Four New 26-Aminocholestane-Type Glycosides from *Solanum abutiloides*

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From the fresh roots of *Solanum abutiloides*, four new 26-aminocholesteryl glycosides were obtained, and their structures were characterized by analysis of their spectra data, including two-dimensional (2D) NMR spectroscopy. These compounds were regarded as key intermediates in the biogenesis of steroidal alkaloids.

Key words cholestane glycoside; abutiloside; *Solanum abutiloides*; biogenesis; steroidal alkaloid

Solanum abutiloides grows wild in South America. In the preceding paper,[1)] we have reported the structural characterization of abutilosides A (**6**) and B (**5**), being 26-aminocholestane-type glycosides, and abutilosides C, D, E, F (**7**) and G, being 26-hydroxycholestane-type glycosides, isolated from the fresh roots of *S. abutiloides*. These compounds were important as a key intermediate in the biogenesis of steroidal alkaloids. In a continuing study on glycosidic constituents, we have now isolated four 26-aminocholestane-type glycosides (**1**—**4**) from the fresh roots of *S. abutiloides*. This paper describes their structural elucidation.

The H_2O fraction of the MeOH extract was separated by MCI gel CHP20P, octadecyl silica gel (ODS) and silica gel column chromatographies, and finally by HPLC to give four new glycosides, named abutilosides H (**1**), I (**2**), J (**3**) and K (**4**).

Abutiloside H (**1**) was obtained as a white powder, $[\alpha]_D$ −107.0° (MeOH), and showed a quasi-molecular ion peak due to $[M+Na+H]^+$ at *m/z* 937 in the positive-ion FAB-MS. The ^{1}H-NMR spectra displayed two quaternary methyls at δ 0.71 and 0.91, three secondary methyls at δ 0.96 (d, J=6.1 Hz), 1.19 (d, J=6.7 Hz) and 1.66 (d, J=6.1 Hz), a NH proton at δ 8.38 (1H, t-like), an olefinic proton at δ 5.31 (1H, br s) and an acetyl methyl at δ 2.06, along with three anomeric protons at δ 4.97 (1H, d, J=7.9 Hz), 5.23 (1H, d, J=7.3 Hz) and 6.02 (1H, br s). The above data indicated that **1** was analogous to abutiloside B (**5**). In the ^{13}C-NMR data of **1**, signals due to the aglycone moiety, except for the signals of the A and B rings, and the sugar moiety were in good agreement with those of **5**. Furthermore, in the heteronuclear multiple bond correlation (HMBC) spectroscopy experiment, the methyl proton signal at δ 0.91 (H-19) showed long-range correlation with δ 140.9 (C-5 of aglycone). From the data presented above, the structure of **1** was elucidated as 26-acetylamino-3β,16α-dihydroxy-cholest-5-en-22-one 3-*O*-β-D-xylopyranosyl-(1→2)-α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranoside.

Abutiloside I (**2**) was obtained as a white powder, $[\alpha]_D$ −38.7° (MeOH). In the NMR data of **2**, signals due to the aglycone moiety were in good agreement with those of **6**, although signals due to the sugar moiety were not identical. Meanwhile, the positive-ion FAB-MS of **2** gave a quasi-molecular ion peak at *m/z* 848, which was lower by 132 mass units than that of **6**. This evidence suggested that its sugar moiety was composed of a rhamnosyl–glucosyl unit. Furthermore, in the NMR data of **2**, signals due to the sugar moiety were almost superimposable on those attached to the C-3 hydroxyl group of abutiloside F (**7**). Consequently, the structure of **2** was elucidated as a 26-aminocholesteryl glycoside with a terminal xylose absent from **6**.

