Steroidal Glycosides from *Solanum dulcamara*[1)]

Yung-Yung LEE,[a] Fumio HASHIMOTO,[a] Shoji YAHARA,[a] Toshihiro NOHARA,*[,a] and Naotoshi YOSHIDA[b]

Faculty of Pharmaceutical Sciences, Kumamoto University,[a] Oe-honmachi 5–1, Kumamoto 862, Japan and Faculty of Pharmaceutical Sciences, Hokkaido University,[b] Kita-ku, Sapporo 060, Japan.
Received August 10, 1993; accepted November 22, 1993

From the aerial parts of *Solanum dulcamara*, two spirostanol glycosides (1 and 2) and two new spirosolane glycosides (3 and 4) were isolated and their chemical structures were characterized as tigogenin 3-*O*-β-solatrioside, degalactotigonin, soladulcidine 3-*O*-β-chacotrioside (named soladulcine A), and soladulcidine 3-*O*-β-lycotetraoside (named soladulcine B), respectively.

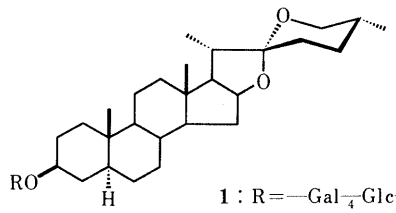
Keywords *Solanum dulcamara*; Solanaceae; spirosolane; steroidal glycoside; soladulcidine; soladulcine

A European crude drug, *Solanum dulcamara* L. has been used to treat cancers, tumours and warts since the time of Galen.[2)] Extensive studies on this plant have been carried out to separate some glycoalkaloids, mainly by German groups[3)] over 30 years ago. In those days there was little spectroscopic data especially nuclear magnetic resonance (NMR) data, to help assign unambiguous structures to these substances. Our systematic studies on the constituents of solanaceous plants required spectroscopic data relating to constituents of this plant. For this reason, we started reinvestigate the chemical constituents of this plant. In an earlier paper, we reported two steroidal glycosides, named soladulcosides A and B.[4)] Here, we have isolated an additional four steroidal glycosidic compounds **1**—**4** from the aerial parts of the plant and we now describe their structural characterization here.

Compound **1**, obtained as colorless needles, mp 252—254 °C, $[\alpha]_D$ −24.4° (pyridine), showed a *quasi*-molecular ion peak at *m/z* 903 $[M+H]^+$ in the positive fast-atom bombardment mass spectrum (FAB-MS). The ^{1}H-NMR spectrum of **1** displayed two singlet signals at δ 0.69 and 0.83 and two doublet signals at δ 0.70 (d, $J=5.3$ Hz) and 1.14 (d, $J=6.7$ Hz) due to steroidal methyls, and three doublet signals at δ 4.90 (d, $J=7.8$ Hz), 5.11 (d, $J=7.4$ Hz) and 5.17 (d, $J=7.1$ Hz) attributable to the anomeric protons of the sugar residue. The above data suggested **1** to be a steroidal triglycoside. The ^{13}C-NMR spectrum (Table I) displayed a total of forty-five signals, among which a signal at δ 109.3 was assignable to the C-22 of spirostanol and those at δ 102.3, 105.1 and 106.8 were attributable to the anomeric carbons of the sugar residue. A comparative study of the ^{13}C-NMR spectrum of **1** with that of tigogenin[5)] suggested that **1** was tigogenin triglycoside. Signals due to the sugar moiety in the ^{13}C-NMR spectrum of **1** were superimposable on those of funkioside D.[6)] Consequently, the structure of **1** was established as tigogenin 3-*O*-β-lucotrioside.

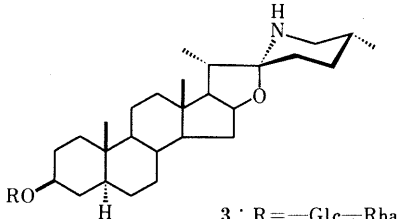
Compound **2**, obtained as colorless needles, mp 287—289 °C, $[\alpha]_D$ −56.0° (pyridine), gave a *quasi*-molecular ion peak at *m/z* 1058 in the positive FAB-MS, which showed signals due to two tertiary methyl groups at δ 0.64 and 0.82, two secondary methyl groups at δ 0.70 (d, $J=5.3$ Hz) and 1.15 (d, $J=7.0$ Hz) and four anomeric protons at δ 4.86 (d, $J=7.4$ Hz), 5.16 (d, $J=7.8$ Hz), 5.20 (d, $J=7.4$ Hz) and 5.53 (d, $J=7.1$ Hz) in ^{1}H-NMR spectrum. Therefore, **2** was deduced to be a spirostanol tetraglycoside. A detailed ^{13}C-NMR study (Table I) of the spirostanol moiety and the linked sugar residue led to the identification of **2** as β-lycotetraosyl[7)] tigogenin, *i.e.* degalactotigonin.

