Bull. Chem. Soc. Ethiop. **2003**, 17(1), 61-66. ISSN 1011-3924
Printed in Ethiopia 

# A NOVEL GLYCOSIDIC STEROIDAL ALKALOID FROM *SOLANUM ACULEASTRUM*

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(Received June 22, 2002; revised February 26, 2003)

**ABSTRACT.** The root bark of *Solanum aculeastrum* Dunal yielded a new steroidal alkaloid glycoside characterised as (25R)-3β-{O-α-L-rhamnopyranosyl-(1→2)-[O-β-D-glucopyranosyl-(1→4)-O-α-L-rhamnopyranosyl-(1→4)]-β-D-glucopyranosyl}-22α-N-spirosol-5-ene. The structure was established by spectroscopic analysis and comparison with published data of similar compounds reported in literature.

**KEY WORDS:** *Solanum aculeastrum* Dunal, (25R)-3β-{O-α-L-Rhamnopyranosyl-(1→2)-[O-β-D-glucopyranosyl-(1→4)-O-α-L-rhamnopyranosyl-(1→4)]-β-D-glucopyranosyl}-22α-N-spirosol-5-ene

## INTRODUCTION

Most of the *Solanum* species have been found to contain steroidal alkaloids [1, 2] and the widely reported biological activities of these plants are attributed to these compounds [3, 4]. High molluscicidal activity shown by compounds isolated from the berries of *Solanum aculeastrum* Dunal prompted further chemical investigation of the root bark of the plant [5]. *S. aculeastrum* is a thorny perennial plant widely distributed in Kenya and grows up to 2-3 m high with white flowers and lemon shaped berries that become yellow-green when ripe. It is not invaded by harmful insects and locally used as hedges. The fresh and boiled froth from the ripe berries is used as a cure for jigger wounds and gonorrhoea, respectively [6]. The present paper describes the isolation and structure elucidation of a new steroidal alkaloid glycoside (**1**) from the alkaloid enriched fraction of the methanol extract of the root bark for which the name solaculine C was proposed.

## RESULTS AND DISCUSSION

Purification of the crude methanol extracts of the root bark extract of *S. aculeastrum* was achieved by repeated droplet counter current chromatography and column chromatography. This led to isolation of **1** as white pellets of melting point 218-221 $^{o}$C.

Compound **1** was identified as solasodine tetraose by comparison of its $^{1}$H NMR and $^{13}$C NMR data with those of solarmargine, a steroidal alkaloid triglycoside isolated from the berries of this plant [5]. FABMS, positive mode, showed an intense peak at *m/z* 1030 due to $[M+H]^{+}$. The peaks at *m/z* 884 and *m/z* 868 were due to loss of a deoxyhexose and a hexose in terminal position [7]. The peak at *m/z* 414 was due to the aglycone and resulted from loss of all the appended sugars. This fragmentation pattern was characteristc of tetraglycoside steroidal alkaloids that have been isolated from *Solanum* species [5, 8, 9]

The $^{1}$H NMR indicated four characteristic steroidal aglycone methyls signals at δ 0.85 (3H, s), 1.05 (3H, s), 0.98 (3H, d $J$ = 6.6 Hz) and 1.18 (3H, d $J$ = 7.3 Hz). A multiplet at δ 1.28 was due to methyls of two rhamnoses while a doublet ($J$ = 4.8 Hz) at δ 5.36 was due to an olefinic proton at

