Studies on the Constituents of Solanaceous Plants, Steroidal Glycosides from *Solanum nodiflorum*

Jun Ando, Akiko Miyazono, Xing-Hua Zhu, Tsuyoshi Ikeda, and Toshihiro Nohara*

Faculty of Pharmaceutical Sciences, Kumamoto University, Oe-honmachi 5–1, Kumamoto 862–0973, Japan.
Received May 28, 1999: accepted August 23, 1999

Our search for bioactive steroidal glycosides among solanaceous plants has led to the isolation and characterization of four new glycosides, β-chacotriosyl (25*R*,26*R*)-spirost-5-ene-3β,17α,26-triol (1), the 26-*O*-methyl derivative (2) of 1, 3-*O*-α-L-rhamnopyranosyl-(1→2)-[β-D-xylopyranosyl-(1→3)] β-D-glucopyranoyl (25*R*,26*R*)-26-*O*-methyl-spirost-5-ene-3β,17α,26- triol (3) and β-chacotriosyl (22Ψ,25R)-furost-5-ene-3β,17α,22,26-tetraol 26-*O*-β-D-glucopyranoside (4) from the fruits of *Solanum nodiflorum*.

Key words *Solanum nodiflorum*; Solanaceae; steroidal saponin; spirostanol glycoside; furostanol glycoside

Our extensive search for bioactive oligoglycosides in solanaceous plants has so far resulted in the isolation of cytotoxic compounds[1] against several tumor cell lines, antifeeding substances[2] against *Thrips palmi* and an important key-intermediate[3] in the biosynthesis of steroidal alkaloids.

Successively, we have planned to search for active anti-herpes compounds among *Solanum* plants and to collect new steroidal specimens, and have also investigated the steroidal constituents of the fruits of *Solanum nodiflorum* to obtain four new steroidal glycosides. This paper deals with their structural elucidation.

The methanolic extract obtained from the fruits of the title plant was subjected to Diaion HP-20, silica gel and Chromatorex octadecyl silica (ODS) chromatography to afford several compounds tentatively named SNF-2-11, among which the structures of SNF-10 (**1**), SNF-5 (**2**), SNF-3 (**3**) and SNF-11 (**4**) are described.

SNF-10 (**1**), obtained as a white powder, showed $[\alpha]_D$ −91.8° (pyridine) and a quasimolecular ion peak $[M+Na+H]^+$ at *m/z* 924 in the positive FAB-MS. The ^{1}H-NMR spectrum displayed signals due to two tertiary methyl groups at δ 0.96 and 1.09, four secondary methyl groups at δ 1.16, 1.29, 1.63 and 1.77 and three anomeric protons at δ 4.93, 5.85 and 6.39. The above spectrum suggested **1** to be a steroidal glycoside, therefore, **1** was acid-hydrolyzed to give D-glucose and L-rhamnose together with unidentified decomposed sapogenols. The ^{13}C-NMR spectrum exhibited a total of 45 carbon signals, among which 18 signals (2×terminal rhamnosyl C-1—6: 102.0, 102.9; 72.5, 72.6; 2×72.8; 73.9, 74.2; 69.5, 70.4; 18.5, 18.7; inner glucosyl C-1—6: 100.3, 78.0, 78.7, 77.8, 76.9, 61.3) could be assigned to a β-chacotriosyl moiety. The remaining 27 signals could be assigned to a spirostanol derivative, in which an oxygen-bearing methylene carbon signal ascribable to C-26 disappeared and a new ketal signal occurred at δ 96.7, suggesting the substitution of a hydroxy group at C-26. Moreover, a signal due to C-17 usually observed around at δ 63 was banished and occurred at δ 90.4, indicating the presence of a hydroxy group at C-17. The substitution at C-17 caused good coincidental α,β,γ-substitution shifts at C-12—16 and C-20—22. Other ^{1}H and ^{13}C signals could be assigned based on ^{1}H–^{1}H, ^{1}H–^{13}C heteronuclear multiple quantum coherence (HMQC) and heteronuclear multiple bond correlation (HMBC) spectra, as shown in the Experimental section. Concerning the stereo-configurations at C-17, 22, and 25, the ^{13}C-NMR spectrum was compared with that of a pennogenin glycoside, Pa-p.[4] Since the signals due to C-13—18, 20, 21, 23, 24 and 27 were almost identical, the configurations at C-17, 22 and 25 were suggested to be *S*, *S* and *R*, respectively. The proton coupling constant of a doublet signal, J=8.6 Hz, at δ 5.19 of H-26 indicated a *trans*-diaxial conformation between H-25 and H-26 suggestive of 25*R*, 26*R* configurations. Consequently, the structure of SNF-10 (**1**) was elucidated to be 3-*O*-α-L-rhamnopyranosyl-(1→2)-[α-L-rhamnopyranosyl-(1→4)]-β-D-glucopyranosyl (β-chacotriosyl) (25*R*,26*R*)-spirost-5-ene-3β,17α,26-triol.

