Two New Soladulcidine Glycosides from *Solanum lyratum*[1)]

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Two new steroid alkaloidal glycosides, named solalyratines A and B, were isolated from *Solanum lyratum* collected in Formosa and their chemical structures were characterized as the diglycoside and triglycoside of soladulcidine by spectroscopy.

Key words *Solanum lyratum*; Solanaceae; soladulcidine glycoside; spirosolane; solalyratine

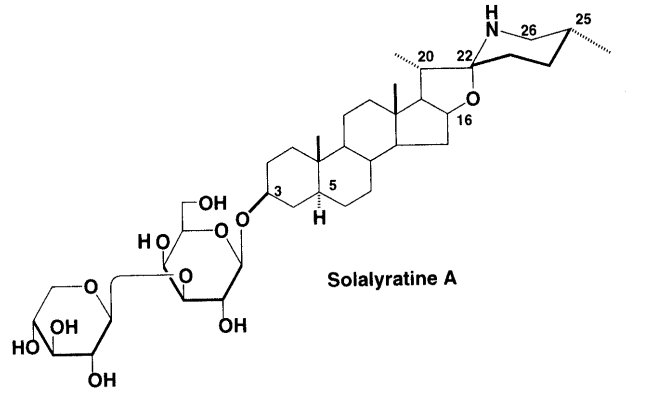
An Eastern crude drug, *Solanum lyratum* L.[2)] has been used to treat cancers, tumours and herpes for centuries, and it corresponds to the European crude anti-cancer drug, *Solaunum dulcamara*.[3)] In an earlier paper, two solanidane glycosides, tentatively named SL-c and SL-d, were isolated as active principles against cultured tumour cells.[4)]

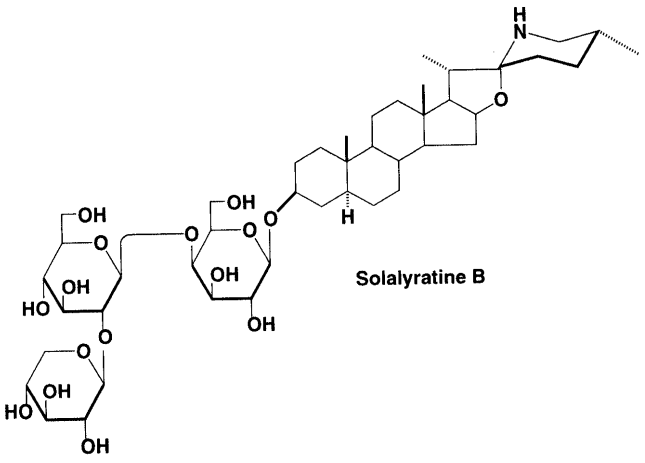
The ingredients in the solanaceous plants can sometimes differ depending on where they grow and, therefore, we have investigated the constituents of the title plant collected in Formosa.

The extract prepared by extraction with MeOH of the fresh aerial part (5 kg) of Formosan *Solanum lyratum* followed by evaporation was subjected to separation procedures using solvent partition and various types of column chromatography to give two glycosides **1** and **2** named solalyratines A and B, respectively, together with several furostanol, spirostanol and spirosolane glycosides. Here, we describe the structural characterization of **1** and **2**.

