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Solanum incanum and *S. heteracanthum* as sources of biologically active steroid glycosides: Confirmation of their synonymy

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ABSTRACT

A new spirostanol saponin (**1**), along with four known saponins, dioscin (**2**), protodioscin (**3**), methyl-protodioscin (**4**), and indioside D (**5**), and one known steroid glycoalkaloid solamargine (**6**) were isolated from the two synonymous species, *Solanum incanum* and *S. heteracanthum*. The structure of the new saponin was established as (23*S*,25*R*)-spirost-5-en-3β,23-diol 3-*O*-{β-D-xylopyranosyl-(1→2)-*O*-α-L-rhamnopyranosyl-(1→4)-[*O*-α-L-rhamnopyranosyl-(1→2)]-β-D-glucopyranoside}, by using a combination of 1D and 2D NMR techniques including ^{1}H, ^{13}C, COSY, TOCSY, NOESY, HSQC and HMBC experiments and by mass spectrometry. The compounds **1**, **3**, **4** and **5** were evaluated for cytotoxicity against five cancer cell lines and for antioxidant and cytoprotective activity.

1. Introduction

The Solanaceae family is one of the best-studied groups of plants, in which there are still many unsolved questions concerning the relations among its members [1]. *Solanum incanum* and *S. heteracanthum* belonging to the subgenus *Leptostemonum* section *Melongena* are considered as synonymous species [2]. *S. incanum* is distributed throughout continental Africa, including South Africa. It is also found growing wild in Madagascar and Mauritius, where the fruits are considered edible. The whole plant of *S. incanum* has been widely used as a folk medicine for treating various diseases in Africa as indicated in the following examples. In Senegal a maceration of the leaves is used as an eye bath to cure ophthalmia. In Malawi, fruit sap is rubbed into scarifications around the eye to treat conjunctivitis. In Uganda, Tanzania and South Africa, extracts of leaves or flowers are used as ear drops to cure inflammations and so on [3]. However, no studies have been reported on *S. heteracanthum*. Numerous *Solanum* species yielded a great variety of steroidal saponins and glycoalkaloids and are of interest from both ecological and human health points of view [4]. In this paper, we will try to see chemically the existence of synonymy between these two species. Thus, we report here the isolation and structure elucidation of a new spirostanol glycoside and five known steroid saponins present in both species. Their structures were unambiguously elucidated by 1D and 2D NMR experiments, including COSY, TOCSY, HSQC, HMBC spectroscopy and mass analyses. Furthermore, we assessed the biological activity of four of them (cytotoxicity and antioxidant activity).

2. Experimental

2.1. General

Optical rotations were recorded on a AA-OR automatic polarimeter. The 1D and 2D NMR spectra (^{1}H-^{1}H COSY,

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TOCSY, NOESY, HSQC, and HMBC) were performed using a UNITY-600 spectrometer at the operating frequency of 600 MHz on a Varian INOVA 600 instrument equipped with a SUN 4 L-X computer system (600 MHz for ^{1}H and 150 MHz for ^{13}C spectra). Conventional pulse sequences were used for COSY, HSQC, and HMBC spectra. TOCSY spectra were acquired using the standard MLEV17 spin-locking sequence 90 ms mixing time. The mixing time in the NOESY experiment was set to 500 ms. The carbon type (CH_3, CH_2, CH) was determined by DEPT experiments. All chemical shifts (δ) are given in ppm, and the samples were solubilized in pyridine-d_5 (δ_C 150.3, 155.9, 123.9). The NMR experiments of certain compounds were recorded in pyridine-d_5 on a Varian VNMR-S 600 MHz [5]. HRESIMS (positive-ion mode) was carried out on a Q-TOF 1-micromass spectrometer. FABMS (negative-ion mode, glycerol matrix) on a JEOL SX 102 mass spectrometer. GC analysis was carried out on a Thermoquest gas chromatograph. Isolations were carried out using vacuum liquid chromatography (VLC) on reversed-phase RP-18 (Spherical C18, 300 A, 75–200 μm, Silicycle) or silica gel 60 (Merck, 15–40 μm), column chromatography (CC) on Sephadex LH-20 (GE Healthcare Bio-Sciences AB) and a medium-pressure liquid chromatographic (MPLC) system [Gilson M 303 pump, Büchi glass column (460 × 15 mm and 230 × 15 mm), Büchi precolumn (110 × 15 mm), silica gel 60 (Merck, 15–40 μm), RP-18 (Spherical C18, 300Å, 75–200 μm, Silicycle)]. Analytical TLC and HPTLC were carried out on silica gel plates 60F254 (Merck). The spray reagent for saponins was vanillin reagent (1% vanillin in EtOH-H_2SO_4, 50:1).