The genuine sapogenol of **1** could not be obtained owing to its rapid decomposition during acid hydrolysis, however, it is worthy to note the hydroxy groups at C-17 and C-26 because the occurrence of the hydroxy group at C-17 produces many complexes such as bethogenin and kryptogenin by acid hydrolysis,[5] whereas, the presence of the hydroxy group at C-26 might cause a transformation into a 26-aldehyde form by opening the F-ring. Therefore, the attachment of two hydroxy groups at C-17 and 26 makes this sapogenol unstable and characteristic.

SNF-5 (**2**), obtained as a white powder, showed $[\alpha]_D$ −121.4° (pyridine) and a quasimolecular ion peak $[M+H]^+$ at *m/z* 915 in the positive FAB-MS. The ^{1}H- and ^{13}C-NMR signals were analogous to those of **1**. Only one difference was recognized in the appearance of a methyl group in **2**. The methyl group resonated at δ_H 3.46 (3H, s) and δ_C 55.8, and a HMBC correlation between δ_C 103.2 at C-26 and δ_H 3.46 was observed. The proton signal at δ 4.56 (1H, d, J=7.9 Hz) assignable to H-26 was indicative of 25*R*, 26*R* configurations. Other signals could be assigned by the ^{1}H–^{1}H, HMQC and HMBC spectra. Namely, the structure of **2** was determined to be a 26-*O*-methyl derivative of SNF-10 (**1**). A glycoside having this sapogenol has already been reported as compound **2** from the bulbs of *Lilium candidum* by Sashida *et al.*[6] SNF-13 (**2**) might be derived from **1** during the extraction and separation procedure.

SNF-3 (**3**), obtained as a white powder, showed $[\alpha]_D$ −69.9° (pyridine) and a quasimolecular ion peak $[M+H]^+$ at *m/z* 901 in the positive FAB-MS. Acid hydrolysis of **3** gave D-glucose, D-xylose and L-rhamnose, together with unidentified artificially decomposed sapogenols. The ^{13}C-NMR signals which originated from the aglycone were superimposed

* To whom correspondence should be addressed.

