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Protodioscin and Pseudoprotodioscin From *Solanum intrusum*

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Abstract: Two furostane type saponin glycosides, protodioscin and pseudoprotodioscin were isolated for the first time from the unripe berries of *Solanum intrusum* (Soria) (family Solanaceae). The structures of both compounds were elucidated using MALSI-TOF mass spectroscopy and one and two dimensional NMR techniques. Protodioscin and related glycosides are reported to have cytotoxic activity on leukemic and HepG2 liver cancer cell lines. The isolation of these compounds from *S. intrusum* will shade the light on the importance of the plant and the need of further studies to be carried out in order to explore its possible therapeutic benefits.

Key words: *Solanum intrusum*, Solanaceae, protodioscin, pseudoprotodioscin, NMR, MS

INTRODUCTION

Solanum intrusum (Soria) herb is known to be used as antiphlogistic, diaphoretic, diuretic, emollient, febrifuge, narcotic, purgative and sedative. The leaves, stems and roots of the plant are used in the treatment of cancerous sores, leucoderma and wounds. Extracts of the plant are analgesic, antispasmodic, anti-inflammatory and vasodilator (http://www.pfaf.org/ index.html). The plant has been used in the manufacture of locally analgesic ointments and the juice of the fruit has been used as an analgesic for toothaches (Chiej, 1984).

Steroidal saponins and glycoalkaloids are widely distributed in genus *Solanum*, a number of which are economically important crop plants. Their importance for plant protection against microbial pathogens or herbivores has been suggested by Valkonen *et al*. (1996) and Osbourn (1996). In addition, many of them showed anti-inflammatory, anthelmintic (Jarald *et al*., 2008), antiviral (Lacaille-Dubois and Wagner, 1996), antifungal (Zhang *et al*., 2006; Grünweller *et al*., 1990), cytotoxic (Shiu *et al*., 2007; Wang *et al*., 2006; Hu *et al*., 1999), immunomodulatory (Marie-Aleth and Lacaille-Dubois, 2005) and hypocholesterolemic activities (Sauvaire *et al*., 1991).

In a previous study, Bitte and Shabana (1969) reported the isolation of dioscin and identification of gracillin (TLC) from the unripe berries of *S. intrusum*.

We report herein the isolation and structure elucidation of protodioscin and pseudoprotodioscin for the first time from the unripe berries of *S. intrusum*.

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MATERIALS AND METHODS

General

Optical rotations were measured using a JASCO DIP-360 automatic polarimeter. IR spectra were measured on a JASCO FT/IR-230 IR spectrometer. ^{1}H- and ^{13}C NMR spectra were measured with a JNM-LA400WB Lambada (JEOL) NMR (^{1}H, 400 MHz; ^{13}C, 100 MHz) and using TMS as an internal standard. MALDI-TOF MS spectra (positive ion mode) were measured on Bruker autoflex mass spectrometer, Bruker, Daltonics Germany. MPLC separation was carried out on Lichroprep RP-18 (Merck, size A) with 5 mL flow rate and 4 mL fractions were collected. HPLC separation was carried on Gelson instrument with 231 XL injector, a 119 UV/VIS detector and TSK-gel ODS-80 TM column (21.5×300 mm, Tosoh).

Plant Materials

Unripe fruits were collected from the plant cultivated in the experimental station, Faculty of Pharmacy, Cairo University, in Jun 2006, from seeds provided by Professor Marawan M. Shabana. The plant was further identified by Dr. Mohamed El-Gebaly, Plant Taxonomy Department, National Research Center, Dokki, Giza 12622, Egypt. A specimen was deposited in the herbarium of Faculty of Pharmacy, Cairo University, Cairo, Egypt (No. SI 10401).

Extraction and Isolation

The methanolic extract of the fresh unripe berries of *S. intrusum* was reexamined following the same scheme previously described by Bitte and Shabana (1969). Five grams of the fraction containing substances E and F as described by Bitte and Shabana (1969) were applied on a Diaion HP-20 (4×15 cm) and eluted with water (500 mL), 50% MeOH (1 l) followed by MeOH (1 l). The methanolic fraction (750 mg) was chromatographed on a silica gel column (4×18 cm) using $CHCl_3$-MeOH-H_2O (73:25:2) as eluting solvent and 75 mL fractions were collected. Fractions eluted between 125-150 (230 mg) were purified on MPLC (RP-18 Si gel column, 2×25 mL) using MeOH-H_2O (7:3) with a flow rate of 5 mL min^{-1} to give compound F (1.40 mg) and a mixture (50 mg). This mixture was separated by HPLC using TSK-gel ODS-80 TM column using MeOH- H_2O (1:1) as mobile phase and flow rate 4 mL min^{-1} to give compound E (2.20 mg).