2.2. Plant material

The roots of *Solanum incanum* (Solanaceae) were collected in October 2008 near University of Antsiranana, Madagascar. Mr. Benja Rakotonirina, botanist of Department of Plant Biology and Ecology, Antananarivo University, Madagascar, authenticated the plant material. A voucher specimen has been deposited n° 15488 in Missouri Botanical Garden Herbarium by W.G. D'Arcy and A. Rakotozafy.

The roots of *Solanum heteracanthum* (Solanaceae) were produced and identified by Sedaherb Company, Saint-Léger sur Deune, Saône et Loire, France. A voucher specimen, n° 5102011, is kept in the Laboratory of Pharmacognosy, Université de Bourgogne, Dijon, France.

2.3. Extraction and isolation

The air-dried plant material of *S. incanum* (200 g) and *S. heteracanthum* (300 g) was extracted three times under reflux by MeOH-H_2O (7:3, 2 L) for 1 h. After evaporation of the solvent in vacuum the resulting extracts of *S. incanum* (24 g) and *S. heteracanthum* (27 g) were obtained (SI and SH, respectively). Of SI, 6 g was then submitted to VLC [silica gel RP-18, H_2O-MeOH gradient (each eluent 100 ml)] to give three fractions (F1–F3). The fraction F3 (900 mg) eluted with MeOH was then separated by CC on Sephadex LH-20 eluting with MeOH to give four subfractions (Frs. 1–4). Fr.1 was fractionated by successive MPLC [silica gel 60, $CHCl_3$:MeOH:H_2O (70:30:5 to 60:32:7)] to give **3** (10 mg). The same procedure is applied to Fr.4 yielding compound **5** (4 mg). To obtain compounds **1**, **2** and **4**, whole Fr. 2 (550 mg) was subjected to MPLC [system A: silica gel, $CHCl_3$:MeOH:H_2O (80:20:2, 70:30:5, 60:32:7) and MeOH] to give 10 subfractions (Fr. 2.1–2.10), one of them Fr. 2.4 (6.8 mg) being the pure compound **2**. Fr. 2.6 (23.1 mg) was further purified by MPLC [system B: silica gel RP-18, H_2O-MeOH gradient], yielding compound **1**. Fraction Fr. 2.7 (94 mg) was subjected to successive MPLC (system A) and MPLC on RP-18 silica gel (system B) yielding compound **4** (27 mg).

An aliquot of SH, 3 g was used to isolate individual compounds. The procedures for isolation and purification are the same as that of SI; VLC on reversed-phase RP-18 and silica gel 60, CC on Sephadex LH-20 and successive MPLC on silica gel 60 and RP-18. The compounds **1** (4 mg), **3** (7.1 mg), **4** (6.4 mg), **5** (4.2 mg) and **6** (5.0 mg) were obtained in a pure form.

2.4. Acid hydrolysis and GC analysis

Each compound (3 mg) was hydrolyzed with 2 N aqueous CF_3COOH (5 ml) for 3 h at 95 °C. After extraction with CH_2Cl_2 (3 x 5 ml), the aqueous layer was neutralized by elimination of the volatile acid using successive evaporations of the milieu in presence of MeOH. The resulting neutral fraction was analyzed by TLC over silica gel ($CHCl_3$:MeOH:H_2O, 8:5:1) in comparison with authentic samples. The trimethylsilyl thiazolidine derivatives of the sugar residue of each compound were prepared and analyzed by GC [6]. The absolute configurations were determined by comparing the retention times with thiazolidine derivatives prepared in a similar way from standard sugars (Sigma-Aldrich).

2.5. Cytotoxicity assays

Assays were done in two companies. One bioassay was carried out by using the MTT assay as described by Carmichael et al. [7], with two human colorectal cancer cell lines, HCT 116 and HT-29, provided by the Oncodesign Society, Dijon, France. Paclitaxel was used as a positive control, and exhibited IC_{50} values of 2.65 and 2.29 nM against HCT 116 and HT-29, respectively. The second one was carried out by using the XTT assay as described by Jost et al. [8] with two human cancer cell line (colorectal SW480 and prostate DU145) and one mouse cancer cell line (mammary EMT6), provided by the Cohiro Society, Dijon, France. Etoposide was used as a positive control, and exhibited IC_{50} values of 13.22 μM, 41.6 μM and > 200 μM against these three cell lines, respectively. Saponins were tested on SW480, DU145, and EMT6 cell lines in the concentration range 0–30 μM.

2.6. Antioxidant and cytoprotective activity

Antioxidant activity was carried out according to the method of Oxygen Radical Absorbance Capacity (ORAC), method described by Cao et al. [9] adapted by COHIRO Society, Dijon, France. This method is based on measuring the decay of the fluorescence of a protein, allophycocyanine (APC) up the presence of a free radical generator 2, 2′-azobis [2-amidinopropane] dihydrochloride (AAPH). The activity is expressed as μM of equivalent Trolox (TE) per gram of sample, which served as positive control. The values correspond to the mean of four independent experiments for