A Furostanol Glycoside from *Allium chinense* G. DON

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From bulbs of *Allium chinense* G. DON (Liliaceae), a new furostanol glycoside, named chinenoside I (1), was isolated and the structure was established to be 26-*O*-*β*-glucopyranosyl 3*β*,22,26-trihydroxy-25(*R*)-5*α*-furostan-6-one 3-*O*-*β*-xylopyranosyl(1→4)-[*α*-arabinopyranosyl(1→6)]-*β*-glucopyranoside.

Keywords *Allium chinense*; chinenoside I; furostanol glycoside; laxogenin; Liliaceae; oligoglycoside ^{13}C-NMR

The isolation and structure determination of new furostanol glycosides from *Allium sativum* (garlic) and *A. ampeloprasum* (elephant garlic) have been reported in the previous papers.[1] As a continuation of our chemical studies on the *Allium* family, the present paper deals with the isolation and structure elucidation of a new furostanol glycoside from *A. chinense* G. DON (Japanese name; rakkyo). *A. chinense* is the original plant of the Chinese crude drug "Xiebei," which has been used for treatment of thoracic pain and diarrhea.[2] There have been reports on various volatile compounds.[3] Recently, Okuyama *et al.* reported that several acid amides isolated as the active principles from this crude drug showed a remarkable inhibitory effect against platelet aggregation.[4]

A crude glycoside fraction of the methanolic extract of bulbs of *A. chinense* was subjected to column chromatography on silica gel and on reversed-phase highly porous polymer, followed by heating in aqueous acetone to afford a new glycoside, named chinenoside I (**1**), $C_{49}H_{80}O_{23}\cdot 3H_2O$, in a 0.01% yield. The field desorption mass spectrum (FD-MS) of **1** exhibited molecular cluster ions at *m/z* 1057 $(M-H_2O+K)^+$ and 1041 $(M-H_2O+Na)^+$. On acid hydrolysis, **1** gave glucose, xylose, and arabinose in the ratio of 2:1:1, and the aglycone, which is identical with laxogenin (**2**),[5] previously isolated from the stem of *Smilax sieboldi* MIQ. as a sapogenin.[6] On standing in methanol, **1** gave glycoside A (**3**), which showed a methoxyl signal at 3.27 ppm in the proton nuclear magnetic resonance (^{1}H-NMR) spectrum. Glycosides **1** and **3** exhibited a purple coloration with the Ehlrich reagent on thin layer chromatography (TLC).[7] In the ^{13}C-NMR spectrum of **1**, inspection of the anomeric carbon signals revealed the presence of four monosaccharide units, and the signals assignable to C-22—C-26 appeared at almost the same positions as those of proto-eruboside-B,[1a] indicating that **1** should be a 22-hydroxyfurostanol tetraglycoside (Tables I and II).

On enzymatic hydrolysis with *β*-glucosidase, **1** gave glycoside B (**4**), $C_{43}H_{68}O_{17}\cdot 2H_2O$, and glucose. The FD-MS of **4** showed the ion at *m/z* 879 $(M+Na)^+$. On acid hydrolysis, **4** gave glucose, xylose, and arabinose, suggesting that **4** is a laxogenin triglycoside. Partial hydrolysis of **4** afforded laxogenin (**2**), and glycosides C (**5**), D (**6**), and E (**7**). These glycosides, **5**, **6**, and **7** were subjected to acid hydrolysis to give glucose, glucose and xylose, and glucose and arabinose, respectively. A comparison of the ^{13}C-NMR spectra of **6** and **7** with that of **5** revealed an additional set of signals due to a terminal *β*-xylopyranosyl and *α*-arabinopyranosyl unit in the respective spectra. Further, on going from **5** to **6**, a carbon signal at 71.9 ppm due to C-4 of the *β*-glucopyranosyl unit was displaced to low-field by 9.0 ppm, while other signals of the sugar moiety of **6** remained almost unshifted. Similarly, deshielding was evidently observed at C-6 of the *β*-glucopyranosyl unit of **7**. As the structures of **5**, **6**, and **7** were determined to be laxogenin 3-*O*-*β*-glucopyranoside, laxogenin 3-*O*-*β*-xylopyranosyl (1→4)-*β*-glucopyranoside, and laxogenin 3-*O*-*α*-arabinopyranosyl(1→6)-*β*-glucopyranoside, it follows that **4** can be represented as laxogenin 3-*O*-*β*-glucopyranosyl-(1→4)-[*α*-arabinopyranosyl(1→6)]-*β*-glucopyranoside.