Compound **3**, obtained as colorless needles, mp 256—258 °C, $[\alpha]_D$ −91.8° (pyridine), gave a positive color with Dragendorff's reagent and a *quasi*-molecular ion at *m/z* 870 $[M+H]^+$ in the positive FAB-MS. The ^{1}H-NMR displayed signals due to Me-18 (δ 0.86), Me-19 (δ 0.86), Me-21 (δ 1.10) and Me-27 (δ 0.81) on the spirosolane skeleton (C-22, δ 98.3). It also suggested the presence of two 6-deoxyhexosyl residues [anomeric protons, δ 6.34, 5.83, 6-methyl groups, δ 1.75, 1.62]. The ^{13}C-NMR spectrum exhibited a total of forty-five signals, among which twenty seven were assigned to soladulcidine[8)] while the rest could be assigned to a β-chacotriosyl moiety.[9)] Consequently, **3** was established as being a soladulcidine



1 : R=—Gal(4)—Glc(2)—Glc

2 : R=—Gal(4)—Glc(3)—Xyl
|2
Glc



3 : R=—Glc(4)—Rha
|2
Rha

4 : R=—Gal(4)—Glc(3)—Xyl
|2
Glc

Chart 1

TABLE I. ^{13}C-NMR Data for **1**, **2**, **3** and **4** (in Pyridine-d_5)

	1	2	3	4
C-1	37.2	37.2	37.2	37.1
C-2	30.0	29.8	29.9	29.8
C-3	77.4	77.3	77.2	77.3
C-4	34.8	34.8	34.6	34.7
C-5	44.7	44.6	44.6	44.6
C-6	28.9	28.9	28.9	28.8
C-7	32.4	32.3	32.5	32.4
C-8	35.2	35.2	35.3	35.2
C-9	54.4	54.4	54.5	54.3
C-10	35.8	35.8	35.9	35.7
C-11	21.3	21.2	21.3	21.2
C-12	40.1	40.1	40.4	40.1
C-13	40.8	40.7	41.0	41.0
C-14	56.4	56.4	56.5	56.3
C-15	32.1	32.1	31.1	32.3
C-16	81.1	81.8	78.8	78.6
C-17	63.2	63.0	63.7	63.3
C-18	16.6	16.6	16.8	16.6
C-19	12.3	12.3	12.4	12.2
C-20	42.0	42.0	41.7	41.7
C-21	15.0	15.0	15.7	15.6
C-22	109.3	109.1	98.3	98.3
C-23	31.8	31.8	34.5	34.2
C-24	29.2	29.2	31.1	30.4
C-25	30.6	30.6	31.6	30.7
C-26	66.9	66.8	48.1	47.4
C-27	17.3	17.3	19.8	19.4
Gal				
C-1	102.3	102.4		102.3
C-2	73.2	73.1		73.1
C-3	75.5	75.0		75.0
C-4	80.9	79.9		79.8
C-5	75.0	76.1		76.1
C-6	60.5	60.7		60.5
Glc (Inner)				
C-1	105.1	104.8		104.7
C-2	85.9	81.3		81.3
C-3	77.5	86.8		86.7
C-4	70.3	70.7		70.6
C-5	78.3	78.6		78.6
C-6	61.6	62.4		62.3
Glc (Terminal)				
C-1	106.8	104.9	99.8	104.8
C-2	76.7	75.3	78.1	75.2
C-3	78.9	78.6	72.5	78.5
C-4	71.8	70.9	77.9	70.9
C-5	78.1	77.7	76.9	77.6
C-6	63.0	63.0	61.4	62.9
Xyl				
C-1		105.1		105.1
C-2		75.5		75.5
C-3		77.5		77.5
C-4		70.4		70.4
C-5		67.3		67.2
Rha (1→4) Glc-				
C-1			102.9	
C-2			72.5	
C-3			72.7	
C-4			73.9	
C-5			70.4	
C-6			18.6	
Rha (1→2) Glc-				
C-1			102.1	
C-2			72.4	
C-3			72.8	
C-4			74.1	
C-5			69.5	
C-6			18.5	

β-chacotrioside, named soladulcine A, which might correspond to one of the α-, β-, γ-soladulcidines.[3a-c]