Abutiloside J (**3**) was obtained as a white powder, $[\alpha]_D$ −54.1° (MeOH), and showed a clustered molecular ion at *m/z* 966.5416 $[C_{48}H_{81}NO_{17}Na]^+$ in the positive high-resolution (HR) FAB-MS. The ^{1}H-NMR spectra displayed two quaternary methyls at δ 0.68 and 0.69, three secondary methyls

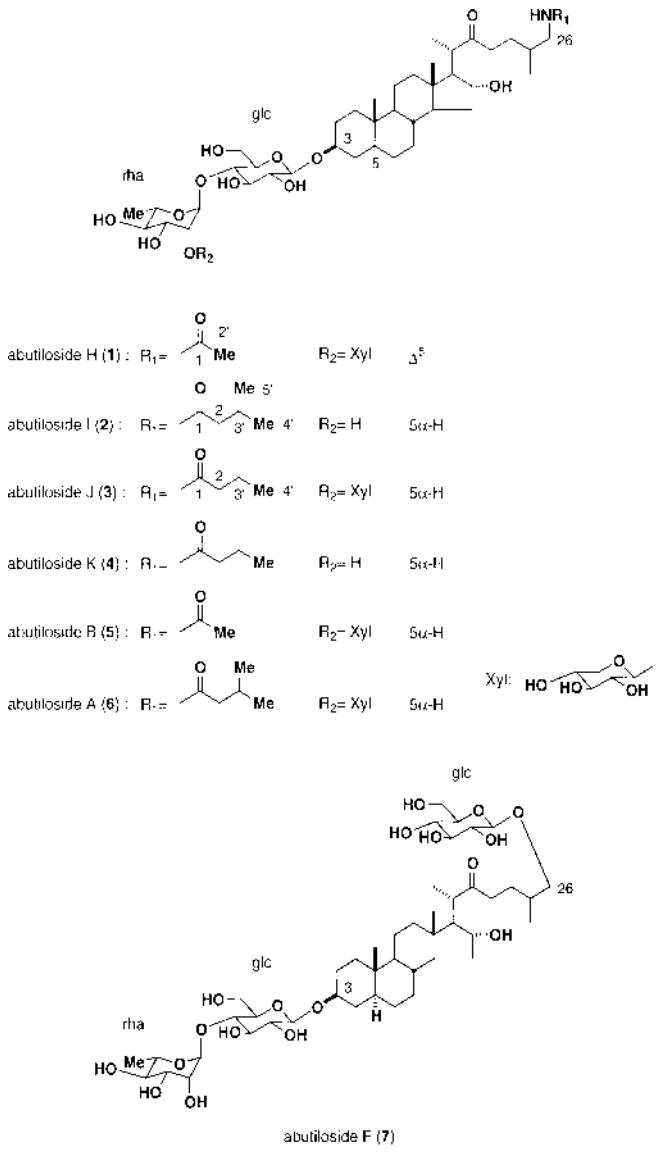

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Table 1. ^{13}C-NMR Data for **1**—**7** (Pyridine-d_5)

	1	**2**	**3**	**4**		**1**	**2**	**3**	**4**
C-1	37.3	37.1	37.0	37.0	C-1′	170.2	172.6	173.1	173.1
C-2	30.2	29.9	29.9	29.9	C-2′	23.1	46.0	38.6	38.6
C-3	78.2	77.4	77.3	77.4	C-3′		26.4	19.7	19.7
C-4	39.3	34.8	34.8	34.8	C-4′		22.7	14.0	14.0
C-5	140.9	44.6	44.5	44.6	C-5′		22.7		
C-6	121.8	28.9	28.9	28.9	Glc-1	102.4	102.7	102.0	102.7
C-7	32.2	32.3	32.2	32.3	Glc-2	75.6	75.5	75.5	75.5
C-8	31.6	35.1	35.1	35.1	Glc-3	76.6	76.7	76.6	76.7
C-9	50.3	54.5	54.4	54.4	Glc-4	77.7	78.5	77.8	78.5
C-10	36.9	35.7	35.7	35.7	Glc-5	77.4	77.2	77.4	77.2
C-11	20.9	21.1	21.0	21.1	Glc-6	61.7	61.6	61.7	61.6
C-12	39.9	40.2	40.1	40.2	Rha-1	101.2	102.1	101.2	102.1
C-13	44.1	44.4	44.4	44.4	Rha-2	81.9	72.6	81.9	72.6
C-14	54.0	53.7	53.7	53.7	Rha-3	72.9	72.8	72.9	72.8
C-15	37.1	37.1	37.0	37.1	Rha-3	72.9	72.8	72.9	72.8
C-16	76.0	76.0	75.9	75.9	Rha-4	74.5	74.0	74.4	74.0
C-17	63.3	63.4	63.4	63.4	Rha-5	70.0	70.4	69.9	70.4
C-18	13.4	13.7	13.6	13.7	Rha-6	18.4	18.5	18.3	18.5
C-19	19.4	12.3	12.2	12.3	Xyl-1	107.6		107.6	
C-20	49.3	49.3	49.3	49.3	Xyl-2	75.6		75.5	
C-21	16.8	16.7	16.7	16.7	Xyl-3	78.5		78.4	
C-22	214.6	214.7	214.7	214.7	Xyl-4	71.0		71.0	
C-23	38.8	38.8	38.7	38.7	Xyl-5	67.4		67.4	
C-24	28.4	28.4	28.3	28.4					
C-25	33.4	33.5	33.4	33.5					
C-26	45.3	45.0	45.0	45.0					
C-27	18.2	18.3	18.3	18.3					

