Soladine glycosides of Solanum unguiculatum (A.) Rich

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**Introduction**

Solanum unguiculatum (A.) Rich is a small, wild, strongly branching, perennial shrub that grows in Yemen [1] and Egypt [2]. An infusion prepared from its fruiting tops is used in folk medicine as a contraceptive. Nothing has been reported in the literature about its chemistry, although many other Solanum species have been studied and shown to contain soladine glycosides.

These alkaloidal glycosides are natural precursors for the commercial synthesis of cortisones, progesterones, and C-21 steroidal contraceptives [3]. We now report the phytochemical investigation of Solanum unguiculatum, with the aim of isolating and identifying its steroidal alkaloidal component.

**Methods**

**Plant material**

Fruiting Solanum unguiculatum were collected from road sides, from waste ground, and from hill sides in the Sana‘a region (Wadi Zahr) in Yemen in May 1991. The identity of the plants was confirmed by Professor M. El-Monayery, Faculty of Science, El-Azhar University, Egypt. A certified specimen has been deposited at the Pharmacognosy Department, Faculty of Pharmacy, Zagazig University, Egypt.

**Extraction**

Air-dried powdered plant (3 kg) was exhaustively extracted with 95% ethanol by cold maceration. The extract (40 g) was subjected to acid-base extraction to yield the alkaloid preparation (12 g). Individual components of this preparation were isolated by rotational locular counter current chromatography (RLCCC), droplet counter current chromatography (DCCC), and preparative thick-layer chromatography (PTLC).

**Apparatus**

Melting points were recorded with a Kofler Microscope (Reicher, Austria; uncorrected). Optical rotation was determined by using a Perkin-Elmer 241 Polarimeter (Norwalk, CT, USA). Nuclear magnetic resonance (NMR) spectra were measured in CD3OD and pyridine-d, with tetramethylsilane (TMS) as internal standard at 300 MHz for 1H-NMR and at 75 MHz for 13C-NMR with a Varian XL 300 (Darmstadt, Germany). Infra-red (IR) spectra were determined with an IR-20 A spectrophotometer (Beckman, Irvine, CA, USA) and a Perkin-Elmer 1420 spectrophotometer (KBr disc). Mass spectrometry (MS) spectra were recorded with an MS 2500 high-resolution spectrometer (Kratos, Manchester, UK) with 70 eV, an ion source temperature of 180°C, and a direct inlet. Precoated thick-layer chromatography (TLC) plates (coated with silica gel 60 F254; the thickness of the layer was 0.25 mm) were obtained from Merck (Darmstadt, Germany).

**Abstract**

Besides soladine, three new glycosides, namely, 3-O-[alpha-L-rhamnopyranosyl-(1→3)]-solasodine, 3-O-[alpha-L-rhamnopyranosyl-(1→2)]-O-[alpha-L-rhamnopyranosyl-(1→4)]-beta-D-galactopyranosyl solasodine, and 3-O-[alpha-L-rhamnopyranosyl-(1→2)]-beta-D-galactopyranosyl solasodine, were isolated from Solanum unguiculatum (A.) Rich. Their structures were determined on the basis of chemical and spectral methods.

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Purification

The separation was carried out using RLCCC-A (Tokyo Rikokai, Japan) with H₂O as stationary phase and gradient elution (ascending) with the mobile phases in the following sequence. The first elution system was n-hexane saturated with H₂O. The second system was ethyl acetate saturated with H₂O; the third system was the upper phase of a mixture of C₂H₅OOCCH₃+C₄H₉OH+H₂O (4+1 +1 ); and the fourth system was the upper layer of a mixture of C₂H₅OOCCH₃+C₄H₉OH+H₂O (2+1+1). The flow rate was 1 ml/min. Fractions were collected in a Ultrarac 7000 from LKB.

A 670 DCC chromatograph system (Buchi, Flawip, Switzerland) was also used. For DCCC a solvent consisting of CHCl₃+CH₃OH+H₂O+C₆H₅OH+NH₂OH (34+65+40+5+1) was used. The stationary phase was the lower phase of this solvent and the mobile phase was the upper phase. The flow rate was 3 ml/h, and fractions were collected with an automatic fraction collector.

The TLC plates were developed in CHCl₃+CH₃OH+ C₆H₅OH+H₂O (60+20+15+6), or CHCl₃+CH₃OH+H₂O (6+9+2) for sugars [4] and treated with vanillin-sulfuric acid and Dragendorff’s reagent. All solvents were purified by using an adaptation of the method of Vogel [5].

Identification

The structure of the isolated compounds was determined on the basis of IR spectroscopy, fast atom bombardment (FAB) MS, ¹H-NMR, ¹³C-NMR with attached proton test (APT) and heteronuclear correlation spectroscopy, which enabled us to assign, unequivocally, the proton and carbon chemical shifts.

Results

Chromatographic separation of the alkaloid preparation yielded three new alkaloids (2, 3, and 4), in addition to choline, trigonilline, and solasonine (1) (Fig. 1).

Compound 2

30 mg 3-O-α-L-rhamnopyranosyl-(1→3)-solasodine was isolated. Crystallization from aqueous methanol yielded needle-shaped crystals with a melting point of 179-181°C and [α]D₀ = -42° (-0.1 mol/l in CH₃OH). The product had an Rf of 0.57 (mobile phase A). Acid hydrolysis gave rhamnose as the sugar moiety.

IR spectroscopy gave the following results (vKBr cm⁻¹): 3600-3240 (OH), 1400-1000 (C-O), 920 and 900 (25 R spiroketal moiety) [8 9].

Positive FAB MS gave the following results (m/z, relative intensity): 559 (M⁺; 59) calculated for C₃₃H₅₃NO₆, 413 (M-Rh⁺ 29), 368 (30), 336 (30), 164 (69), and 146 (72).

¹³C-NMR spectra recorded in CD₃OD are given in Table 1.

Compound 3

68 mg 3-O-α-L-rhamnopyranosyl-(1→2)-α-L-rhamnopyranosyl-(1→4)-β-D-galactopyranosyl solasodine was isolated. Crystallization from aqueous methanol yielded needle-shaped crystals with a melting point of 306°C (under decomposition) and [α]D₀ = -110° (1.05 mol/l in pyridine), [α]D₀ = -102° (1.08 mol/l in CH₃OH). The product had an Rf of 0.33 (mobile phase A). Acid hydrolysis gave galactose and rhamnose as sugar moieties.

IR spectroscopy gave the following results (vKBr cm⁻¹): 3400 (OH), 2950 (C-H), 1640 (C-C), 1200-1000 (C-O), 960, 920, 900, 870, 840, and 820 with the absorption band at 900 cm⁻¹ being of greater intensity than the band at 920 cm⁻¹ (25 R spiroketal moiety).

Positive FAB MS gave the following results (m/z, relative intensity): 868 (M⁺+H⁺; 100) for C₆₁H₇₉O₄N, 750 (6), 722 (3), 704 (3), 604 (0.1), 444 (23), 412 (22), 396 (66), 357 (6), 324 (36), and 307 (83). Negative FAB MS gave the following results (m/z, relative intensity): 866 (M-H-, 9), 720 (M-H-methylpentose, 3.6), 643 (3), 412 (M-H-methylpentose-methylpentose-hexose, 5), 367 (8), 339 (14), 275 (18), 219 (11), 183 (100), and 162 (18).

¹³C-NMR spectra recorded in pyridine-d₅ are given in Table 1.

Compound 4

21 mg 3-O-α-L-rhamnopyranosyl-(1→2)-β-D-galactopyranosyl solasodine was isolated. Crystallization