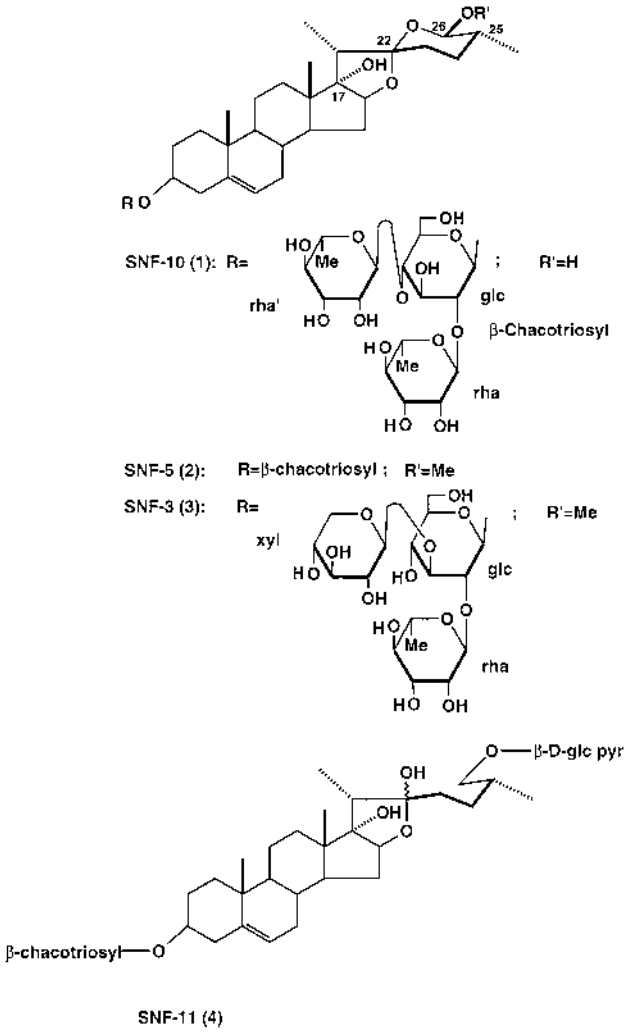

Fig. 1

on those of **2**. On the other hand, signals due to the sugar moiety suggested the presence of a terminal rhamnopyranosyl moiety (δ 102.4, 72.4, 72.8, 74.1, 69.6, 18.7), a terminal xylopyranosyl moiety (δ 105.4, 74.7, 78.3, 70.7, 67.2) and a hexosyl moiety (δ 99.9, 77.2, 88.2, 69.5, 77.7 and 62.4), the latter of which correlated to H-1—H-5 and H_2-6, respectively, of the β-D-glucopyranosyl moiety, as shown in the Experimental section. Moreover, the HMBC spectrum revealed connectivities between a xylosyl anomeric proton (overlapped) at around δ 4.95 and the C-3 of the glucosyl moiety at δ 88.2, between a rhamnosyl anomeric proton at δ 6.32 (1H, br s) and the C-2 of the glucosyl moiety at δ 77.7, and a glucosyl anomeric proton at δ 4.98 (1H, d, J=7.9 Hz) and the C-3 of the sapogenol moiety at δ 77.9. Therefore, the structure of **3** was represented as 3-*O*-α-L-rhamnopyranosyl-(1→2)-[β-D-xylopyranosyl-(1→3)] β-D-glucopyranoyl (25*R*,26*R*)-26-*O*-methyl-spirost-5-ene-3β,17α,26-triol.

SNF-11 (**4**), obtained as a white powder, showed $[\alpha]_D$ −82.1° (pyridine) and a quasimolecular ion peak $[M-H]^-$ at *m/z* 1063 in the negative FAB-MS. The ^{13}C-NMR signals which arose from the sugar residue revealed the attachment of the β-chacotriosyl moiety at C-3 of the aglycone, as well as another β-D-glucopyranosyl residue. The appearance of the signals at δ 75.3, 111.4 and 90.8 were assigned to C-26 and C-22 on the furostanol skeleton and C-17 with a hydroxy group, respectively. Consequently, the structure of **4** was deduced to be β-chacotriosyl (22Ψ,25*R*)-furost-5-ene-3β,17α,22,26-tetraol 26-*O*-β-D-glucopyranoside.

Table 1. ^{13}C-NMR Spectral Data for SNF-10 (**1**), SNF-13 (**2**), SNF-3 (**3**) and SNF-11 (**4**)

