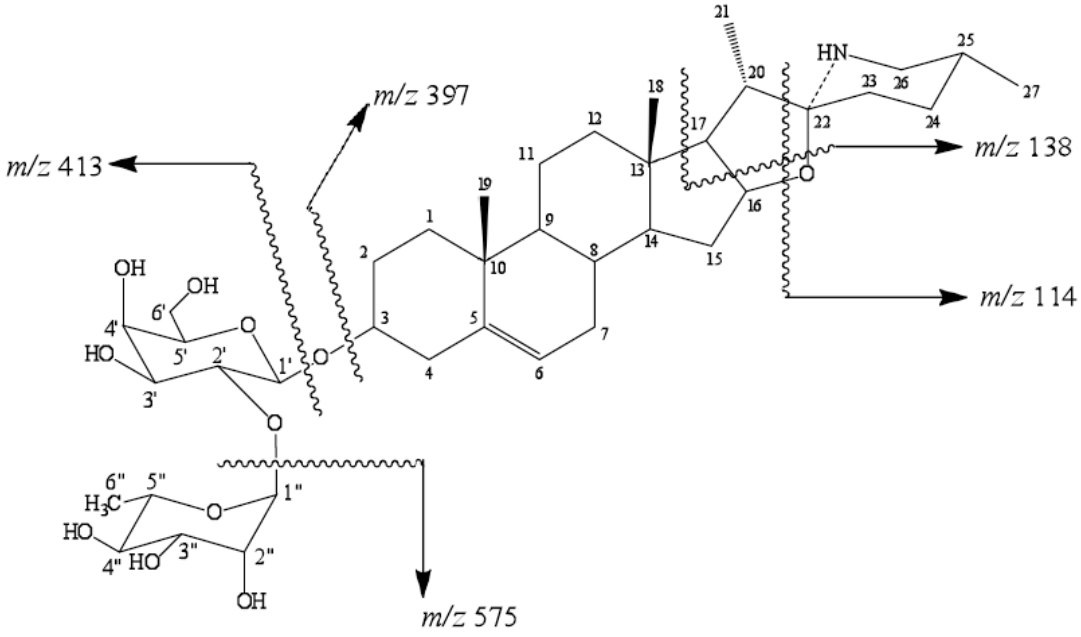

Figure 2: Important EIMS Fragments of *Solaxanine* (**1**).

rhamnose. H-6 of methine carbon exhibited as one down field broad singlet at δ 5.28 due to the presence of double bond at C-6 [23]. It was further confirmed by ^{1}H-^{1}H COSY spectrum (Figure **3**) which indicated the vicinal coupling of H-6 proton at (δ 5.28) with H-7 proton at (δ 1.95), the other vicinal couplings observed between H-20 (δ 2.04) with H-21(δ 1.18) and H-25(δ 1.51) with H-27(δ 0.87).

HMBC (heteronuclear multiple bond correlation) spectrum of this compound (Figure **4**), showed cross-peak between H-1″ δ 4.26 (d, J=1.4Hz) to C-2′ of galactose δ 75.16 ($^{3}J_{CH}$ coupling) while anomeric proton of an inner galactose at δ 4.95 (d, J=7.8Hz) to C-3 of aglycone δ 70.65 ($^{3}J_{CH}$ coupling) which confirmed the sequence and connectivity of disaccharide chain with aglycone. While H-19 (δ 0.91)

