Cholestane Glycosides from *Solanum abutiloides*[1)]

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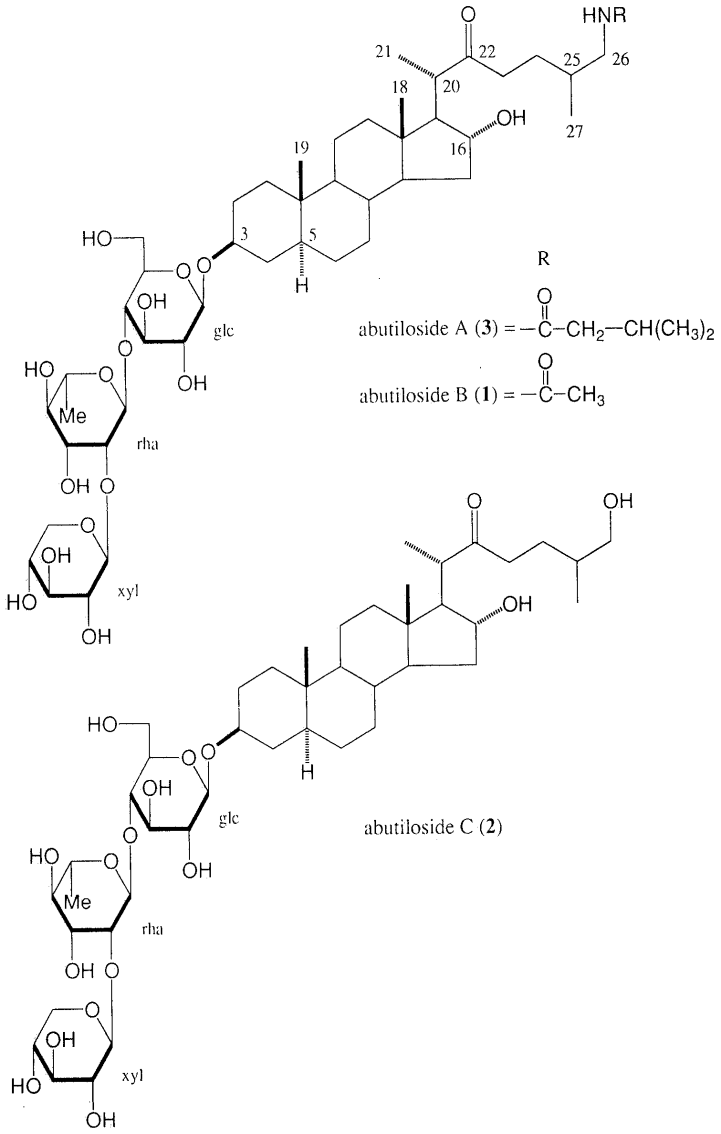
From the roots of *Solanum abutiloides*, glycosides of 26-hydroxy- and 26-aminocholestane were obtained and their structures were characterized. Both of them were regarded as key intermediates in the biogenesis of steroidal alkaloids.

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

In the preceding paper,[2)] we reported the chemical structure of a 26-aminocholesteryl glycoside, abutiloside A, isolated from the roots of *Solanum abutiloides*, and its importance as a key intermediate in the biogenesis of steroidal alkaloids. Successively, we obtained two related compounds named abutilosides B and C, and we deal with their chemical characterization in this paper.

Abutiloside B (**1**), a white powder, $[\alpha]_D$ −41.9° (MeOH), showed a *quasi*-molecular peak $[M+Na+H]^+$ at *m/z* 939 in the FAB-MS, and strong absorption due to a hydroxyl group at 3413 cm^{-1}, a carbonyl group at 1697 cm^{-1} and an amide group at 1643 cm^{-1} in the IR spectrum. The ^{1}H-NMR spectrum of **1** displayed signals due to two tertiary methyl groups at δ 0.68 and 0.69, two secondary methyl groups at δ 0.96 (d, J=6.71 Hz) and 1.19 (d, J=7.32 Hz), a NH group at δ 8.33 (1H, t-like)