Acid Hydrolysis of Compound 1 and 2

Each compound (3 mg) was dissolved in 1M HCl (dioxane-H_2O, 1:1, 10 mL) and heated at 100°C for 2.5 h in a sealed glass tube. After dioxane was removed, the solution was extracted with EtOAc (3×5 mL). The EtOAc extract was washed with H_2O and evaporated to dryness under vacuum. The residue was analysed by TLC against authentic diosgenin. The aqueous portion was neutralised by passing through an ion exchange resin (Amberlite MB-3, Sigma) column and concentrated. The sugars were identified by TLC analysis against authentic sugars.

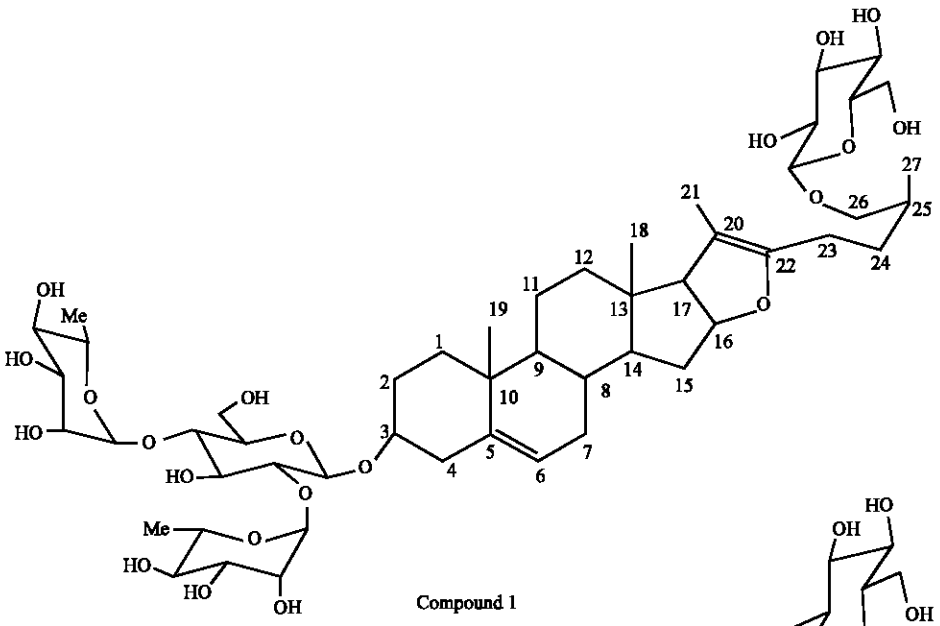
Compound 1: $C_{51}H_{82}O_{21}$, an amorphous white powder; $[\alpha]^{25}_{D}$ – 55.6° (MeOH, *c*. 0.34); ^{1}H-NMR (400 MHz): 5. 37 (1H, brd, H-6), 5.28 (1H, brs, H-1``), 4.85 (1H, brs, H-1```), 4.75 (1H, m, H-16), 4.47 (1H, d, J = 7.7 Hz, H-1`), 4.25 (1H, d, J=7.7 Hz, H-1`````), 3.62 (1H, m, H-3), 3.70 (1H, m, H-26a), 3.40 (1H, m, H-26b), 1.60 (3H, s, H-21), 1.30 (3H, d, J = 6.3 Hz, H-1```),1.27 (3H, d, J = 6.3 Hz, H-1``), 1.03 (3H, s, H-19), 0.70 (3H, s, H-18) 0.95 (3H, d, J = 6.6 Hz, H-27). MALDI-TOF MS, m/z (positive ion mode): 1053 $[M + Na]^{+}$.

Compound 2: $C_{51}H_{84}O_{22}$, an amorphous white powder; $[\alpha]^{25}_{D}$ – 8.4° (*c*. 0.02, 75 % MeOH); ^{1}H-NMR (400 MHz): 6.39 (1H, brs, H-1```), 5.85 (1H, brs, H-1``), 5. 30 (1H, brd, H-6), 3.95 (1H, m, H-3), 3.68 (1H, brd, J = 9.8 Hz, H-26a), 3.50 (1H,t, J = 10.0 Hz, H-26b), 1.59 (3H, d, J = 6.3 Hz,