Solalyratine A (**1**), obtained as colorless needles, mp 252—254 °C, $[\alpha]_D$ −89.2°(pyridine), gave a positive color with Dragendorff's reagent and a quasi-molecular ion peak at *m/z* 732 $[M+Na]^+$ along with sugar-liberated ion peaks at *m/z* 600 $[M+Na\text{-pentose}]^+$ and 438 $[M+Na\text{-pentose-hexose}]^+$ in the positive FAB-MS. The ^{1}H-NMR spectrum of **1** displayed two singlet signals at δ 0.65 and 0.83 and two doublet signals at δ 0.79 (d, $J=5.3$ Hz) and 1.06 (d, $J=7.1$ Hz) due to steroidal methyls, and two doublet signals at δ 5.01 (d, $J=7.7$ Hz) and 5.27 (d, $J=7.6$ Hz) attributable to the anomeric protons of the sugar residue. The EI-MS of **1** showed charcteristic fragment ion peaks at *m/z* 114 and 138 due to $[C_6H_{12}NO]^+$ and $[C_9H_{16}N]^+$, respectively, for spirosolane derivatives.[5)] The above data suggested **1** to be a spirosolane diglycoside. Acid hydrolysis of **1** provided a sapogenol identical with soladulcidine, and xylose and galactose on TLC. The sugar part was converted to trimethylsilyl ethers of the corresponding methyl 2-(polyhydroxyalkyl)-thiazolidine-4(*R*)-carboxylates followed by gas-liquid chromatogaphy (GLC) analysis,[6)] and **1** was found to be composed of D-galactose and D-xylose. The ^{13}C-NMR spectrum (in Experimental) displayed a total of thirty-eight signals, among which were attributed signals originating from soladulcidine and the remaining signals were assigned to a β-D-xylopyranosyl-(1→3)-β-D-galactopyranosyl moiety. In the NMR spectra of **1**, heteronuclear multiple bond correlations (HMBC) were observed from an anomeric proton of a terminal xylose at δ 5.27 (d, $J=7.6$ Hz) to C-3 of galactose at δ 87.0 and from an anomeric proton of an inner galactose at δ 5.01 (d, $J=7.7$ Hz) to C-3 of aglycone at δ 77.6, supporting the deduced sugar connectivities. Therefore, the structure was determined to be as shown in the formula.

Solalyratine B (**2**), obtained as colorless needles, mp 274—277 °C, $[\alpha]_D$ −17.9° (pyridine), gave a positive color with Dragendorff's reagent and a molecular ion peak at *m/z* 871 $[M]^+$ along with sugar liberated ion peaks at *m/z* 739 $[M-\text{pentose}]^+$, 577 $[M-\text{pentose}-\text{hexose}]^+$ and 415 $[M-\text{pentose}-2\times\text{hexose}]^+$ in the positive FAB-MS. The ^{1}H-NMR spectrum of 2 displayed two singlet signals at δ 0.61 and 0.83 and two doublet signals at δ 0.79 (d, $J=5.3$ Hz) and 1.06 (d, $J=7.1$ Hz) due to 18-, 19-, 27- and 21-methyl groups, respectively, on the steroidal skeleton and three doublet signals at δ 4.87 (d, $J=7.4$ Hz), 5.16 (d, $J=7.2$ Hz) and 5.23 (d, $J=7.2$ Hz) attributable to the anomeric protons of the sugar residue. The above data indicated **2** to be a spirosolane triglycoside. Acid hydrol-





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ysis of **2** provided soladulcidine together with a sugar residue. The sugar part was converted into thiazolidine derivatives and checked by GLC.[6] Glycoside **2** was found to consist of D-galactose, D-glucose and D-xylose. The ^{13}C-NMR spectrum showed a total of forty-four signals, and the signals due to soladulcidine were subtracted to give those due to the sugar moiety. The remainder indicated the presence of a β-D-xylopyranosyl-(1→2)-β-D-glucopyranosyl-(1→4)- β-D-galactopyranosyl moiety. The HMBC correlations revealed the connectivities of the sugar linkages as follows: from H-1 of a terminal xylose at δ 5.23 (d, J=7.2 Hz) to C-2 of glucose at δ 86.3, from H-1 of glucose at δ 5.16 (d, J=7.2 Hz) to C-4 of an inner galactose at δ 81.3 and from H-1 of an inner galactose at δ 4.87 (d, J=7.4 Hz) to C-3 of soladulcidine at δ 77.4. Therefore, the structure of **2** was as shown in the formula.

While the steroidal alkaloid glycosides obtained from Formosan *Solanum lyratum* were spirosolane derivatives, ones obtained from Japanese plants were solanidane derivatives: this might be due to the different places of origin.

