A New Spirostanol Glycoside from Fruits of *Solanum indicum* L.

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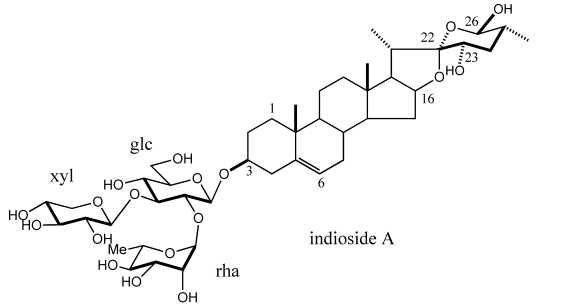
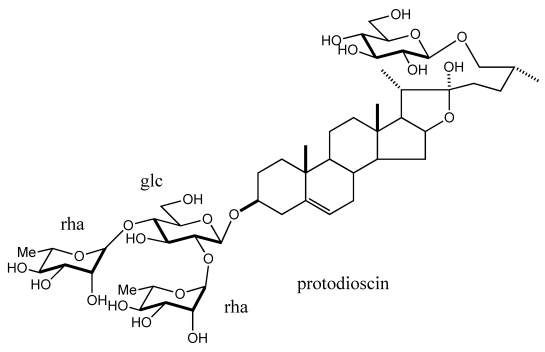
A new characteristic steroidal glycoside of the 23*S*,26*R*-hydroxylated spirostanol-type named indioside F was isolated from the fruit of *Solanum indicum*, along with indioside A and protodioscin. On the basis of spectroscopic analysis, the structure of indioside F was found to be 3-*O*-α-L-rhamnopyranosyl-(1→2)-[α-L-rhamnopyranosyl-(1→4)]-β-D-glucopyranosyl (β-chacotriosyl) (22*R*,23*S*,25*R*,26*R*)-spirost-5-ene-3β,23,26-triol.

Key words *Solanum indicum*; fruit; steroidal glycoside; spirostanol

Solanum indicum L. belongs to the Solanaceae family. It has been used in Chinese folk medicine as antiinflammatory and wound-healing agents, as an analgesic, and for the treatment of rhinitis, cough, and breast cancer.[1)] In Thailand, fruits of *S. indicum* and *S. torvum* are available in the markets; these are used as vegetables and as essential ingredients in anticarcinogens. Previously, Nohara *et al.* isolated new steroidal glycosides from the fruits (indiosides A and B) and roots (indiosides C, D and E) of *S. indicum*.[2)] Indioside D was the induced feeding preference of larvae of the moth *Manduca sexta* (tobacco hornworm).[3)] In our recent study on the constituents of the fruits cultivated in the botanical garden of Sojo University, we successfully achieved the isolation and structural characterization of a new steroidal glycoside, indioside F, along with indioside A and protodioscin.

The methanolic extract obtained from the fruits was subjected to Diaion HP-20, silica gel, and octadecyl silica (ODS) chromatographies to afford three compounds **1**—**3**. Compounds **2** and **3** were well-known steroidal glycosides and were identified as indioside A[1—3)] and protodioscin,[4,5)] respectively. This paper deals with the structure elucidation of the new compound **1**.