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and an acetyl methyl group at δ 2.05 along with three anomeric proton signals at δ 4.98 (1H, d, J=7.33 Hz), 5.23 (1H, d, J=7.93 Hz) and 6.02 (1H, s). A comparative study of the above ^{1}H-NMR spectrum of **1** with that of abutiloside A (**3**)[2] led to the assignments of signals as shown in the experimental section. The ^{13}C-NMR signals exhibited a total of 46 carbon signals, in which characteristic signals were attributed as follows by comparison with those of **3**: δ 77.3 (C-3), 76.0 (C-16), 13.7 (C-18), 12.3 (C-19), 214.6 (C-22), 45.3 (C-26), 23.1, 170.1 (acetyl group). Moreover, signals due to a glycosidic moiety were superimposed on those of **3**, indicating the presence of β-D-xylopyranosyl-(1→2)-α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranoside. Based on the above evidence, the structure of **1** was elucidated as a 26-aminocholesteryl glycoside with the configurations at C-3 and -16 being the same as those of **3**, and replaced by an acetyl group instead of an isopentyl group attached to the amino group at C-26 in **3**, as shown in the formula.

Abutiloside C (**2**) was obtained as a white powder, $[\alpha]_D$ −54.2° (MeOH) and showed strong absorption due to a hydroxyl group at 3409 cm^{-1} and a carbonyl group at 1693 cm^{-1} in the IR spectrum and signals due to two tertiary methyl groups at δ 0.68 and 0.69, and two secondary methyl groups at δ 1.08 (d, J=6.71 Hz) and 1.20 (d, J=6.72 Hz) together with three anomeric protons at δ 4.98 (1H, d, J=7.33 Hz), 5.22 (1H, d, J=7.33 Hz), and 6.01 (1H, s) in the ^{1}H-NMR. The time-of-flight (TOF)-MS exhibited *quasi*-molecular ion peaks at m/z 913 $[M+K]^+$ and 898 $[M+Na]^+$. In comparing the ^{13}C-NMR spectrum of **2** with that of **1**, a signal appeared at δ 67.3 instead of the disappearance of a methylene signal

Table 1. ^{13}C-NMR Data for **1**, **2** and **3**

	1	2	3		1	2	3
C-1	37.0	37.0	37.1	C-26	45.3	67.3	45.0
C-2	29.9	29.9	29.9	C-27	18.2	17.3	18.3
C-3	77.3	77.3	77.3	C-1′	170.1		172.6
C-4	34.8	34.8	34.8	C-2′	23.1		46.0
C-5	44.5	44.5	44.6	C-3′			26.4
C-6	28.9	28.9	28.9	C-4′			22.7
C-7	32.3	32.2	32.3	C-5′			22.7
C-8	35.1	35.1	35.1	glc-1	102.0	102.0	102.0
C-9	54.4	54.4	54.4	glc-2	75.6	75.5	75.5
C-10	35.7	35.7	35.7	glc-3	76.6	76.6	76.6
C-11	21.1	21.0	21.1	glc-4	77.8	77.8	77.8
C-12	40.2	40.1	40.2	glc-5	77.4	77.3	77.3
C-13	44.4	44.4	44.4	glc-6	61.7	61.7	61.7
C-14	53.7	53.7	53.7	rha-1	101.2	101.2	101.2
C-15	37.0	36.9	37.0	rha-2	81.9	81.9	81.9
C-16	76.0	75.9	76.0	rha-3	73.0	72.8	72.9
C-17	63.4	63.4	63.4	rha-4	74.5	74.4	74.4
C-18	13.7	13.7	13.7	rha-5	70.0	69.9	69.9
C-19	12.3	12.3	12.3	rha-6	18.4	18.4	18.4
C-20	49.3	49.3	49.3	xyl-1	107.6	107.5	107.6
C-21	16.7	16.8	16.8	xyl-2	75.6	75.5	75.5
C-22	214.6	215.0	214.7	xyl-3	78.5	78.4	78.4
C-23	38.8	39.1	38.8	xyl-4	71.0	71.0	71.0
C-24	28.4	27.9	28.4	xyl-5	67.4	67.4	67.4
C-25	33.4	36.1	33.4				