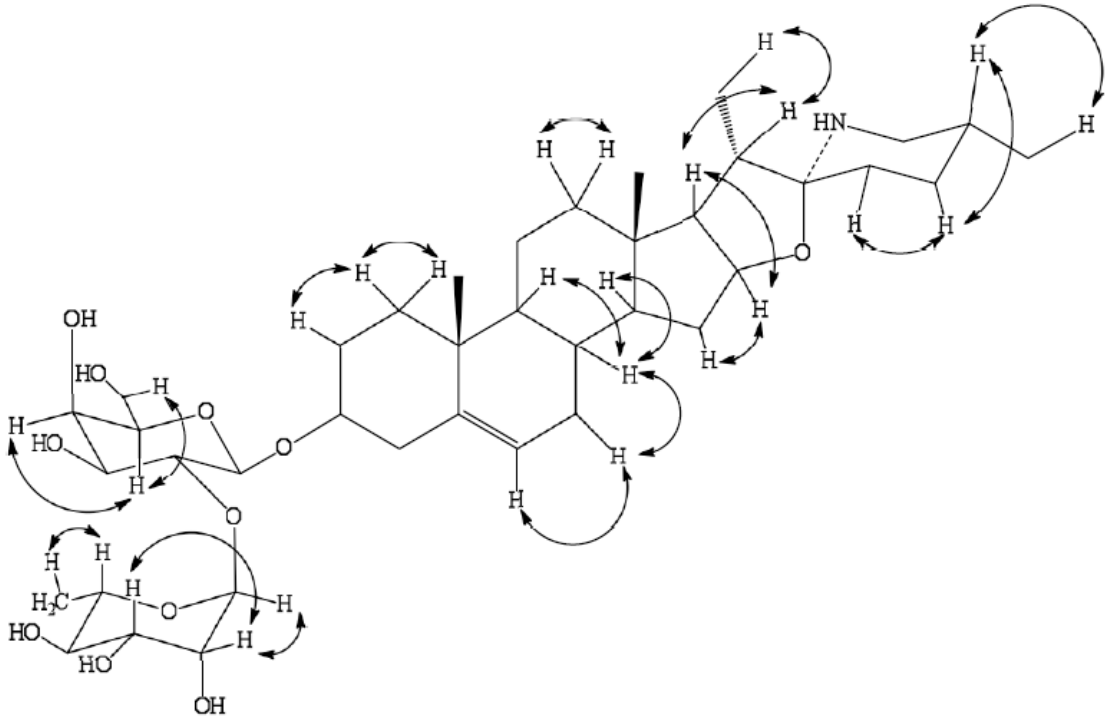

Figure 3: Selective ^{1}H-^{1}H Cosy correlation of *Solaxanine* (**1**).

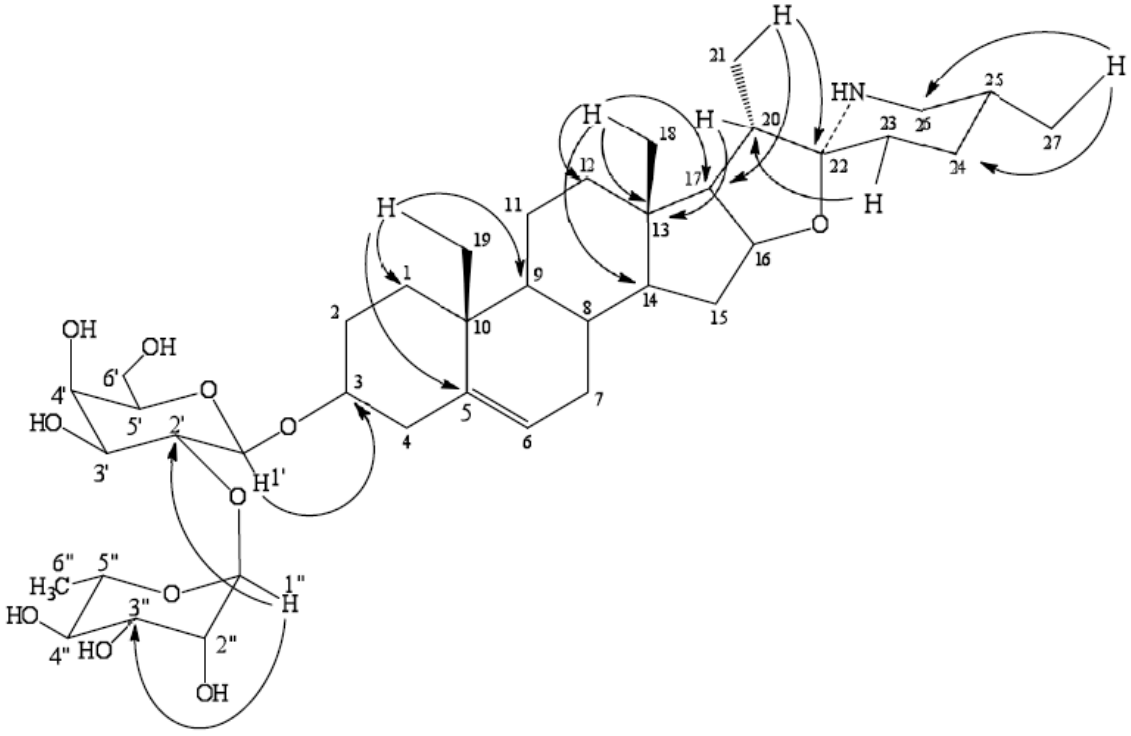

Figure 4: Selective HMBC correlation of *Solaxanine* (**1**).

showed correlation with C-1 (δ 37.10) ($^{3}J_{CH}$ coupling), C-9 (δ 49.80) ($^{3}J_{CH}$ coupling) and C-5 (δ 140.21) ($^{3}J_{CH}$ coupling).

On the basis of above mentioned chemical evidences and spectral analysis, the structure of compound **1** was established as *O*-(3){α-L-

rhamnopyranosyl-(1→2$_{gal}$)-β-D-galactopyranosyl}-solasodine. It is a new compound named solaxanine.

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