	1	Pa-p	**2**	**3**	**4**
C-1	37.6	37.4	37.5	37.5	37.6
2	30.2	30.0	30.1	30.1	30.2
3	78.1	77.6	77.8	77.9	78.1
4	39.0	38.8	38.9	38.7	39.0
5	140.8	140.7	140.8	140.8	140.8
6	121.8	121.6	121.8	121.9	121.0
7	32.4	32.2	31.6	32.5	32.0
8	32.3	31.6	32.3	32.8	34.3
9	50.3	50.1	50.2	50.2	50.2
10	37.2	37.0	37.1	37.2	37.2
11	20.1	20.8	20.9	21.0	21.0
12	32.1	32.0	31.7	32.2	36.9
13	45.1	45.0	45.2	45.2	45.1
14	53.0	52.9	53.1	53.1	53.1
15	32.1	32.2	32.3	31.7	32.5
16	90.2	89.9	90.3	90.3	90.5
17	90.4	90.1	90.2	90.3	90.8
18	17.1	17.1	16.7	17.1	17.3
19	19.5	19.3	19.4	19.5	19.4
20	45.0	44.7	44.8	44.8	43.6
21	9.8	9.3	9.5	9.6	10.5
22	112.7	109.8	112.4	112.5	111.4
23	32.0	32.0	32.1	31.8	32.3
24	28.2	28.6	27.9	28.0	28.0
25	37.7	30.3	35.3	35.3	32.1
26	96.7	66.7	103.2	103.3	75.3
27	17.5	17.2	17.1	16.7	17.5
26-OMe			55.8	55.9	
Glc					
1	100.3		100.2	99.9	100.3
2	78.0		78.0	77.7	78.1
3	78.7		78.6	88.2	78.6
4	77.8		77.9	69.5	77.7
5	76.9		76.8	77.7	76.9
6	61.3		61.3	62.4	61.3
Rha					
1	102.0		102.2	102.4	102.9
2	72.5		72.4	72.4	72.6
3	72.8		72.7	72.8	72.7
4	73.9		73.8	74.1	73.9
5	69.5		70.4	69.6	70.4
6	18.5		18.6	18.7	18.5
Rha′					
1	102.9		102.0		101.9
2	72.6		72.4		72.6
3	72.8		72.8		72.8
4	74.2		74.1		74.1
5	70.4		69.5		69.5
6	18.7		18.4		18.6
Xyl					
1				105.4	
2				74.7	
3				78.3	
4				70.7	
5				67.2	
26-*O*-Glc					
1					104.9
2					75.2
3					78.1
4					71.7
5					78.5
6					62.9

Experimental

Optical rotations were determined on a JASCO DIP-1000 KUY polarimeter (l=0.5). FAB-MS were obtained in a glycerol matrix in the positive ion mode using a JEOL JMS-DX300 and JMS-DX 303HF. NMR spectra were measured in pyridine-d_5 on a JEOL α-500 spectrometer and chemical shifts were referenced to tetramethylsilane (TMS). GLC was performed on a HP5890A gas chromatograph with a flame ionization detector. Column chromatography (CC) was carried out with silica gel 60 (230—400 mesh, Merck), Sephadex LH-20 (25—100 μm, Pharmacia Fine Chemicals), MCI gel CHP-20P (75—150 μm, Mitsubishikasei), Chromatorex ODS (30—50 μm, Fuji Silysia Chemical, Ltd.), and TLC was performed on precoated silica gel 60F_{254} (0.2 mm, Merck) and RP-18 F_{254S} (Merck).

Plant Material The seeds of the title plant were provided by Dr. Masaharu Matsui (National Research Institute of Vegetables, Ornamental Plants and Tea, Ministry of Agriculture, Forestry and Fisheries, Ano, Mie, Japan) and cultivated at the botanical garden of Kumamoto University.

Isolation The fruits (2.91 kg) were collected and extracted with hot MeOH and the resulting extract (159.6 g) was treated with hexane. The hexane insoluble portion (109.0 g) was successively column-chromatograhed on Diaion HP-20 (water and MeOH, with gradient increases in MeOH), Sephadex LH-20 (MeOH), silica gel ($CHCl_3$–MeOH–water=7 : 3 : 0.5), Chromatorex ODS (70% and 80% MeOH) and silica gel ($CHCl_3$–MeOH–water=8 : 2 : 0.2 and 7 : 3 : 0.5) to give SNF-10 (**1**, 110 mg), SNF-5 (**2**, 165 mg), SNF-3 (**3**, 63 mg) and SNF-11 (**4**, 65 mg).