Since **1** is a furostanol glycoside corresponding to **4**, it was established to be 26-*O*-*β*-glucopyranosyl 3*β*,22,26-trihydroxy-25(*R*)-5*α*-furostan-6-one 3-*O*-*β*-xylopyranosyl-(1→4)-[*α*-arabinopyranosyl(1→6)]-*β*-glucopyranoside.

It is noteworthy that **1** is the first example of a furostanol glycoside having laxogenin as a spirostanol sapogenin. On

TABLE I. ^{13}C-NMR Chemical Shifts: Aglycone Moiety (in C_5D_5N)

	2[a]	5	6	7	4	1	3
C-1	36.6	36.7	36.7	36.8	36.7	36.7	36.7
C-2	30.7	29.6	29.5	29.6	29.4	29.4	29.5
C-3	70.6	76.8	76.6	76.8	77.0	77.0	77.0
C-4	30.0	27.1	27.1	27.0	27.0	27.0	27.0
C-5	56.8[b]	56.4[b]	56.5	56.5	56.5	56.5[b]	56.5[b]
C-6	210.4	209.5	209.5	209.7	209.5	209.7	209.6
C-7	46.7	46.8	46.8	46.8	46.7	46.8	46.8
C-8	37.3	37.4	37.4	37.4	37.4	37.4	37.3
C-9	53.9	53.7	53.7	53.7	53.7	53.7	53.7
C-10	40.9	41.1	41.1	41.1	41.1	41.4	41.4
C-11	21.3	21.6	21.6	21.6	21.6	21.5	21.5
C-12	39.5	39.7	39.7	39.6	39.6	39.7	39.5
C-13	40.9	40.8	40.8	40.9	40.9	40.9	40.9
C-14	56.5[b]	56.5[b]	56.5	56.5	56.5	56.4[b]	56.4[b]
C-15	31.3	31.8	31.8	31.8	31.8	32.1	31.8
C-16	80.4	80.9	81.0	80.9	80.9	80.8	81.1
C-17	62.0	62.8	62.8	62.9	62.8	63.8	64.1
C-18	16.4	16.5	16.5	16.5	16.5	16.6	16.4
C-19	13.2	13.1	13.1	13.1	13.1	13.1	13.1
C-20	41.6	42.0	42.0	42.0	42.0	40.6	40.5
C-21	14.4	15.0	15.0	15.0	15.0	16.4	16.3
C-22	109.3	109.3	109.3	109.3	109.3	110.7	112.7
C-23	31.5	31.8	31.8	31.8	31.8	37.1	30.8
C-24	28.7	29.2	29.2	29.2	29.2	28.4	28.2
C-25	30.2	30.6	30.6	30.6	30.6	34.3	34.2
C-26	66.8	66.9	66.9	66.5	66.9	75.3	75.2
C-27	17.1	17.3	17.3	17.3	17.3	17.4	17.2
OCH_3							47.3

a) Measured in $CDCl_3$. *b*) Values in any column may be reversed, though those given here are preferred.