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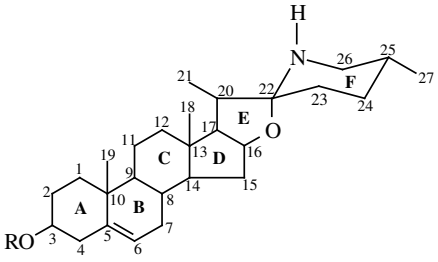
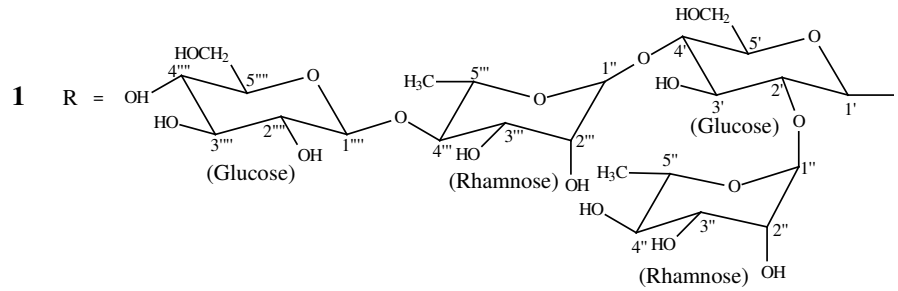
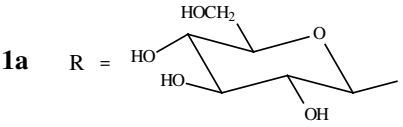
C-6. Four anomeric proton signals were observed at δ 5.22, 4.93, 4.67 (d, *J* = 8.0) and 4.49 (d, *J* = 7.6 Hz). $^{13}$C NMR spectrum of **1** showed 51 carbon signals of which 27 arose from aglycone moiety [10]. The DEPT experiment of **1** indicated four anomeric carbon signals at δ 103.4, 102.1, 100.3 and 100.1 with 4 quarternary, 12 methylene, 29 methine and 6 methyls carbon signals (Table 1). These splitting patterns are similar to those exhibited by other steroidal tetraglucoalkaloids isolated from *Solanum* species [1, 5, 11].





**1** R =



**1a** R =

**1b** R = H

The signal of C-22 at δ 99.4 for **1** indicated the presence of spirosolane type of alkaloid and hence signals at δ 141.2 and 122.0 were ascribed to the olefinic carbons at C-5 and C-6 [5, 8, 9]. The absence of carbon signals at *ca* 64-67 indicates that there are no methylene carbons due to a pentose sugar [12, 13]. This spectral data and comparisons with similar compounds in literature indicated that **1** consisted of $\Delta^5$steroidal aglycone [8, 10]. Carbon signal of C-23 of **1** was observed at δ 33.1 while that of C-26 at δ 46.5. This suggested that **1** has identical stereochemistry at C-22 and C-25 with solasodine [1, 5, 7, 9]. Hence, the presence of solaculinetetranoside of solasodine(25R)-22αN-spirosol-5-en-3β-ol. These considerations were in agreement with HMBC experiment (Table 2) as well as comparison of $^{13}$C NMR data of **1** with those of similar tetraglycosides isolated from *Solanum* species [1, 5, 10, 11]. Six carbon signal each at 100.1, 78.4, 77.1, 81.1, 77.1, 61.4; 100.3, 71.5, 80.1, 83.5, 69.6, 17.7 and 102.1, 71.7, 73.6, 73.2, 69.8,

Table 1. $^{13}$C NMR spectral data for compounds **1**, **1a** and **1b** and $^{1}$H NMR for **1**.