SNF-10 (1) A white powder, $[\alpha]_D^{26}$ −91.8° (c=0.5, pyridine). Positive FAB-MS *m/z*: 924 $[M+Na+H]^+$. ^{1}H-NMR (pyridine-d_5) δ: 0.96 (3H, s, Me-18), 1.09 (3H, s, Me-19), 1.16 (3H, d, J=6.7 Hz, Me-27), 1.29 (3H, d, J=7.3 Hz, Me-21), 1.63 (3H, d, J=6.1 Hz, rha Me-6), 1.77 (3H, d, J=6.7 Hz, rha′ Me-6), 4.57 (1H, t-like, J=7.0 Hz, H-16), 4.93 (overlapped, glc H-1), 5.19 (1H, d, J=8.6 Hz, H-26), 5.29 (1H, br s, H-6), 5.85 (1H, br s, rha H-1), 6.39 (1H, br s, rha′ H-1). ^{13}C-NMR (pyridine-d_5) δ: Table 1.

Analyses of Sugars Respective compounds **1**—**4** were hydrolyzed with 2 mol/l HCl in H_2O for 4 h at 80 °C. The reaction mixture was neutralized with 2 mol/l NaOH in H_2O and extracted with $CHCl_3$. The aqueous layer was concentrated to dryness *in vacuo* to give a residue which was dissolved in dry pyridine and added to L-cysteine methyl ester hydrochloride.[7] The reaction mixture was heated for 2 h at 60 °C and concentrated to dryness by blowing N_2 gas. To the residue was added trimethylsilylimidazole, and the mixture was heated for 1 h at 60 °C. The reaction mixture was concentrated to dryness by blowing N_2 gas. To the residue was extracted with hexane and H_2O, and the organic layer was analyzed by gas liquid chromatography (GLC): column, OV-17 (0.32 mm×30 m); detector, flame ionization detector (FID); column temp., 230 °C; detector temp., 270 °C; injector temp., 270 °C; carrier gas, He (2.2 kg/cm^2). One peak was observed at t_R (min); 17′12″ (D-Glc), 11′62″ (L-Rha) 9′71″ (D-Xyl). The standard monosaccharides were subjected to the same reaction and GLC analysis under the same condition.

SNF-5 (2) A white powder, $[\alpha]_D$ −121.4° (c=0.5, pyridine). Positive FAB-MS *m/z*: 915 $[M+H]^+$. ^{1}H-NMR (pyridine-d_5) δ: 0.96 (3H, s, Me-18), 0.97 (3H, d, J=6.1 Hz, Me-27), 1.10 (3H, s, Me-19), 1.25 (3H, d, J=6.7 Hz, Me-21), 1.62 (3H, d, J=6.1 Hz, rha Me-6), 1.76 (3H, d, J=6.7 Hz, rha′ Me-6), 3.46 (3H, s, OMe), 3.64 (1H, m, glc H-5), 3.86 (1H, m, H-3), 4.16, 4.20 (1H each, m, glc H_2-6), 4.20 (overlapped, glc H-2, glc H-4), 4.36 (3H, overlapped, glc H-3, rha H-4, rha′ H-4), 4.55 (2H, overlapped, H-16, rha H-3), 4.56 (1H, d, J=7.9 Hz, H-26), 4.62 (1H, dd, J=3.2, 9.1 Hz, rha′ H-3), 4.68 (1H, br s, rha H-2), 4.82 (1H, br s, rha′ H-2), 4.93 (3H, overlapped, glc H-1, rha H-5, rha′ H-5), 5.33 (1H, br s, H-6), 5.83 (1H, br s, rha′ H-1), 6.29 (1H, br s, rha H-1). ^{13}C-NMR (pyridine-d_5) δ: Table 1.