Compound **1**, which was obtained as an amorphous powder $[\alpha]_D$ −25.8° (MeOH), exhibited a quasimolecular ion peak at *m/z* 924 due to $[M+Na+H]^+$ in the positive FAB-MS. The molecular formula was estimated as $C_{45}H_{72}O_{18}$. The ^{1}H-NMR (pyridine-d_5) spectrum of **1** indicated two tertiary methyl groups at δ 0.94 (3H , s, H_3-18) and 0.95 (3H, s, H_3-19) and two secondary methyl groups at δ 1.09 (3H, d, J=5.7 Hz, H_3-27) and 1.26 (3H, d, J=6.9 Hz, H_3-21), which is characteristic of a typical steroidal glycoside: the three anomeric proton signals at δ 4.85 (1H, d, J=7.7 Hz), 5.76, and 6.29 (each 1H, s) suggested that **1** constituted of three molecules having a glycosidic moiety. The ^{13}C-NMR (pyridine-d_5) spectrum showed 27 signals that originated from the aglycone of the glycoside; these signals indicated the presence of a hemiacetal (δ 96.0), a ketal (δ 113.4), three oxygenated methines (δ 67.0, 77.7, 81.6), a tri-substituted double bond (δ 121.6, 140.6), eight methylenes (δ 37.3, 30.0, 40.9, 32.2, 19.3, 38.2, 31.6, 37.3), six methines (δ 31.4, 50.1, 56.5, 62.4, 36.1, 38.8), four methyls (δ 16.4, 19.2, 14.7, 17.2), and two quaternary carbons (δ 37.0, 40.8). In addition, the spectrum revealed that the sapogenol moiety was a spirostanol derivative containing one hemiacetal, one ketal, and three oxygen-bearing carbons. After acid hydrolysis of **1**, the sugar moieties in the hydrolysate were checked by HPLC equipped with optical rotation detector. On the other hand, the ^{13}C-NMR spectrum indicated 18 carbon signals that originated from the sugar moiety and two terminal α-L-rhamnopyranosyl moieties; the remaining carbon signals were attributed to the 2,4-di-substituted β-D-glucopyranosyl moiety. The heteronuclear multiple bond correlation (HMBC) spectrum (Fig. 1) showed correlations from H_3-27 (3H, d, J=5.7 Hz) at δ 1.09 to C-26 at δ 96.0, from H_3-21 (3H, d, J=6.9 Hz) at δ 1.26 to C-22 at δ 113.4, and from H-23 (1H, dd, J=3.0, 9.2 Hz) at δ 3.96 to C-22, suggesting that the aglycone of **1** was a 3,23,26-trihydroxyspirost-5-ene derivative. The signal due to H-26 appeared as a doublet at δ 5.10 (J=8.0 Hz), indicating *trans*-diaxial coupling between H-26 and H-25. Moreover, in the nuclear Overhauser effect spectroscopy (NOESY) spectrum (Fig. 1), correlations were observed between H-20 (δ 3.00) and H-23 (δ 3.96), and between H-23 and H-25 (δ 2.03), indicating that C-22 and C-25 were both in the *R* configuration, while in the sugar region, HMBC was observed from the rhamnosyl H-1 at δ

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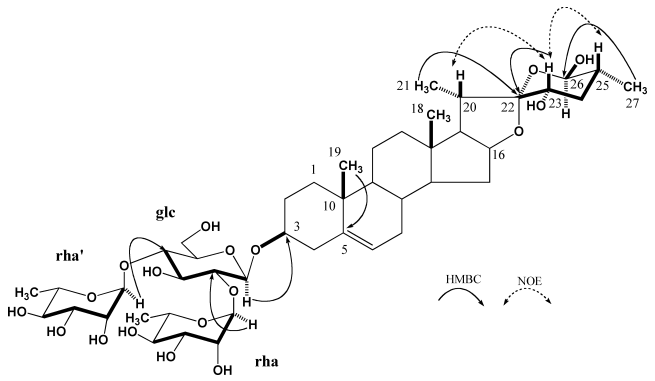

Fig. 1. Structure of Compound **1** with Key 2D-NMR Correlations

5.76 to the glucosyl C-2 at δ 78.0, from the 2nd rhamnosyl H-1 at δ 6.29 to the glucosyl C-4 at δ 77.7, and from the glucosyl H-1 (1H, d, J=7.7 Hz) at δ 4.85 to C-3 of the aglycone moiety at δ 77.7. Consequently, the structure of **1** was characterized as 3-*O*-α-L-rhamnopyranosyl-(1→2)-[α-L-rhamnopyranosyl-(1→4)]-β-D-glucopyranosyl (β-chacotriosyl) (22*R*,23*S*,25*R*,26*R*)-spirost-5-ene-3β,23,26-triol.

Experimental

General Procedure Optical rotations were measured with a JASCO P-1020 (l=0.5) automatic digital polarimeter. FAB-MS were obtained with a glycerol matrix in the positive ion mode using a JEOL JMS-DX300 and a JMS-DX 303 HF spectrometer. The ^{1}H- and ^{13}C-NMR spectra were measured in pyridine-d_5 with JEOL α-500 spectrometer, and chemical shifts are given on a δ (ppm) scale with tetramethylsilane (TMS) as the internal standard. Column chromatographies were carried out on a Diaion HP-20 (Mitsubishi Chemical Ind.), silica gel 60 (230—400 mesh, Merck), and ODS (Wako Pure Chemical Industries, Ltd.). TLC was performed on silica gel plates (Kieselgel 60 F_{254}, Merck) and RP C_{18} silica gel plates (Merck). The spots on TLC were visualized by UV light (254/366 nm) and sprayed with 10% H_2SO_4, followed by heating.

Plant Material The fruits of *Solanum indicum* L. were provided from the botanical garden of Sojo University, Japan. A voucher specimen was deposited in the Herbarium of the Sojo University.