| Carbon | **1** | **1a** | **1b** | DEPT | $^{1}$H NMR (**1**) | |
|---|---|---|---|---|---|---|
| | | | | | α | β |
| 1 | 37.9 | 37.9 | 37.2 | $CH_2$ | 0.91 | 1.90 |
| 2 | 31.5 | 30.1 | 32.1 | $CH_2$ | 1.79 | 1.42 |
| 3 | 79.0 | 80.1 | 71.7 | CH | 3.65 | |
| 4 | 38.6 | 39.2 | 42.2 | $CH_2$ | 2.50 | 2.31 |
| 5 | 141.2 | 141.2 | 140.8 | C | - | |
| 6 | 122.0 | 122.1 | 121.3 | CH | 5.36 | |
| 7 | 32.5 | 32.6 | 32.1 | $CH_2$ | 1.59 | 2.03 |
| 8 | 32.1 | 32.1 | 30.2 | CH | 1.59 | |
| 9 | 50.7 | 50.8 | 50.1 | CH | 0.91 | |
| 10 | 37.5 | 37.4 | 37.6 | C | - | |
| 11 | 21.3 | 21.4 | 20.9 | $CH_2$ | 1.37 | 1.49* |
| 12 | 39.9 | 40.3 | 39.9 | $CH_2$ | 1.15 | 1.70 |
| 13 | 41.5 | 41.3 | 40.5 | C | - | |
| 14 | 57.0 | 57.0 | 56.5 | CH | 1.01 | |
| 15 | 32.7 | 32.6 | 31.2 | $CH_2$ | 1.83 | 1.30 |
| 16 | 78.4 | 79.5 | 78.9 | CH | 4.40 | |
| 17 | 62.5 | 62.3 | 62.7 | CH | 1.64 -1.69* | |
| 18 | 16.5 | 16.7 | 16.4 | $CH_3$ | 0.85 | |
| 19 | 19.6 | 19.7 | 19.4 | $CH_3$ | 1.05 | |
| 20 | 42.3 | 42.1 | 41.3 | CH | 1.79-1.94* | |
| 21 | 15.2 | 15.1 | 14.8 | $CH_3$ | 1.18 | |
| 22 | 99.4 | 99.0 | 98.3 | C | - | |
| 23 | 33.1 | 34.0 | 34.0 | $CH_2$ | 1.59 | |
| 24 | 30.2 | 30.1 | 31.6 | $CH_2$ | 1.36-1.54* | |
| 25 | 30.1 | 30.0 | 31.4 | $CH_2$ | 1.52 | |
| 26 | 46.5 | 47.4 | 47.6 | $CH_2$ | 2.25- 2.23* | |
| 27 | 18.8 | 19.3 | 19.3 | $CH_3$ | 0.98 | |
| 1' | 100.1 | 101.8 | | CH | 4.49 | |
| 2' | 78.4 | 70.5 | | CH | 3.60 | |
| 3' | 77.1 | 77.2 | | CH | 3.86 | |
| 4' | 81.1 | 76.8 | | CH | 3.45 | |
| 5' | 77.1 | 74.2 | | CH | 3.92 | |
| 6' | 61.4 | 62.2 | | $CH_2$ | 3.20 | |
| 1" | 102.1 | | | CH | 4.93 | |
| 2" | 71.7 | | | CH | 3.86 | |
| 3" | 73.6 | | | CH | 3.67 | |
| 4" | 73.2 | | | CH | 3.26 | |
| 5" | 69.8 | | | CH | 4.01 | |
| 6" | 17.9 | | | $CH_3$ | 1.28 | |
| 1''' | 100.3 | | | CH | 5.22 | |
| 2''' | 71.5 | | | CH | 4.05 | |
| 3''' | 80.1 | | | CH | 3.65 | |
| 4''' | 83.5 | | | CH | 3.86 | |
| 5''' | 69.6 | | | CH | 4.05 | |
| 6''' | 17.7 | | | $CH_3$ | 1.28 | |
| 1'''' | 103.4 | | | CH | 4.67 | |
| 2'''' | 74.3 | | | CH | 3.25 | |
| 3'''' | 76.5 | | | CH | 3.90 | |
| 4'''' | 71.3 | | | CH | 3.45 | |
| 5'''' | 77.2 | | | CH | 3.60 | |
| 6'''' | 61.4 | | | $CH_2$ | 3.45 | |

* Overlapped proton signals.

17.9 were assigned to inner glucosyl (Gluc-1), inner rhamnosyl (Rham-2) and outer rhamnosyl (Rham-1) carbons, respectively, by comparison with chemical shift values of the corresponding inner glucosyl, inner and outer rhamnosyl carbons of solarmagine and sycophantine and those of the methyl-O-α-L-rhamnopyranosides [5, 10, 11]. Similarly the six carbon signals at δ 103.4, 74.3, 76.5, 71.3, 77.2 and 61.4 were assigned to the terminal glucosyl (Gluc-2) carbons by comparison with chemical shift values of methyl-O-β-D-glucopyranosides [12, 13]. The sugar carbon signals of C-2' (78.4), C-4' (81.1) and C-4''' (83.5) are shifted to lower field because they are points of interglycosidic linkages [13, 14].