Compound **4** was obtained as colorless needles, mp 264—266 °C, $[\alpha]_D$ −58.4° (pyridine), and gave a positive color with Dragendorff's reagent and a *quasi*-molecular ion at *m/z* 1034 $[M+H]^+$ in the positive FAB-MS and fragment ion peaks at *m/z* 138 ($C_9H_{16}N^+$) and 114 ($C_6H_{12}ON^+$) with electron impact (EI)-MS, being characteristic for spirosolane.[10] The ^{1}H-NMR spectrum disclosed signals due to Me-18 (δ 0.63), Me-19 (δ 0.85), Me-21 (δ 1.16) and Me-27 (δ 0.80) on the spirosolane skeleton and four anomeric protons at δ 4.86, 5.17, 5.21 and 5.54, originating from the sugar residue. In addition, the ^{13}C-NMR spectrum revealed a total of fifty carbons, among which twenty seven were assigned to soladulcidine while the remainder were attributable to the β-lycotetraosyl moiety. Therefore, **4** was concluded to be soladulcidine 3-*O*-β-lycotetraoside, named soladulcine B, as shown, identical to soladulcidine tetraoside.[3d-f]

Experimental

The ^{1}H-NMR and ^{13}C-NMR spectra were recorded on a JEOL JNM-GX 400 NMR spectrometer and chemical shifts are given using a δ (ppm) scale with tetramethylsilane (TMS) as an internal standard. MS were measured on a JEOL DX-303 HF spectrometer. The EI-MS were measured on a Finnegan-4510 instrument. TLC was performed on precoated Kieselgel 60 F_{254} plate (0.2 mm, Merck) using $CHCl_3$–MeOH–water (7:3:0.5, 8:2:0.2) as mobile phase and detection was carried out by spraying with 10% H_2SO_4 followed by heating. Column chromatography (CC) was carried out on colums of Kieselgel 60 (270—400 mesh, Merck), MCI gel CHP 20P (75—100 mm, Mitsubishi Chem. Ind. Co., Ltd.), Sephadex LH-20 (25—100 mesh, Pharmacia Fine Chemical Co., Ltd.) and Bonda-PAK 500/C_{18} (Waters Associates, Inc.) Melting points were determined using a Yanagimoto micro-melting point apparatus and were uncorrected. Optical rotations were obtained with a JASCO DIP-360 digital polarimeter (l=0.5).

Extraction and Separation The fresh aerial parts (4.0 kg) of *Solanum dulcamara* L. (solanaceae), cultivated at the Botanical Garden of Hokkaido University, were extracted with MeOH to give a extract ($CHCl_3$), which was partitioned between $CHCl_3$ and H_2O. The organic layer (49.7 g) was subjected to silica-gel column chromatography and eluted with $CHCl_3$–MeOH (30:1→10:1)→$CHCl_3$–MeOH–H_2O (9:1:0.1→7:3:0.5) to provide fractions 1 to 10. Fractions 4, 8 and 9 were respectively subjected to the silica-gel column chromatography to afford four compounds **1**—**4** in yields of 0.06%, 0.78%, 0.21% and 0.57%, respectively.

Compound 1 Colorless needles, mp 252—254 °C, $[\alpha]_D^{24}$ −24.4° (c=0.48, pyridine), Dragendorff's reagent: negative, *Anal.* Calcd for $C_{45}H_{74}O_{18}\cdot 2H_2O$: C, 57.55; H, 8.37. Found: C, 57.41; H, 8.35. Positive FAB-MS (*m/z*): 904 $[M+H]^+$, 741 $[M+H-Glc]^+$, 579 $[M+H-2\times Glc]^+$. Negative FAB-MS (*m/z*): 902 $[M-H]^-$. ^{1}H-NMR (pyridine-d_5) δ: 0.69 (3H, s, Me-18), 0.70 (3H, d, J=5.3 Hz, Me-27), 0.83 (3H, s, Me-19), 1.14 (3H, d, J=6.7 Hz, Me-21), 3.47 (1H, m, H-26), 3.54 (1H, m, H-26), 4.90 (1H, d, J=7.8 Hz, Gal H-1), 5.11 (1H, d, J=7.4 Hz, Glc′ H-1), 5.17 (1H, d, J=7.1 Hz, Glc H-1). ^{13}C-NMR (pyridine-d_5): Table I.

Compound 2 Colorless needles, mp 287—289 °C, $[\alpha]_D^{25}$ −56.0° (c=0.49, pyridine). Dragendorff reagent: negative. *Anal.* Calcd for $C_{50}H_{82}O_{22}\cdot 3/2H_2O$: C, 56.53; H, 8.07. Found: C, 56.71; H, 8.11. Positive FAB-MS (*m/z*): 1058 $[M+Na]^+$, 923 $[M+Na-Xyl]^+$, 579 $[M-Xyl-2\times Glc]^+$. ^{1}H-NMR (pyridine-d_5) δ: 0.64 (3H, s, Me-18), 0.70 (3H, d, J=5.3 Hz, Me-27), 0.82 (3H, s, Me-19), 1.15 (3H, d, J=7.0 Hz, Me-21), 4.86 (1H, d, J=7.4 Hz, Glc H-1), 5.16 (1H, d, J=7.8 Hz, Gal H-1), 5.20 (1H, d, J=7.4 Hz, Xyl H-1), 5.53 (1H, d, J=7.1 Hz, Glc H-1). ^{13}C-NMR (pyridine-d_5): Table I.