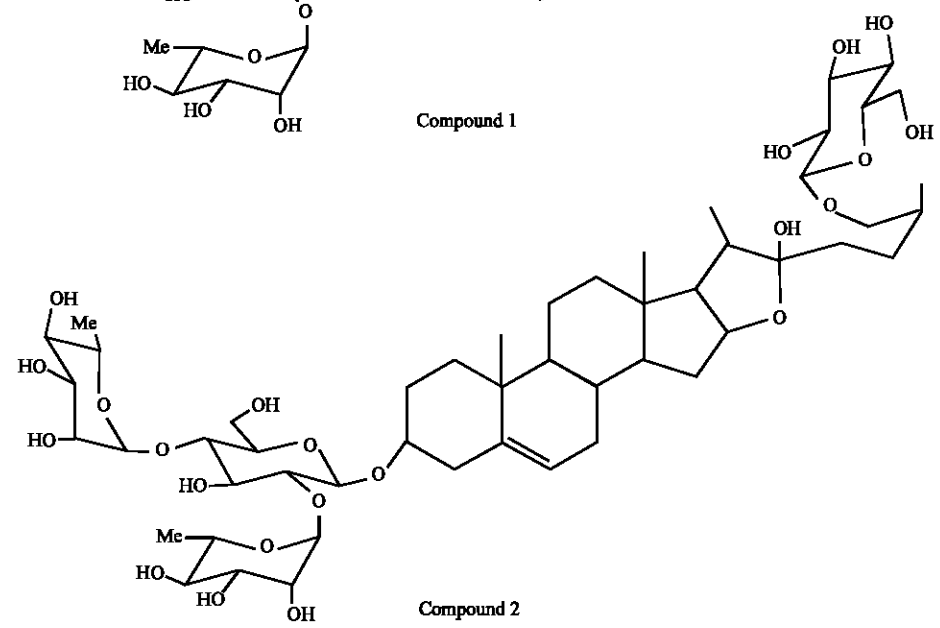
H-1```),1.73 (3H, d, *J* =6.3 Hz, H-1``), 1.30 (3H, d, *J* = 7.0 Hz, H-21), 1.04 (3H, s, H-19), 0.88 (3H, s, H-18) 0.97 (3H, d, *J* = 6.6 Hz, H-27). MALDI-TOF MS, m/z (positive ion mode) : 1071 $[M + Na]^+$, 1053 $[M + Na- H_2O]^+$.

RESULTS AND DISCUSSION

The reinvestigation of the crude saponin fraction of the unripe berries of *S. intrusum* using open column, MPLC and HPLC chromatography led to the isolation of two furostane type saponin glycosides 1 and 2.



Compound 1



Compound 2

Compound 1 was obtained as white amorphous powder, gave positive Liebermann-Burchard, Molish and Ehrlich reactions, which suggested that compound 1 is a furostanol saponin glycoside. The molecular formula of 1 was determined to be $C_{51}H_{82}O_{21}$ from the presence of a quasi-molecular ion

peak $[M+Na]^+$ at m/z 1053 in MALDI-TOF-MS and from ^{13}C-NMR spectrum. ^{1}H- and ^{13}C-NMR spectra revealed the presence of four anomeric proton signals at δ_H 5.28, 4.85, 4.47, 4.25 directly correlated to carbon signals at δ_C 102.0, 103.1, 100.5 and 104.4 in HMQC spectrum. The sugar moieties were identified as two glucose and two rhamnose units from the inspection of 1D 1H- and ^{13}C-NMR spectra and further confirmed from the result of acid hydrolysis. Compound 1 was a bisdesmosidic glycoside with sugars moieties attached to C-3 and C-26 as revealed from strong long range correlation observed of C-3/H-1` (Glc) and C-26/H-1```` (Glc) in HMBC spectrum. The attachment of the two rhamnose units at C-2` and C-4` of glucose at C-3 of the aglycone part was deduced from the downfield shift of the corresponding carbons (79.6 and 79.7, respectively) relative to the same carbons of glucose attached to C-26 (Table 1). This was further confirmed from strong correlation observed in HMBC spectrum of H-1′′/C-2′ and H-1′′′/C-4′. Now, the sugars in compound 1 was as follow one glucose attached to C-26 and a trisacharride *O*-α-L-rhamnopyranosyl (1′′′→2′)-[α-L-rhamnopyranosyl (1′′′→4′)] β-D-glucopyranoside attached to C-3. ^{13}C NMR (DEPT) spectrum of 1 revealed a total of 51 carbon signals, of which 27 were ascribed to aglycone part. These signals were due to the presence of 4 methyls, 10 methylenes, 8 methines and 5 quaternary carbons. Signals due to two double bonds were observed in ^{13}C NMR of 1. The signals at δ_C104.8 and 154.2 are due to C-20/C-22 double bond while signals at δ_C 141.0 and 122.1 are due to C-5/C-6 double bond. From MS and NMR data (1D and 2D), compound 1 could be identified as pseudoprotodioscin and further confirmed by comparison of its spectral data with those reported in literature (He *et al*., 2005).