Table 2. The $^1$H NMR and HMBC spectral data for compound **1**.

| Proton | $^1$H NMR (*J* in Hz) | | Correlated C-atom HMBC |
|---|---|---|---|
| H-18 | 0.85 | *s* | C-14, C-13, C-12 |
| H-19 | 1.05 | *s* | C-10, C-9, C-5, C-1 |
| H-21 | 1.18 | *d* (7.3) | C-20, C-22, C-17 |
| H-27 | 0.98 | *d* (6.6) | C-26, C-25, C-24 |
| H-1' | 4.49 | *d* (7.6) | C-3 |
| H-2' | 3.60 | *d* (7.6) | C-1'' |
| H-4' | 3.45 | *s* | C-1''' |
| H-1'' | 5.22 | *s* | C-2' |
| H-1''' | 5.09 | *s* | C-4' |
| H-2''' | 3.86 | s | C-1'''' |
| H-1'''' | 4.67 | *d* (8.0) | C-2''' |

Establishment of the type of sugars appended to **1** was achieved by complete and mild hydrolysis. Glucose and rhamnose were detected by co-spotting with authentic sugars on complete hydrolysis of **1**. A mono-glycoside, **1a** after partial hydrolysis of **1** had $[M+H]^+$ at *m/z* 576 and 33 carbon signals in the $^{13}$C NMR experiment. The aglycone, **1b**, obtained after complete acid hydrolysis of a mono-glycoside, indicated $[M+H]^+$ at *m/z* 414 and 27 carbon signals in $^{13}$C NMR experiment (Table 1). The pyranoside on **1a** was established to be glucose. Interglycosidic linkages were ascertained from FABMS fragmentation patterns and NMR studies using $^1$H-$^1$H COSY and HMBC spectra (Table 2) as well as comparison with data of similar compounds reported in literature [1, 5, 7, 12, 13].

The assignment of proton and carbon chemical shifts of **1** and the corresponding sugar moieties were established by analysis of NMR (Table 1). The coupling constants for non-anomeric protons on sugars could not easily be ascertained from $^1$H NMR due to superimposition. However, the splitting patterns of the sugar moieties were recognized. The β-linkages for glucose were established from the coupling constants of the anomeric protons while the α-configuration of rhamnose was ascertained from comparison with similar sugars in literature [12, 13]. The D, D, L and L configuration for glucosyl and rhamnosyl residues, respectively were assumed as these usually occur in glycosides of higher plants [7].

## EXPERIMENTAL

*General.* Mps uncorr. TLC precoated kieselgel 60$F_{254}$ (0.25 mm, Merck). CC silica gel (mesh 230-400) eluted with MeOH-$CHCl_3$ (3:2) or MeOH-$CHCl_3$-cyclohexane (3:2:1) unless otherwise stated. Dragendorff's reagent and anisaldehyde were used for detection of alkaloids and glycosides, respectively. IR: KBr. $[\alpha]_D$ in MeOH-$CHCl_3$, 1:1, *c* 0.2) at 24 °C unless stated. Positive FABMS with Xe at 8 keV from glycerol matrix. $^1$H and $^{13}$C NMR (Brucker DPX 75.5 MHz and 300 MHz,

respectively) using solvent $CD_3OD$:$CDCl_3$ (1:1, TMS). DCCC (Tokyo, Rikakikai Co. Ltd.) fitted with 9 racks each with 25 of 4 mL (i.d. 2 mm). Pump pressure of between 5-10 mbars and temperature of 30 °C (ascending mode).

*Plant materials.* Fresh root bark (5 kg) of *S. aculeastrum* was obtained from Mount Elgon, in Western Province, Kenya. The identity of the plant was verified by Mr. Simon Mathenge of Botany Department, Nairobi University and voucher specimen SAc/0198/2000 deposited at the University of Nairobi Herbarium.

*Extraction and isolation of the alkaloid*. The root bark was cut into small pieces and extracted with cold MeOH four times (5 L x 4). The MeOH extract was concentrated under reduced pressure and the residue freeze-dried to give dark-brown extract (80 g). Part of this (3 g) extract was dissolved in a mixture of mobile and stationary phases (1:1) and injected in the DCCC. Elution was done with MeOH-$CHCl_3$-$H_2O$ (7:13:8) and saturated $CHCl_3$ as stationary phase. Alkaloid rich fractions were combined and concentrated under reduced pressure giving CR1 (833 mg). This was injected again in the DCCC and eluted with MeOH-$CHCl_3$-$H_2O$-$NH_3$ (8:13:6:1) and after alkaloidal fractions being pooled gave CRR1 (440 mg). This was further injected in the DCCC and eluted with MeOH-$CHCl_3$-$H_2O$-$NH_3$-iso-PrOH (65:35:40:1:5) resulting in fractions CR40 (240 mg). This was subjected to CC on silica gel repeatedly eluting with MeOH-$CHCl_3$ (3:2) and MeOH-$CHCl_3$-$NH_3$ (14:11:1) to give **1** (60 mg) of $R_f$ 0.45 in the same solvent.