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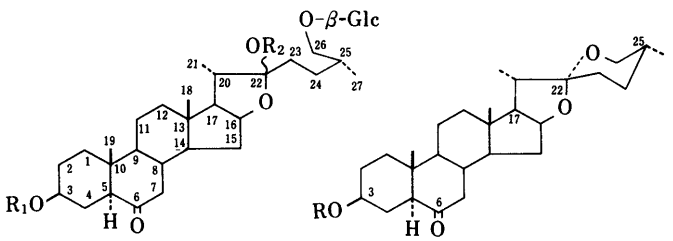
TABLE II. ^{13}C-NMR Chemical Shifts: Sugar Moiety

		5	6	7	4	1	3
C-3 sugars							
Glucose	1	102.3	102.1	102.2	102.1	102.1	102.1
	2	75.4	75.1[a]	75.2	74.9[a]	74.9[a]	74.9[a]
	3	78.7[a]	78.4	78.6	78.5	78.4[b]	78.5[b]
	4	71.9	80.9	72.4[a]	79.9	79.9	79.9
	5	78.6[a]	77.0[b]	77.1	75.0[a]	75.2[a]	75.2[a]
	6	63.0	62.0	69.8	68.1	68.1	68.1
Xylose	1		105.6		105.7[b]	105.1[c]	105.1[c]
	2		75.0[a]		74.9[a]	75.0[a]	75.0[a]
	3		76.3[b]		76.3	76.3	76.3
	4		70.9		71.1	71.1	71.1
	5		67.4		67.3	67.3	67.3
Arabinose	1			105.5	105.1[b]	104.9[c]	105.0[c]
	2			71.9[a]	72.6	72.5	72.5
	3			74.5	74.6[a]	74.9[a]	74.9[a]
	4			69.2	69.8	69.8	69.8
	5			66.9	67.3	67.3	67.3
C-26 sugar							
Glucose	1					105.7[c]	105.7[c]
	2					74.5[a]	74.6[a]
	3					78.6[b]	78.6[b]
	4					71.7	71.8
	5					78.4[b]	78.5[b]
	6					62.8	62.9

a—*c*) Values in any column may be reversed, though those given here are preferred.



R1 R2

1 : -β-Glc(4→β-Xyl, 6→α-Ara) -H

3 : -β-Glc(4→β-Xyl, 6→α-Ara) -CH3

R

2 : -H

4 : -β-Glc(4→β-Xyl, 6→α-Ara)

5 : -β-Glc

6 : -β-Glc(4→β-Xyl)

7 : -β-Glc(6→α-Ara)

Chart 1

acid hydrolysis of the crude saponin fraction, tigogenin, neotigogenin, and gitogenin were also isolated. Further studies on several minor glycosides are in progress.

Experimental

General Procedure The NMR spectra were taken on a JEOL JNM GX-270 spectrometer using tetramethylsilane as an internal standard. The FD-MS spectra were recorded on a JEOL JMS DX-300 mass spectrometer with an emitter heating current of 28—35 mA. Gas liquid chromatography (GLC) was run on a Shimadzu GC-9AM gas chromatograph. Identifications of resulting monosaccharides after acid hydrolysis were carried out as described in a previous paper.[8]

Extraction and Isolation of 1 Frozen, freshly bulbs of *A. chinense*, 1.7 kg (collected in Hiroshima, Japan), were crushed in MeOH and twice extracted with hot MeOH (3 l). A suspension of the MeOH extract in H_2O was applied to a column of MCI gel CHP20P (Mitsubishi Chem. Ind., Ltd.) (stepwise elution with H_2O, 20% aqueous MeOH, and MeOH). The fraction (2.5 g) eluted with MeOH was subjected to a combination of chromatographies on silica gel (solvent: $CHCl_3$–MeOH–H_2O (7:3:0.5, homogeneous)) and on MCI gel CHP20P (solvent: 63% aqueous MeOH) to afford a mixture of **1** and **3**. The mixture was heated with 30% aqueous acetone at 100 °C for 4 h, then the solution was concentrated to dryness to give **1** (yield: 0.01%).