at δ 0.98 (d, J=6.7 Hz), 1.19 (d, J=6.7 Hz) and 1.66 (d, J=6.1 Hz), a NH proton at δ 8.28 (1H, t-like) and propyl protons (3H, t, J=7.3 Hz at δ 0.92, 2H, m, at δ 1.83 and 2H, t, J=7.3 Hz at δ 2.32), along with three anomeric protons at δ 4.98 (1H, d, J=7.3 Hz), 5.22 (1H, d, J=7.3 Hz) and 6.01 (1H, br s). A comparative study of the above ^{1}H-NMR spectrum of **3** with that of **6** led to the assignment of signals as shown in the experimental section. In the ^{13}C-NMR spectrum of **3**, signals due to the aglycone moiety, except for those signals owed to a butyl group, and the sugar moiety were also in good agreement with those of **6**. Consequently, the structure of **3** was determined to involve the replacement of a butyl group instead of an isopentyl group attached to the amino group at C-26 in **6**.

Abutiloside K (**4**) was obtained as a white powder, $[\alpha]_D$ −50.4° (MeOH), and showed a quasi-molecular ion peak due to $[M+Na]^-$ at m/z 834, which was lower by 132 mass units than that of **3**, in the positive-ion FAB-MS. In the NMR data of **4**, signals due to an aglycone moiety were consistent with those of **3**, while signals due to a sugar moiety were almost superimposable on those of **2**. From the above evidence, the structure of **4** was elucidated to be a 26-aminocholesteryl glycoside with a terminal xylose absent from **3**.

Kaneko *et al.* presented a hypothetical biogenetic pathway of solanidine in *Veratrum*.[2] These compounds (**1**—**4**) were regarded as comparable to the key intermediate just after the incorporation of nitrogen into dormantinone to change into verazine. Further, **1** was a corresponding 5-ene type compound to **5**, and was a very important key intermediate in the biogenesis of 5-ene type steroidal alkaloids. Consequently, we first confirmed the existence of a 5-ene type compound in the roots of *S. abutiloides*.

Experimental

General Procedures Optical rotations were taken with a JASCO DIP-1000 automatic digital polarimeter. The NMR spectra were measured with a JEOL alpha 500 NMR spectrometer, and chemical shifts are given on a δ (ppm) scale with tetramethylsilane (TMS) as an internal standard. The FAB-MS was measured with a JEOL DX-303 HF spectrometer. HPLC was carried out using a TSK gel-120A (7.8 mm i.d.×30 cm) column with a Tosoh CCPM pump and Tosoh RI-8010 differential refractometer as a detector. TLC was performed on pre-coated Kieselgel 60 F_{254} (Merck), and detection was achieved by spraying it with 10% H_2SO_4, followed by heating. Column chromatography was carried out on Kieselgel (230—400 mesh, Merck), ODS (PrePAK-500/C_{18}, Waters) and MCI gel CHP20P (Mitsubishi Chemical Ind.).

Plant Material *S. abutiloides* were cultivated at the Botanical Garden of Kumamoto University. The roots (2.2 kg) were harvested in October,1996, and identified by Dr. T. Yoshida. A voucher specimen is deposited in the National Research Institute of Vegetables, Ministry of Agriculture, Forestry and Fisheries, Ano, Mie in Japan.