*(25R)-3β-{O-α-L-Rhamnopyranosyl-(1→2)-[O-β-D-glucopyranosyl-(1→4)-O-α-L-rhamnopyranosyl-(1→4)]-β-D-glucopyranosyloxy}-22αN-spirosol-5-ene (**1**)*. White pellets, m.p. 218-221 °C, $[\alpha]_D^{24}$ -49.5° ($CH_3OH$-$CHCl_3$, *c* 0.2). IR (KBr) $\nu_{max}$ $cm^{-1}$: 3394 (OH or NH), 1655 (C=C) and 1026 (C-O or C-N). FAB-MS *m/z* (%): 1030 (85), 884 (6), 868 (6), 414 (31). $^1$H NMR (400 MHz, $CDCl_3$-$CD_3OD$, 1:1): *cf*. Table 1. $^{13}$CNMR (100 MHz, $CDCl_3$-$CD_3OD$, 1:1): *cf*. Table 1.

*(25R)-3β-{O-β-D-Glucopyranosyloxy}-22αN-spirosol-5-ene (**1a**)*. Light yellow pellets, m.p. 236-238 °C, $[\alpha]_D^{24}$ -78.5° ($CH_3OH$-$CHCl_3$, *c* 0.2). FAB-MS *m/z* (%): 576 (100), 414 (16). $^1$H NMR (300 MHz, $CDCl_3$-$CD_3OD$, 1:1): *cf*. Table 1. $^{13}$C NMR (75.5 MHz, $CDCl_3$): *cf*. Table 1.

*Solasodine or (25R)-3β-hydroxy-22αN-spirosol-5-ene (**1b**)*. White powder, m.p. 298-300 °C, $[\alpha]_D^{24}$ -73° ($CH_3OH$-$CHCl_3$, *ca* 0.2). FAB-MS *m/z* (%): 414 (49). $^1$H NMR (300 MHz, $CDCl_3$): *cf*. Table 1. $^{13}$C NMR (75.5 MHz, $CDCl_3$): *cf*. Table 1

*Sugar analysis of* **1**. Compound **1** (5 mg) was refluxed in 5 mL of 5% HCl-MeOH soln for 2 hours. The solution was then diluted with distilled water and extracted with $CHCl_3$ and the aglycones separated as organic layer. The aqueous filtrate was neutralized with $BaCO_3$ and evaporated. The resulting concentrated solution was chromatographed on TLC ($CHCl_3$-MeOH-$Me_2CO$-$H_2O$, 3:3:3:1) against reference sugars [15]. The sugar components were identified as glucose and rhamnose.

*Partial and complete acid hydrolysis of* **1**. Compound **1** (30 mg) was partially hydrolysed by refluxing in 10 mL of 0.5 M HCl for 30 minutes. The resulting solution was diluted and extracted with $CHCl_3$. This was put on CC and eluted with MeOH-$CHCl_3$ (4:1). Fractions 18-32 of $R_f$ 0.52 and showing green colouration on being sprayed with anisaldehyde-sulphuric acid and heated were combined to give **1a** (15 mg). Compound **1a** (10 mg) was further refluxed with 10 mL of 5% HCl-MeOH solution for 2 hours and extracted with $CHCl_3$. CC of this extract afforded an aglycone, **1b** (4 mg). The sugar appended to the aglycone was established to be glucose.

## ACKNOWLEDGEMENTS

This work was supported by German Academic Exchange Service (DAAD). We are very grateful to the following: Prof. Dr. Udo Eilert, of Pharmaceutical Biology, Technical University of Braunschweig, for the laboratory space; Ms. Doring of Chemistry Department, Technical University of Braunschweig and Dr. Victor Wray of GBF, Germany, for running FABMS and NMR, respectively. Mr. Liani Soboi, of Chemistry Department, Kenyatta University, Nairobi, for pretreatment of samples.

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