Cholestane Glycosides from *Solanum abutiloides*. III

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Four new cholestane glycosides, named abutilosides D (1), E (2), F (3) and G (4), were isolated from the fresh roots of *Solanum abutiloides*. By chemical and spectroscopic evidence, their structures were elucidated as 26-*O*- β -D-glucopyranosyl 3 β ,16 α ,26-trihydroxy-5 α -cholestan-22-one 3-*O*- β -D-xylopyranosyl-(1 $\rightarrow$ 2)- α -L-rhamnopyranosyl-(1 $\rightarrow$ 4)- β -D-glucopyranoside and its desxylosyl compound (1 and 3, respectively), 26-*O*- β -D-glucopyranosyl 3 β ,16 α ,26-trihydroxycholest-5-en-22-one 3-*O*- β -D-xylopyranosyl-(1 $\rightarrow$ 2)- α -L-rhamnopyranosyl-(1 $\rightarrow$ 4)- β -D-glucopyranoside and its desxylosyl compound (2 and 4, respectively). These compounds were regarded as precursors of a dormantinone type compound on a hypothetical pathway of steroid biogenesis.

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

In the preceding paper,¹⁾ we reported the isolation and structural determination of three cholestane glycosides, named abutilosides A, B and C (5), from the fresh roots of *Solanum abutiloides*. These compounds were important as a key intermediate in the biogenesis of steroidal alkaloids. In a continuing study on glycosidic constituents, we obtained four additional new cholestane glycosides, named abutilosides D (1), E (2), F (3) and G (4). This paper describes their structural characterization.

The methanol extract of the fresh roots of *Solanum abutiloides* was partitioned into an AcOEt–water solvent system. MCI gel CHP-20P column chromatography of the water soluble portion provided the glycosidic constituents, which were purified by using a combination of Sephadex LH-20, silica gel and octadecyl silica gel (ODS) column chromatographies to furnish four abutilosides (1–4).

Abutiloside D (1) was obtained as a white powder, $[\alpha]_D^{25}$ –56.6° (MeOH), and showed an ion peak due to $[M-H]^-$ at m/z 1035 in the neg. FAB-MS. The ¹H-NMR spectrum of 1 displayed two tertiary methyls at δ 0.67 and 0.68, three secondary methyls at δ 0.98 (d, $J=6.1$ Hz), 1.19 (d, $J=6.7$ Hz) and 1.66 (d, $J=6.7$ Hz), and four anomeric protons at δ 4.82 (1H, d, $J=7.9$ Hz), 4.98 (1H, d, $J=7.3$ Hz), 5.23 (1H, d, $J=7.9$ Hz) and 6.02 (1H, br s). The above ¹H-NMR spectrum of 1 was similar to that of abutiloside C (5). In the ¹³C-NMR spectrum of 1, the signals due to the aglycone moiety, except for C-24, C-25 and C-26 at the side chain, and the sugar moiety were also in good agreement with those of 5. Meanwhile, the neg. FAB-MS of 1 gave a quasi-molecular peak at m/z 1035, which was higher by 162 mass units than that of 5. Furthermore, a comparative study of the ¹³C-NMR spectrum of 1 with that of 5 indicated the presence of an additional glucosyl unit in 1, which was linked to the C-26 hydroxy group of an aglycone moiety. On acid hydrolysis, 1 afforded D-glucose, D-xylose and L-rhamnose, together with several unidentified artificial sapogenoles.²⁾ The ¹H- and ¹³C-NMR spectrum of 1, which could be assigned with the aid of ¹H–¹H correlation spectroscopy (COSY), heteronuclear multiple quantum coherence (HMQC), total correlation spectroscopy (TOCSY) and heteronuclear multiple bond correlation spectroscopy (HMBC) techniques, showed signals due to a tetrasaccharide moiety consisting of two glucopyranosyl moieties, one xylopyranosyl moiety and one rhamnopyra-

nosyl moiety. The HMBC experiment showed that the trisaccharide and monosaccharide moieties were linked to the C-3 and C-26 hydroxy groups of the aglycone, respectively. Moreover, the anomeric proton signals at δ 4.82, 4.98, 6.02 and 5.23 showed long-range correlations with the ¹³C signals at δ 75.1 (C-26 of aglycone), 77.3 (C-3 of aglycone), 77.8 (C-4 of 4-substituted glucose) and 81.9 (C-2 of 2-substituted rhamnose), respectively. From the above evidence, the structure of 1 was concluded to be 26-*O*- β -D-glucopyranosyl 3 β ,16 α ,26-trihydroxy-5 α -cholestan-22-one 3-*O*- β -D-xylopyranosyl-(1 $\rightarrow$ 2)- α -L-rhamnopyranosyl-(1 $\rightarrow$ 4)- β -D-glucopyranoside.