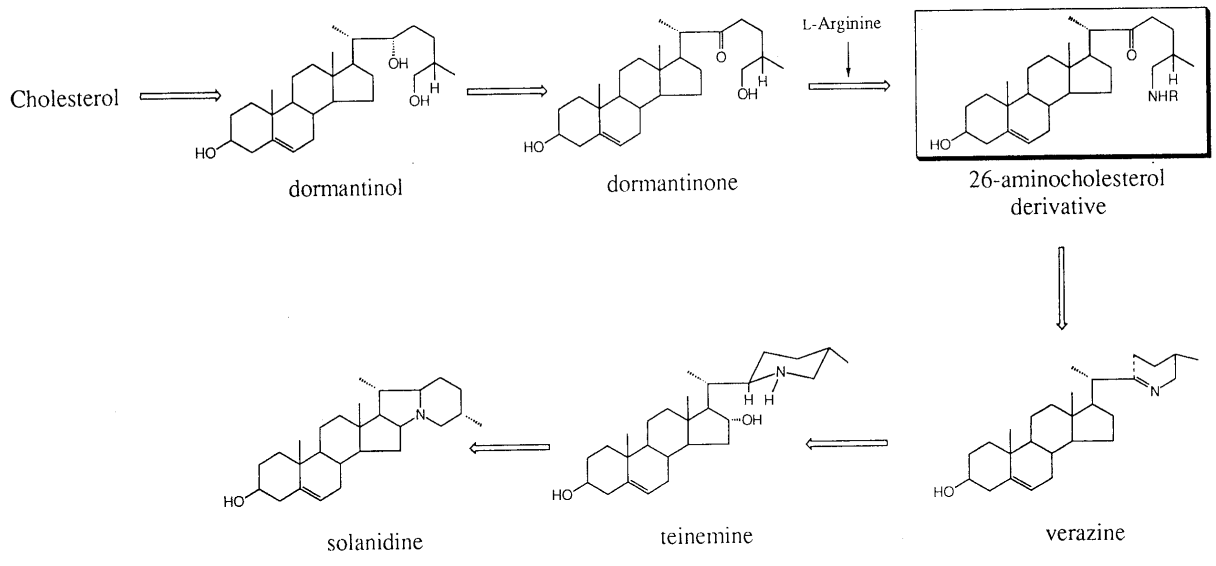

Chart 1

at δ 45.3 assignable to C-26 in **1**, suggesting the occurrence of a hydroxylmethyl group at C-25 in **2**. Except for the disappearance of the acetyl group and the difference of the chemical shift at C-26, all chemical shifts of **1** and **2** were almost superimposable on each other, including a sugar residue; thus, the chemical structure of **2** was determined to be 3-*O*-β-D-xylopyranosyl-(1→2)-α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl 3β,16α,26-trihydroxy-5α-cholestan-22-one. Regarding the configuration at C-25 in **1**—**3**, it was deduced to be *R* due to the coexistence of dioscin and solamargine in this plant, however, this remains to be solved.

Kaneko *et al.* presented a hypothetical biogenetic pathway of solanidine in *Veratrum*, as shown in Chart 1,[3] in which they indicated the possibility that L-arginine was incorporated in the production of verazine. Compounds **1** and **2** obtained here were regarded as comparable to the key intermediate just after the incorporation of nitrogen into dormantinone to change into verazine and dormantinone, respectively. Probably, **1** and **3** couldn't participate in cyclization to the imino-compound owing to acylation prior to cyclization into the verazine-type. The occurrence of the different sugar residues in **1**—**3** with that of dioscin and solamargine may be due to a difference in enzymes for glycosidation to the cholestane and furostane/spirostane sapogenols. Futhermore, the coexistence of corresponding 5-ene compounds to **1**—**3** is also certified.