Extraction and Separation The plant material was extracted with MeOH at room temperature for six months, and the extract (92.6 g) was partitioned in AcOEt and water (1 : 1). The water-soluble portion (87.6 g) was subjected to MCI gel CHP20P column chromatography with MeOH–H_2O (20→30→40→50→60→70→80→90→100%) to afford ten fractions (fr. 1—10). Fraction 5 (1.7 g) was further separated by ODS column chromatography with MeOH–H_2O (60→70%) and by silica gel column chromatography with $CHCl_3$–MeOH–H_2O (7 : 3 : 0.5), followed by HPLC with MeOH–H_2O (13 : 7), to furnish abutiloside H (**1**) (5 mg). Fraction 6 (2.8 g) was subjected to ODS column chromatography with MeOH–H_2O (60→70→80%) to afford five fractions (fr. 11—15). Fraction 12 was further separated by silica gel column chromatography with $CHCl_3$–MeOH–H_2O (7 : 3 : 0.5), followed by HPLC with MeOH–H_2O (7 : 3), to furnish abutiloside J (**3**) (22 mg). Fraction 14 was further separated by silica gel column chromatography with $CHCl_3$–MeOH–H_2O (8 : 2 : 0.2), followed by HPLC with MeOH–H_2O (7 : 3), to furnish abutilosides I (**2**) (4 mg) and K (**4**) (5 mg).

Abutiloside H (**1**): A white powder, $[\alpha]_D^{25}$ −107.0° (c=0.20, MeOH). Pos. FAB-MS (m/z): 937 $[M+Na+H]^+$. ^{1}H-NMR (pyridine-d_5): δ 0.71 (3H, s, H_3-18), 0.91 (3H, s, H_3-19), 0.96 (3H, d, J=6.1 Hz, H_3-27), 1.19 (3H, d, J=6.7 Hz, H_3-21), 2.06 (3H, s, H_3-2′), 3.28 (1H, m, H_a-26), 3.46 (1H, m, H_b-26), 3.82 (1H, br d, J=9.8 Hz, H-3), 5.31 (1H, br s, H-6), 8.38 (1H, t-like,

NH); glc-1 to glc-6, 4.97 (1H, d, J=7.9 Hz), 3.98 (1H, dd, J=8.5, 8.5 Hz), 4.32 (1H, dd, J=9.2, 9.2 Hz), 4.46 (1H, dd, J=9.2, 9.2 Hz), 3.87 (1H, m), 4.29 (1H, dd, J=5.5, 11.4 Hz), 4.39 (1H, br d, J=11.6 Hz); rha-1 to rha-6, 6.02 (1H, br s), 4.68 (1H, br s), 4.58 (1H, br d, J=9.2 Hz), 4.32 (1H, dd, J=9.2, 9.2 Hz), 5.00 (1H, m), 1.66 (3H, d, J=6.1 Hz); xyl-1 to xyl-5, 5.23 (1H, d, J=7.3 Hz), 4.07 (1H, dd, J=7.9, 8.5 Hz), 4.12 (1H, dd, J=8.5, 8.5 Hz), 4.15 (1H, m), 3.66 (1H, dd, J=9.8, 10.3 Hz), 4.29 (1H, overlapped). ^{13}C-NMR (pyridine-d_5): Table 1.

Abutiloside I (**2**): A white powder, $[\alpha]_D^{25}$ −38.7° (c=0.15, MeOH). Pos. FAB-MS (m/z): 848 $[M+Na]^+$. ^{1}H-NMR (pyridine-d_5): δ 0.69 (3H, s, H_3-18), 0.68 (3H, s, H_3-19), 0.97 (6H, t, J=7.3 Hz, H_3-4′ and H_3-5′), 0.98 (3H, d, J=6.1 Hz, H_3-27), 1.19 (3H, d, J=6.7 Hz, H_3-21), 2.23 (2H, t, J=6.7 Hz, H_2-2′), 2.33 (2H, m, H_2-3′), 3.31 (1H, m, H_a-26), 3.49 (1H, m, H_b-26), 3.80 (1H, br d, J=9.2 Hz, H-3), 8.28 (1H, t-like, NH); glc-1 to glc-6, 4.97 (1H, d, J=7.3 Hz), 3.98 (1H, dd, J=8.5, 8.5 Hz), 4.25 (1H, dd, J=8.5, 9.2 Hz), 4.45 (1H, dd, J=9.2, 9.2 Hz), 3.90 (1H, m), 4.17 (1H, dd, J=3.7, 12.2 Hz), 4.32 (1H, br d, J=9.8 Hz); rha-1 to rha-6, 5.89 (1H, br s), 4.71 (1H, d, J=3.7 Hz), 4.59 (1H, dd, J=3.7, 9.2 Hz), 4.35 (1H, dd, J=9.2, 9.2 Hz), 5.03 (1H, m), 1.73 (3H, d, J=6.1 Hz). ^{13}C-NMR (pyridine-d_5): Table 1.