Compound 2 was isolated as amorphous powder and had a molecular formula $C_{51}H_{84}O_{22}$ as shown by MALDI-TOF-MS (m/z 1071. $[M+Na]^+$) and ^{13}C-NMR. The NMR data of 2 showed great similarity to those of 1, except the absence of the double bond at C-20/C-22. The ^{1}H- and ^{13}C-NMR spectral data of sugar moieties were undistinguished from those of 1, indicating the presence of a glucose at C-26 and a trisaccharide *O*-α-L-rhamnopyranosyl (1′′′→2′)-[α-L-rhamnopyranosyl

Table 1: ^{13}C NMR spectral data of compounds 1 and 2

C	1	2	C	1	2
1	37.6, t	37.8, t	C-26 Glc````		
2	30.1, t	30.1, t	1	104.9	103.6
3	78.1, d	78.1, d	2	75.2	75.2
4	40.0, t	38.9, t	3	77.8	78.4
5	140.8, s	140.8, s	4	71.7	71.7
6	121.8, d	121.8, d	5	78.4	78.8
7	32.4, t	32.7, t	6	61.3	61.3
8	31.8, d	31.8, d	C-3 Glc`		
9	50.8, d	50.3, d	1	100.3	100.2
10	37.1, s	37.4, s	2	78.6	77.3
11	21.51, t	21.1, t	3	76.9	77.9
12	39.6, t	39.95, t	4	78.6	78.5
13	43.4, s	40.6, s	5	77.9	76.9
14	55.0, d	56.6, d	6	62.8	62.9
15	31.4, t	34.5, t	C-2` Rh``		
16	84.9, d	81.1, d	1	102.0	102.0
17	64.5, d	63.8, d	2	72.5	72.5
18	14.3, q	16.4, q	3	72.7	72.7
19	19.6, q	19.4, q	4	73.9	73.9
20	104.8, s	40.8, d	5	69.5	69.5
21	11.8, q	16.4, q	6	18.5	18.2
22	152.4, s	110.7, d	C-4` Rh```		
23	34.5, t	31.3, t	1	102.9	102.9
24	23.66, t	28.3, t	2	72.5	72.5
25	33.5, d	33.5, d	3	72.8	72.8
26	74.8, t	75.2, t	4	74.1	74.1
27	17.7, q	17.4, q	5	70.4	70.4
			6	18.6	18.3

(1‴→4′)] β-D-glucopyranoside attached to C-3, which was confirmed in similar way as in 1. From the previous data and from the study of ^{1}H- and ^{13}C-NMR data, compound 2 could be identified as protodioscin and confirmed from the comparison with data in literature (Hibasami *et al*., 2003; Ikeda *et al*., 2004).

Steroidal saponin glycosides with diosgenin as aglycone are used as plant estrogens or phytoestrogens (Murkies *et al*., 1998). They are naturally occurring plant compounds that are similar to mammalian estrogens. Dietary phytoestrogens can provide a wide range of health benefits, including protection from the development of some cancers, cardiovascular disease, osteoporosis and menopausal symptoms (Gardiner and Ramberg, 2001). In addition, the aphrodisiac properties of plants that contain protodioscin are documented. *Tribulus terrestris* containing protodioscin or its products have been extensively used both in Chinese and Indian traditional medicine for the treatment of various ailments such as urinary, cardiovascular and gastrointestinal disorders (Anand *et al*., 1994; Wang *et al*., 1990; Chemexcil, 1992). Administration of plant products containing protodioscin was found to improve libido and spermatogenesis (Tomova *et al*., 1981). Protodioscin and related glycosides are reported to have cytotoxic activity on leukemic and HepG2 liver cancer cell lines.

Protodioscin, isolated from fenugreek (*Trigonella foenum graecum* L.), was reported to induce cell death and morphological change indicative of apoptosis in leukemic cell line H-60 (Hibasami *et al*., 2003) and methyl protodioscin was reported to induce G2/M cell cycle arrest and apoptosis in HepG2 liver cancer cells (Wang *et al*., 2006).

The isolation of dioscin, protodioscin and pseudoprotodioscin from *S. intrusum* will shade the light on the importance of the plant and the need of further studies to be carried out to explore its possible therapeutic benefits.

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