Compound 3 Colorless needles, mp 256—258 °C, $[\alpha]_D^{24}$ −91.8° (c=0.33, pyridine). Dragendorff's reagent: positive. *Anal.* Calcd for $C_{45}H_{75}NO_{15}\cdot H_2O$: C, 60.86; H, 8.74; N, 1.58. Found: C, 61.03; H, 8.60; N, 1.60. Positive FAB-MS (*m/z*): 870 $[M+H]^+$. ^{1}H-NMR (pyridine-d_5) δ: 0.81 (3H, d, J=5.9 Hz, Me-27), 0.86 (6H, s, Me-18, Me-19),

1.10 (3H, d, J=7.0 Hz, Me-21), 1.62 (3H, d, J=5.9 Hz, Rha Me-6), 1.75 (3H, d, J=6.2 Hz, Rha Me-6), 4.96 (1H, d, J=7.7 Hz, Glc H-1), 5.83 (1H, s, Rha H-1), 6.34 (1H, s, Rha H-1). ^{13}C-NMR (pyridine-d_5): Table I.

Compound 4 Colorless needles, mp 264—266 °C, $[\alpha]_D^{24}$ −58.4° (c=0.50, pyridine), Dragendorff's reagent: positive. *Anal.* Calcd for $C_{50}H_{82}NO_{21}\cdot 2H_2O$: C, 56.11; H, 8.19; N, 1.31. Found: C, 56.31; H, 8.01; N, 1.30. Positive FAB-MS (m/z): 1034 $[M+H]^+$, 900 $[M-Xyl]^+$, 577 $[M-Xyl-2\times Glc]^+$. EI-MS (m/z): 138 ($C_9H_6N^+$), 114 ($C_6H_{12}ON^+$). ^{1}H-NMR (pyridine-d_5) δ: 0.63 (3H, s, Me-18), 0.80 (3H, d, J=6.2 Hz, Me-27), 0.85 (3H, s, Me-19), 1.16 (3H, d, J=7.0 Hz, Me-21), 4.86 (1H, d, J=7.7 Hz, Glc H-1), 5.17 (1H, d, J=8.0 Hz, Gal H-1), 5.21 (1H, d, J=8.0 Hz, Xyl H-1), 5.54 (1H, d, J=7.3 Hz, Glc H-1). ^{13}C-NMR (pyridine-d_5): Table I.

References and Notes

1) Part 29 in the series of studies on the solanaceous plants.
2) S. M. Kupchan, S. J. Barboutis, J. R. Knox, C. A. Lau-Can, *Sciences*, **150**, 1827 (1965).
3) *a*) K. Schreiber, *Planta Med.*, **6**, 94 (1958); *b*) P. M. Boll, B. Andersen, *Planta Med.*, **10**, 421 (1962); *c*) K. Schreiber, H. Roensch, *Arch. Pharm.*, **298**, 285 (1965); *d*) M. Alkemeyer, H. Sander, *Naturwiss.*, **46**, 207 (1959); *e*) H. Sander, M. Alkemeyer, R. Haensel, *Arch. Pharm.*, **295**, 6 (1962); *f*) H. Sander, *Planta Med.*, **11**, 303 (1963).
4) T. Yamashita, T. Matsumoto, S. Yahara, N. Yoshida, T. Nohara, *Chem. Pharm. Bull.*, **39**, 1626 (1991).
5) K. Tori, S. Seo, Y. Terui, J. Nishikawa, F. Yasuda, *Tetrahedron Lett.*, **22**, 2405 (1981).
6) S. Okamura, K. Shingu, S. Yagara, H. Kohoda, T. Nohara, *Chem. Pharm. Bull.*, **40**, 2981 (1992).
7) Y. Hirai, T. Konishi, S. Sanada, Y. Ida, J. Shoji, *Chem. Pharm. Bull.*, **30**, 3476 (1982).
8) R. Radeglic, G. Adam, H. Ripperger, *Tetrahedron Lett.*, **1977**, 903.
9) S. B. Mahato, N. P. Sahu, A. N. Ganguly, R. Kasai, O. Tanaka, *Phytochemistry*, **19**, 2017 (1980).
10) K. Nakano, M. Miyamura, M. Ohe, Y. Yoshioka, T. Nohara, T. Tomimatsu, *Yakugaku Zasshi*, **104**, 1140 (1984).