Abutiloside J (**3**): A white powder, $[\alpha]_D^{25}$ −54.1° (c=0.95, MeOH). HR-FAB-MS (m/z): 966.5416 $[M+Na]^+$ (Calcd for $C_{48}H_{81}NO_{17}Na$ 966.5403). ^{1}H-NMR (pyridine-d_5): δ 0.69 (3H, s, H_3-18), 0.68 (3H, s, H_3-19), 0.92 (3H, t, J=7.3 Hz, H_3-4′), 0.98 (3H, d, J=6.7 Hz, H_3-27), 1.19 (3H, d, J=6.7 Hz, H_3-21), 1.83 (2H, m, H_2-3′), 2.33 (2H, t, J=7.3 Hz, H_2-2′), 3.32 (1H, m, H_a-26), 3.48 (1H, m, H_b-26), 3.88 (1H, br d, J=10.4 Hz, H-3), 8.28 (1H, t-like, NH); glc-1 to glc-6, 4.98 (1H, d, J=7.3 Hz), 3.97 (1H, dd, J=8.5, 8.5 Hz), 4.24 (1H, dd, J=9.2, 9.2 Hz), 4.45 (1H, dd, J=9.2, 9.2 Hz), 3.91 (1H, m), 4.25 (1H, overlapped), 4.44 (1H, br d, J=9.2 Hz); rha-1 to rha-6, 6.01 (1H, br s), 4.68 (1H, br s), 4.58 (1H, br d, J=9.2 Hz), 4.26 (1H, dd, J=9.2, 9.2 Hz), 5.01 (1H, m), 1.66 (3H, d, J=6.1 Hz); xyl-1 to xyl-5, 5.22 (1H, d, J=7.3 Hz), 4.06 (1H, dd, J=7.9, 7.9 Hz), 4.12 (1H, dd, J=8.5, 8.5 Hz), 4.14 (1H, m), 3.66 (1H, t, J=10.4 Hz), 4.27 (1H, overlapped). ^{13}C-NMR (pyridine-d_5): Table 1.

Abutiloside K (**4**): A white powder, $[\alpha]_D^{25}$ −50.4° (c=0.25, MeOH). Pos. FAB-MS (m/z): 834 $[M+Na]^+$. ^{1}H-NMR (pyridine-d_5): δ 0.69 (3H, s, H_3-18), 0.68 (3H, s, H_3-19), 0.92 (3H, t, J=7.3 Hz, H_3-4′), 0.97 (3H, d, J=6.1 Hz, H_3-27), 1.19 (3H, d, J=6.7 Hz, H_3-21), 1.83 (2H, m, H_2-3′), 2.31 (2H, t, J=7.3 Hz, H_2-2′), 3.31 (1H, m, H_a-26), 3.47 (1H, m, H_b-26), 3.80 (1H, br d, J=9.2 Hz, H-3), 8.26 (1H, t-like, NH); glc-1 to glc-6, 4.96 (1H, d, J=7.3 Hz), 3.98 (1H, dd, J=7.9, 8.5 Hz), 4.24 (1H, dd, J=8.5, 9.2 Hz), 4.45 (1H, dd, J=9.2, 9.2 Hz), 3.90 (1H, m), 4.16 (1H, dd, J=3.7, 12.2 Hz), 4.32 (1H, br d, J=9.2 Hz); rha-1 to rha-6, 5.89 (1H, br s), 4.70 (1H, d, J=3.7 Hz), 4.58 (1H, dd, J=3.7, 9.2 Hz), 4.34 (1H, dd, J=9.2, 9.2 Hz), 5.02 (1H, m), 1.73 (3H, d, J=6.1 Hz). ^{13}C-NMR (pyridine-d_5): Table 1.

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