Chinenoside I (**1**): White powder (from aqueous acetone), $[\alpha]_D^{25}$ −42.2° (*c*=0.66, pyridine). *Anal.* Calcd for $C_{49}H_{80}O_{23}\cdot 3H_2O$: C, 53.93; H, 7.94. Found: C, 53.98; H, 7.58. ^{1}H-NMR (pyridine-d_5) δ: 0.65 (3H, s), 0.84 (3H, s), 0.99 (3H, d, *J*=6.2 Hz), 1.35 (3H, d, *J*=7.0 Hz), 4.83 (1H, d, *J*=7.7 Hz), 4.95 (1H, d, *J*=7.7 Hz), 5.01 (1H, d, *J*=7.7 Hz), 5.50 (1H, d, *J*=7.7 Hz). FD-MS *m/z*: 1057 $(M-H_2O+K)^+$, 1041 $(M-H_2O+Na)^+$, 925 (1057 − pentosyl)$^+$, 909 (1041 − pentosyl)$^+$.

Formation of 3 A methanol solution (4 ml) of **1** (30 mg) was heated at 70 °C for 1 h, and then concentrated to dryness to afford **3**. **3**: ^{1}H-NMR (pyridine-d_5) δ: 0.64 (3H, s), 0.76 (3H, s), 1.01 (3H, d, J=6.6 Hz), 1.21 (3H, d, *J*=6.6 Hz), 3.27 (3H, s), 4.87 (1H, d, *J*=7.7 Hz), 4.95 (1H, d, *J*=7.7 Hz), 5.08 (1H, d, *J*=7.7 Hz), 5.51 (1H, d, *J*=7.7 Hz).

Acid Hydrolysis of 1 Glycoside **1** (50 mg) was heated with 2 N HCl–dioxane (1:1, 5 ml) in a sealed tube at 100 °C for 4 h. The reaction mixture was poured into H_2O, and then extracted with $CHCl_3$. After evaporation of $CHCl_3$, the residue was separated by silica gel chromatography (solvent: $CHCl_3$–MeOH (50:1)) to give **2** (15 mg). **2**: Colorless needles (from benzene), mp 206—208 °C (lit.[6] 210—212 °C). $[\alpha]_D^{25}$ −79.1° (*c*=0.34, $CHCl_3$). ^{1}H-NMR ($CDCl_3$) δ: 0.78 (3H×2, s, 18-, 19-CH_3), 0.79 (3H, d, *J*=6.7 Hz, 27-CH_3), 0.97 (3H, d, *J*=6.7 Hz, 21-CH_3), 3.57 (1H, tt, *J*=9.2, 4.6 Hz).

Enzymatic Hydrolysis of 1 A mixture of **1** (72 mg) and β-glucosidase (from almond, P. L. Biochemicals, 0.13 g) in acetate buffer (pH 4.1, 30 ml) was incubated at 37 °C for 5 h. The reaction mixture was diluted with H_2O and applied to a column of MCI gel CHP20P. The column was washed with H_2O and then eluted with MeOH. After evaporation of the MeOH, the residue was reprecipitated from MeOH–AcOEt to give **4**. **4**: White powder. *Anal.* Calcd for $C_{43}H_{68}O_{17}\cdot 2H_2O$: C, 57.83; H, 8.13. Found: C, 57.65; H. 8.03. ^{1}H-NMR (pyridine-d_5) δ: 4.95 (1H, d, *J*=8.1 Hz), 5.08 (1H, d, *J*=7.7 Hz), 5.51 (1H, d, *J*=8.0 Hz). FD-MS *m/z*: 879 $(M+Na)^+$.

Partial Hydrolysis of 4 Glycoside B (**4**, 25 mg) was heated with 5% aqueous sulfuric acid–EtOH (5:2, 3 ml) at 80 °C for 20 min. The reaction mixture was diluted with H_2O and applied to a column of MCI gel CHP20P (stepwise elution with H_2O and MeOH). The fraction eluted with MeOH was separated by chromatography on silica gel (solvent: $CHCl_3$–MeOH–H_2O (50:10:1, homogeneous)) to afford **2** (2 mg), **5** (2 mg), **6** (3 mg), and **7** (5 mg). **5**: FD-MS *m/z*: 615 $(M+Na)^+$. **6**: FD-MS *m/z*: 747 $(M+Na)^+$. **7**: FD-MS *m/z*: 747 $(M+Na)^+$.

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