Extraction and Isolation The fresh fruits of *S. indicum* (39.7 g) were crushed and extracted with hot MeOH. The resulting MeOH extract (2.3 g) was refluxed with *n*-hexane, and the insoluble *n*-hexane portion was partitioned between *n*-butanol and water to give *n*-butanol fraction (0.9 g) and aqueous fraction (1.44 g). The *n*-butanol fraction (0.9 g) was successively chromatographed on silica gel ($CHCl_3$: MeOH : H_2O=8 : 2 : 0.2) to afford 6 fractions (1—6). Fr. 6 was compound **1** (86.0 mg). Fr. 5 (69.0 mg) was subjected to ODS eluted with 60% MeOH to give compound **2** (30.6 mg). The aqueous fraction was subjected to Diaion HP-20 eluting with H_2O and MeOH. The eluted fraction with MeOH (227.0 mg) was subjected to silica gel column ($CHCl_3$: MeOH : H_2O=7 : 3 : 0.5) to provide 5 fractions (1—5). Fr. 4 was compound **3** (42.4 mg).

Compound 1 An amorphous powder, $[\alpha]_D^{23}$ −25.8° (c=1.78, MeOH). Positive FAB-MS m/z: 924 $[M+Na+H]^+$. ^{1}H-NMR spectrum (in pyridine-d_5) δ: 0.94 (3H , s, H_3-18), 0.95 (3H, s, H_3-19), 1.09 (3H, d , J=5.7 Hz, H_3-27), 1.26 (3H, d , J=6.9 Hz, H_3-21), 1.60 (3H, d, J=5.5 Hz, rha H_3-6), 1.74 (3H, d, J=4.9 Hz, rha′ H_3-6), 3.63 (1H, m, glc H-5), 3.86 (1H, m, H-3), 3.96 (1H, J=3.0, 9.2 Hz, H-23), 4.07 (1H, br d, J=11.6 Hz, glc H-6), 4.18 (overlapped, glc H-2, glc H-3, glc H′-6), 4.34 (overlapped, glc H-4, rha H-4, rha′ H-4), 4.51 (1H, br d, J=9.1 Hz, H-16), 4.59 (1H, m, rha H-3), 4.66 (1H, br s, rha H-2), 4.70 (1H, dd, J=3.1, 7.8 Hz, rha′ H-3), 4.80 (1H, br s, rha′ H-2), 4.85 (1H, d, J=7.7 Hz, glc H-1), 4.93 (overlapped, rha H-5, rha′ H-5), 5.10 (1H, d, J=8.0 Hz, H-26), 5.30 (1H, m, H-6), 5.76 (1H, s, rha H-1), and 6.29 (1H, s, rha′ H-1). ^{13}C-NMR (in pyridine-d_5) δ: 37.3 (C-1), 30.0 (C-2), 77.7 (C-3), 40.9 (C-4), 140.6 (C-5), 121.6 (C-6), 32.2 (C-7), 31.4 (C-8), 50.1 (C-9), 37.0 (C-10), 19.3 (C-11), 38.2 (C-12), 40.8 (C-13), 56.5 (C-14), 31.6 (C-15), 81.6 (C-16), 62.4 (C-17), 16.4 (C-18), 19.2 (C-19), 36.1 (C-20), 14.7 (C-21), 113.4 (C-22), 67.0 (C-23), 37.3 (C-24), 38.8 (C-25), 96.0 (C-26), 17.2 (C-27). β-D-Glucopyranosyl moiety; 100.1 (C-1), 78.0 (C-2), 78.5 (C-3), 77.7 (C-4), 76.7 (C-5), 61.1 (C-6), α-L-rhamnopyranosyl moiety; 101.9 (C-1), 72.5 (C-2), 72.6 (C-3), 73.7 (C-4), 70.3 (C-5), 18.3 (C-6), and α-L-rhamnopyranosyl′ moiety; 102.7 (C-1), 72.3 (C-2), 72.5 (C-3), 73.9 (C-4), 69.4 (C-5), 18.5 (C-6).

Sugar Analysis A solution of compound (**1**) (1.0 mg) in 2 M HCl/dioxane (1 : 1, 2 ml) was heated at 100 °C for 1 h. The reaction mixture was diluted with H_2O and evaporated to remove dioxane. The solution was neutralized with Amberlite MB-3 and passed through a SEP-PAK C_{18} cartridge to give a sugar fraction. The sugar fraction was concentrated to dryness *in vacuo* to give a residue, which was dissolved in CH_3CN/H_2O (3 : 1, 250 μl). The sugar fraction was analyzed by HPLC under the following conditions: column, Shodex RS-Pac DC-613 (6.0 mm i.d.×150 mm, Showa-Denko, Tokyo, Japan); solvent, CH_3CN/H_2O (3 : 1); flow rate; 1.0 ml/min; column temperature, 70 °C; detection, refractive index (RI) and optical rotation (OR). The t_R (min) of sugars were as follow: L-rhamnose 4.4 (−), D-glucose 7.2 (+). [reference: L-rhamnose 4.4 (negative optical rotation: −), D-glucose 7.2 (positive optical rotation: +)].

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