Experimental

Optical rotations were measured on a JASCO DIP-360 automatic digital polarimeter. IR spectra were recorded with a JEOL FT-IR spectrometer, JIR-6500W. The ^{1}H- and ^{13}C-NMR spectra were measured with a JEOL JUM-GX 270, 400 NMR spectrometer, and chemical shifts are given using δ (ppm) values with tetramethylsilane as an internal standard. The FAB-MS was recorded with a JEOL DX-300 spectrometer. TLC was performed on pre-coated Kieselgel 60 F_{254} plates (Merck). Column chromatography was carried out with MCI gel CHP 20P (Mitsubishi Chemical Ind.), Kieselgel 60 (70—230 mesh and 230—400 mesh, Merck) and Chromatorex ODS-DU 3050MT (Fuji Silysia).

Isolation of Abutilosides B (1) and C (2) The seeds identified as *Solanum abutiloides* at the National Research Institute of Vegetables, Ministry of Agriculture, Forestry and Fisheries, Ano, Mie in Japan were cultivated at the Botanical Garden of Kumamoto University in 1993. The harvested roots (2.72 kg) of this plant were extracted with refluxing methanol, and the resulting extract was then evaporated to dryness to give a residue (106.2 g) which was subsequently partitioned between benzene and water. The aqueous layer was evaporated to dryness to give a residue (93.8 g) which was subjected to CHP-20P column chromatography and eluted with water to MeOH and MeOH+2.8% NH_4OH to provide six fractions. These fractions were further separated and purified using silica gel with a $CHCl_3$–MeOH–water solvent system and Chromatorex ODS with 40% MeOH to MeOH, gradiently, to afford abutilosides A (**3**, 138 mg), B (**1**, 40 mg) and C (**2**, 38 mg) together with solamargine (337 mg) and dioscin (11 mg), which was obtained in a corresponding spirostanol style after the treatment of β-glucosidase during a separation procedure due to a complex mixture.

Abutiloside B (1) A white solid, $[\alpha]_D^{26}$ −41.9° (*c*=0.49, MeOH). IR ν_{max}^{KBr} cm^{-1}: 3413 (OH), 1697 (C=O), 1643 (amide). Positive FAB-MS *m/z*: 939 [M+Na+H$^+$]. ^{1}H-NMR (in pyridine-d_5) δ: 0.68 (3H, s, Me-18), 0.69 (3H, s, Me-19), 0.96 (3H, d, *J*=6.71 Hz, Me-27), 1.19 (3H, d, *J*=7.32 Hz, Me-21), 1.67 (3H, d, *J*=6.10 Hz, rha Me-6), 2.05 (3H, s, Me-2′), 3.28 (1H, m, H_a-26), 3.46 (1H, m, H_b-26), 4.98 (1H, d, *J*=7.33 Hz, glc H-1), 5.23 (1H, d, *J*=7.93 Hz, xyl H-1), 6.02 (1H, s, rha H-1), 8.33 (1H, t-like, NH). ^{13}C-NMR (in pyridine-d_5): Table 1.

Abutiloside C (2) A white solid, $[\alpha]_D^{32}$ −54.2° (*c*=0.53, MeOH). IR ν_{max}^{KBr} cm^{-1}: 3409 (OH), 1693 (C=O). TOF-MS *m/z*: 913 [M+K]$^+$, 897 [M+Na]$^+$. ^{1}H-NMR (in pyridine-d_5) δ: 0.68 (3H, s, Me-18), 0.69 (3H, s, Me-19), 1.08 (3H, d, *J*=6.71 Hz, Me-27), 1.20 (3H, d, *J*=6.72 Hz, Me-21), 1.66 (3H, d, *J*=6.10 Hz, rha Me-6), 3.61—3.69 (2H, m, H_2-26), 4.98 (1H, d, *J*=7.33 Hz, glc H-1), 5.22 (1H, d, *J*=7.33 Hz, xyl H-1), 6.01 (1H, s, rha H-1). ^{13}C-NMR (in pyridine-d_5): Table 1.

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References and Notes

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