Abutiloside E (2) was obtained as a white powder, $[\alpha]_D^{25}$ –60.5° (MeOH), and showed an ion peak due to $[M-H]^-$ at m/z 1033, which was lower by 2 mass units than that of 1, in the neg. FAB-MS. The ¹H-NMR spectrum of 2 displayed two tertiary methyls at δ 0.70 and 0.91, three secondary methyls at δ 0.98 (d, $J=6.7$ Hz), 1.20 (d, $J=6.7$ Hz) and 1.66 (d, $J=6.1$ Hz), an olefinic proton at δ 5.30 (1H, br s) and four anomeric protons at δ 4.81 (1H, d, $J=7.9$ Hz), 4.98 (1H, d, $J=7.3$ Hz), 5.23 (1H, d, $J=7.9$ Hz) and 6.02 (1H, br s). Based on the above ¹H-NMR data, the structure of 2 was considered to be analogous to that of 1. In the ¹³C-NMR spectrum of 2, the chemical shifts of the aglycone moiety, except for the signals owing to the A and B rings, and the sugar moiety showed coincidence with those of 1. This evidence indicated the presence of a double bond in the B ring. In the HMBC experiment, the methyl proton signal at δ 0.91 (H-19) showed long-range correlations with δ 140.9 (C-5 of aglycone). Consequently, the structure of 2 was determined to be 26-*O*- β -D-glucopyranosyl 3 β ,16 α ,26-trihydroxycholest-5-en-22-one 3-*O*- β -D-xylopyranosyl-(1 $\rightarrow$ 2)- α -L-rhamnopyranosyl-(1 $\rightarrow$ 4)- β -D-glucopyranoside.

Abutiloside F (3) was obtained as a white powder, $[\alpha]_D^{25}$ –51.8° (MeOH), and showed an ion peak due to $[M-H]^-$ at m/z 903, which was lower by 132 mass units than that of 1, in the neg. FAB-MS. The ¹H-NMR spectrum of 3 displayed two tertiary methyls at δ 0.68 and 0.69, three secondary methyls at δ 0.98 (d, $J=6.1$ Hz), 1.19 (d, $J=6.7$ Hz) and 1.73 (d, $J=6.1$ Hz), and three anomeric protons at δ 4.82 (1H, d, $J=7.9$ Hz), 4.98 (1H, d, $J=7.3$ Hz) and 5.90 (1H, br s). In the ¹³C-NMR spectrum of 3, signals due to the aglycone moiety were in good agreement with those of 1, although the signals

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Table 1. ^1H -NMR Chemical Shifts for Oligosaccharide Moieties of 1—4 (Pyridine- d_5)

	1	2	3	4
3-O-				
Glc-1	4.98 d (7.3)	4.98 d (7.3)	4.98 d (7.3)	4.98 d (7.3)
Glc-2	3.99 dd (7.3, 9.1)	3.99 dd (8.5, 8.5)	4.01 dd (7.3, 9.1)	4.00 dd (7.3, 9.1)
Glc-3	4.22	4.22 dd (8.5, 8.5)	4.17 dd (9.1, 9.1)	4.15 dd (9.1, 9.1)
Glc-4	4.46 dd (9.1, 9.1)	4.46 dd (9.2, 9.2)	4.45 dd (9.1, 9.1)	4.46 dd (9.1, 9.1)
Glc-5	3.92 m	3.87 m	3.90 m	3.87 m
Glc-6	4.25 dd (4.3, 12.0)	4.25	4.23 dd (5.5, 11.8)	4.23 dd (4.3, 11.9)
	4.44 br d (12.0)	4.38 br d (12.0)	4.35 br d (11.6)	4.36 br d (11.6)
Rha-1	6.02 br s	6.02 br s	5.90 br s	5.90 br s
Rha-2	4.69 br d (3.0)	4.68 br d (3.1)	4.71 br s	4.71 br s
Rha-3	4.58 dd (3.0, 9.1)	4.67 dd (3.1, 9.2)	4.58 br d (10.0)	4.58 br d (10.4)
Rha-4	4.27 dd (9.1, 9.1)	4.27	4.37 dd (9.1, 9.1)	4.39 dd (9.1, 9.1)
Rha-5	5.02 m	5.02	5.02 m	5.01 m
Rha-6	1.66 d (6.7)	1.66 d (6.1)	1.73 d (6.1)	1.73 d (6.1)
Xyl-1	5.23 d (7.9)	5.23 d (7.9)		
Xyl-2	4.07 dd (7.9, 8.5)	4.07 dd (7.9, 7.9)		
Xyl-3	4.12 dd (8.5, 8.5)	4.12 dd (8.5, 8.5)		
Xyl-4	4.15	4.14 dd (7.3, 7.3)		
Xyl-5	3.67 dd (10.4, 10.4)	3.66 dd (9.8, 10.6)		
	4.29	4.28		
26-O-				
Glc-1	4.82 d (7.9)	4.81 d (7.9)	4.82 d (7.9)	4.82 d (7.9)
Glc-2	4.02 dd (7.9, 8.5)	4.02 dd (7.9, 8.5)	4.02 dd (7.9, 8.5)	4.01 dd (7.9, 8.5)
Glc-3	4.25	4.25	4.26	4.26
Glc-4	4.25	4.25	4.26	4.26
Glc-5	3.94 m	3.94 m	3.92 m	3.93 m
Glc-6	4.39 br d (11.8)	4.39 dd (5.5, 11.6)	4.33 br d (11.2)	4.34 br d (11.2)
	4.56 br d (11.6)	4.56 dd (3.6, 12.6)	4.56 br d (12.2)	4.56 br d (12.3)