Experimental

Melting points were determined on a Yanagimoto micro-melting point apparatus and were uncorrected. Optical rotations were obtained with a JASCO DIP-360 digital polarimeter. Elemental analyses were carried out using a Perkin–Elmer 2400 CHN elemental analyzer. GLC analysis was performed on a HP-5890A gas chromatograph with an H_2 flame ionization detector, the column was 3% OV-1 (0.32 mm × 30 m); column temperature, 160 °C; detection temperature, 270 °C; injection temperature, 270 °C; carrier gas, He (2.2 kg/cm^2). The FAB-MS were measured with a JEOL JMS-HX 110 spectrometer. The ^{1}H and ^{13}C-NMR spectra were recorded on a Bruker AVANCE DMX 600 (600.10 MHz for ^{1}H-NMR and 150.92 MHz for ^{13}C-NMR) or JEOL α-500 (500.00 MHz for ^{1}H-NMR and 125.65 MHz for ^{13}C-NMR), respectively. Chemical shifts are reported in δ (ppm scale) using tetramethylsilane (TMS) as an internal standard. The ^{1}H–^{1}H, ^{1}H–^{13}C correlation spectroscopy (COSY) and HMBC spectra were recorded with standard Bruker and JEOL software. Chromatography was carried out using Kieselgel 60 (70—230 or 230—400 mesh, Merck), Sephadex LH-20 (25—100 m, Pharmacia Fine Chemical Co., Ltd.), MCI gel CHP-20P (Mitsubishi Chemical Ind.), Bondapak C_{18} (Water Associates), Chromatorex ODS (Fuji-Silysia Chemical Ltd.). TLC was performed on precoated Kiselgel 60 F254 plates (0.2 mm, Merck) and detection was achieved by spraying with 10% H_2SO_4 followed by heating. TLC of sugars was performed on Kieselgel 60 plates (Merck Art 5553) with *n*-PrOH–acetone–water (5 : 3 : 1) and spots were visualized by spraying with *o*-aminobenzenesulfonic acid–H_3PO_4,[7] followed by heating.

Extraction and Separation The fresh aerial parts (5 kg) of *Solanum lyratum* collected in Formosa (collection and identification of the plant material were carried out by Mr. Hsien-Chang Chang, Chief of Pharmacognosy Section, Brion Research Institute, Taiwan) were extracted with MeOH to give a extract which was partitioned between *n*-BuOH and water. The aqueous layer (20 g) was subjected to a variety of types of column chromatography on silica-gel $CHCl_3$–MeOH–H_2O=9 : 1 : 0.1→8 : 2 : 0.2→7 : 3 : 0.5, gradient elution) MCI CHP 20P (H_2O→MeOH, gradient elution) and Bondapak C_{18} (H_2O→MeOH, gradient elution) to provide solalyratines A (**1**, 96 mg) and B (**2**, 200 mg), together with other glycosides.

Solalyratine A (1) Colorless needles, mp 252—254 °C, $[\alpha]_D$ −89.2° (c=0.50, pyridine). Dragendorff's reagent: positive. *Anal.* Calcd for $C_{38}H_{63}NO_{11}\cdot 3H_2O$: C, 59.74; H, 9.10; N, 1.83. Found: C, 59.43; H, 9.35; N, 1.86. EI-MS m/z: 138 $[C_9H_{16}N]^+$, 114 $[C_6H_{12}NO]^+$. Positive FAB-MS m/z: 732 $[M+Na]^+$, 600 $[M+Na-pentose]^+$, 438 $[M+Na-pentose-hexose]^+$. ^{1}H-NMR (pyridine-d_5) δ: 0.65 (3H, s, Me-18), 0.79 (3H, d, J=5.3 Hz, Me-27), 0.83 (3H, s, Me-19), 1.06 (3H, d, J=7.1 Hz, Me-21), 5.01 (1H, d, J=7.7 Hz, gal. H-1), 5.27 (1H, d, J=7.6 Hz, xyl. H-1). ^{13}C-NMR (pyridine-d_5) δ: 37.3, 30.0, 77.6, 34.7, 44.8, 29.0, 32.5, 35.1, 54.5, 35.9, 21.4, 40.3, 41.1, 56.5, 30.8, 78.8, 63.5, 16.8, 12.4, 41.6, 15.5, 98.5, 34.5, 30.8, 31.2, 47.9, 19.8 (aglycone C-1-27), 102.5, 71.2, 87.0, 70.4, 76.2, 60.7 (gal. C-1-6), 105.0, 75.1, 77.8, 71.0, 67.4 (xyl. C-1-5).