SNF-3 (3) A white powder, $[\alpha]_D$ −69.9° (c=0.5, pyridine). Positive FAB-MS *m/z*: 901 $[M+H]^+$. ^{1}H-NMR (pyridine-d_5) δ: 0.96 (3H, s, Me-18), 0.97 (3H, d, J=6.1 Hz, Me-27), 1.11 (3H, s, Me-19), 1.25 (3H, d, J=7.3 Hz, Me-21), 1.76 (3H, d, J=6.1 Hz, rha Me-6), 3.45 (3H, s, 26-OMe), 3.68 (1H, t-like, J=10.4 Hz, xyl H-5), 3.84 (1H, m, glc H-5), 3.94 (2H, t-like, J=8.2 Hz, glc H-4, xyl H-2), 4.08 (1H, t-like, J=8.8 Hz, xyl H-3), 4.17 (4H, overlapped, glc H-2, glc H-3, xyl H-4, xyl H-5′), 4.26 (1H, dd, J=5.5, 10.4 Hz, glc H-6), 4.33 (1H, t-like, J=9.2 Hz, rha H-3), 4.45 (1H, br d, J=10.4 Hz, glc H′-6), 4.55 (1H, d, J=8.5 Hz, H-26), 4.58 (2H, overlapped, H-16, rha H-3), 4.88 (br s, rha H-2), 4.95 (overlapped, xyl H-1, rha H-5), 4.98 (1H, d, J=7.9 Hz, glc H-1), 5.33 (1H, br s, H-6), 6.32 (1H, br s, rha H-1).
^{13}C-NMR (pyridine-d_5) δ: Table 1.

SNF-11 (4) A white powder, $[\alpha]_D$ −82.1° (c=0.5, pyridine). Negative FAB-MS *m/z*: 1063 $[M-H]^-$. ^{1}H-NMR (pyridine-d_5) δ: 1.00 (3H, s, Me-18), 1.02 (3H, d, J=6.1 Hz, Me-27), 1.09 (3H, s, Me-19), 1.38 (3H, d, J=6.7 Hz, Me-21), 1.63 (3H, d, J=6.1 Hz, rha Me-6), 1.76 (3H, J=6.1 Hz, rha′ Me-6), 4.08 (1H, d, J=8.6 Hz, glc H-6), 4.22 (1H, m, glc H′-6), 4.37 (1H, m, 26-*O*-glc H-6), 4.82 (1H, d, J=7.9 Hz, 26-*O*-glc H-1), 4.93 (1H, overlapped, glc′ H-1), 5.30 (1H, br s, H-6), 5.85 (1H, br s, rha H-1), 6.39 (1H, s, rha′ H-1). ^{13}C-NMR (pyridine-d_5) δ: Table 1.

Acknowledgements We are grateful to Prof. H. Okabe of Fukuoka University, and Mr K. Takeda and Mr T. Iriguchi of the Analytical Center of Kumamoto University for NMR and MS measurements.

References and Notes

1) Nakamura T., Komori C., Lee Y.-Y., Hashimoto F., Yahara S., Nohara T., Ejima, A., *Biol. Pharm. Bull*., **19**, 564—566 (1996).
2) Matsui M., Monma S., Koyama K., *Bull. Natl. Res. Inst. Veg*., *Ornam. Plants and Tea*, *Jpn., Ser. A*, **10**, 13—24 (1995).
3) Ohmura E., Nakamura T., Tian R.-H., Yahara S., Yoshimitsu H., Nohara T., *Tetrahedron Lett*., **36**, 8443—8444 (1995).
4) The ^{13}C-NMR data for the sapogenol part (Pa-p) of Pa: Nohara T., Yabuta H., Suenobu M., Hida R., Miyahara K., Kawasaki T., *Chem. Pharm. Bull*., **21**, 1240—1247 (1973).
5) Nohara T., Miyahara K., Kawasaki T., *Chem. Pharm. Bull*., **22**, 1772—1780 (1974).
6) Mimaki Y., Satou T., Kuroda M., Sashida Y., Hatakeyama Y., *Chem. Pharm. Bull*., **46**, 1829—1832 (1998).
7) Hara S., Okabe H., Mihashi, K., *Chem. Pharm. Bull*., **35**, 501—507 (1987).