Acid Hydrolysis of 1 A solution of **1** (25 mg) in 2 N HCl (dioxane–H_2O=1 : 1) was refluxed for 2 h. The reaction mixture was diluted with water to give a precipitate which was recrystallized from MeOH to provide an aglycone, colorless needles (7 mg), mp 197—199 °C, EI-MS m/z: 413 $[M]^+$, 138 $[C_9H_{16}N]^+$, 114 $[C_6H_{12}NO]^+$. ^{13}C-NMR (pyridine-d_5) δ: 37.5, 32.4, 70.6, 37.8, 44.7, 28.9, 32.3, 35.2, 54.4, 35.4, 21.1, 39.9, 40.5, 56.6, 31.8, 78.9, 62.7, 16.6, 12.1, 41.2, 15.0, 98.3, 34.1, 30.1, 31.3, 47.5, 19.3, C-1-27). The filtrate was treated with Amberlite IRA-400 to give a sugar mixture, which was checked by TLC, Rf 0.43 (gal), 0.67 (xyl.).

A mixture of the sugar residue and L-cysteine methyl ester hydrochloride in pyridine was warmed at 60 °C for 1 h. After the product was dried and mixed with *N*-trimethylsilyl-1*H*-imidazole, it was heated at 60 °C for 30 min. The reaction product was partitioned between hexane and water. The organic layer was subjected to GLC. t_R (min): 9 min 57 s (D-xylose) and 18 min 16 s (D-galactose).

Solalyratine B (2) Colorless needles, mp 274—279 °C, $[\alpha]_D$ −17.9° (c=0.50, pyridine). Dragendorff's reagent: positive. *Anal.* Calcd for $C_{44}H_{73}NO_{16}\cdot 2H_2O$: C, 58.19; H, 8.55; N, 1.54. Found C, 58.43; H, 8.35; N, 1.56. EI-MS m/z: 138 $[C_9H_{16}N]^+$, 114 $[C_6H_{12}NO]^+$. Positive FAB-MS m/z: 871 $[M]^+$, 739 $[M-pentose]^+$, 577 $[M-pentose-hexose]^+$, 415 $[M-pentose-2\times hexose]^+$. ^{1}H-NMR (pyridine-$d_5$) δ: 0.61 (3H, s, Me-18), 0.79 (3H, d, J=5.3 Hz, Me-27), 0.83 (3H, s, Me-19), 1.06 (3H, d, J=7.1 Hz, Me-21), 4.87 (1H, J=7.4 Hz, gal. H-1), 5.16 (1H, d, J=7.2 Hz, glc. H-1), 5.23 (1H, d, J=7.2 Hz, xyl. H-1). ^{13}C-NMR (pyridine-d_5) δ: 37.3, 29.9, 77.4, 34.9, 44.6, 28.8, 32.3, 35.3, 54.3, 35.7, 21.1, 40.6, 41.0, 56.5, 30.7, 78.8, 63.2, 16.8, 12.4, 41.9, 15.6, 98.3, 34.5, 30.7, 31.2, 47.4, 19.8 (aglycone C-1-27), 102.5, 73.2, 75.6, 81.3, 75.4, 60.7 (gal. C-1-6), 105.0, 86.3, 77.8, 72.0, 77.8, 63.4 (glc. C-1-6), 107.1, 76.2, 78.6, 70.5, 67.3 (xyl. C-1-5).

Acid hydrolysis of **2** (12 mg) in the same way as **1** gave an aglycone identical with soladulcidine (3 mg) and a mixture of sugars, which was monitored by TLC, *Rf* 0.43 (gal.), 0.52 (glc.), and 0.67 (xyl.). The sugar mixture was further converted into the methyl 2-(polyhydroxyalkyl)thiazolidine-4(*R*)-carboxylates and monitored by GLC, t_R (min): 10 min 02 s (D-xylose), 17 min 10 s (D-glucose), and 18 min 22 s (D-galactose).

References and Notes

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