Coupling constants (J in Hz) are given in parentheses.

due to the sugar moiety were not identical. On acid hydrolysis, 3 afforded D-glucose and L-rhamnose, together with several unidentified artificial sapogenoles.²⁾ The ^1H - and ^{13}C -NMR data of the sugar moiety were assigned by ^1H - ^1H COSY and HMQC techniques. In the HMBC experiment, long-range correlations were observed between the anomeric proton [δ 4.82 (d, $J=7.9$ Hz)] of terminal glucose and the C-26 (δ 75.1) of aglycone, the anomeric proton [δ 4.98 (d, $J=7.3$ Hz)] of 4-substituted glucose and the C-3 (δ 77.4) of aglycone and the anomeric proton [δ 5.90 (br s)] of terminal rhamnose and the C-4 (δ 78.5) of 4-substituted glucose. Based on the above evidence, the structure of 3 was elucidated as a cholestane glycoside with a terminal xylose absent from 1.

Abutiloside G (4) was obtained as a white powder, $[\alpha]_D$

-81.3° (MeOH), and showed an ion peak due to $[\text{M}-\text{H}]^-$ at m/z 901, which was lower by 2 mass units than that of 3, in the neg. FAB-MS. In the ^1H - and ^{13}C -NMR spectrum of 4, signals due to an aglycone moiety were consistent with those of 2, while signals due to a sugar moiety were almost superimposable on those of 3. From the above evidence, the structure of 4 was concluded to have a double bond at the C-5 of 3. The configuration at C-25 in 1—4 was deduced to be R due to the coexistence of dioscin and solamargine in this plant. However, this presumption was a moot point.

Kaneko *et al.* presented a hypothetical biogenetic pathway of solanidine in *Veratrum*.³⁾ 1—4 were regarded as precursors of a dormantinone type compound on a hypothetical pathway of steroid biogenesis. Further, 2 and 4 were corresponding 5-ene compounds to 1 and 3. Consequently, the coexistence of a 5-ene type compound was confirmed in the roots of *Solanum abutiloides*.

Experimental

Optical rotations were taken with a JASCO DIP-1000 automatic digital polarimeter. The ^1H - and ^{13}C -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 and taken in a 3-nitrobenzyl alcohol matrix. HPLC was carried out using a TSK gel-120T (7.8 mm i.d. $\times$ 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 F₂₄₅ (Merck), and detection was achieved by spraying them with 10% H_2SO_4 followed by heating. Column chromatography was carried out on Kieselgel (230—400 mesh, Merck), Sephadex LH-20 (Pharmacia), ODS (PrePAK-500/C₁₈, Waters) and MCI gel CHP-20P (Mitsubishi Chemical Ind.)

Extraction and Separation The seeds identified as *Solanum abutiloides* at the National Research Institute of Vegetables, Ministry of Agri-

Table 2. ^{13}C -NMR Data for 1–5 (Pyridine- d_5)

	1	2	3	4	5
C- 1	37.1	37.3	37.1	37.4	37.0
C- 2	29.9	30.2	30.0	30.2	29.9
C- 3	77.3	78.2	77.4	78.4	77.3
C- 4	34.8	39.3	34.9	39.3	34.8
C- 5	44.6	140.9	44.6	140.9	44.5
C- 6	28.9	121.8	28.9	121.8	28.9
C- 7	32.2	32.1	32.4	32.1	32.2
C- 8	35.1	31.6	35.1	31.8	35.1
C- 9	54.4	50.3	54.4	50.3	54.4
C-10	35.7	36.9	35.7	36.9	35.7
C-11	21.1	20.9	21.1	20.9	21.0
C-12	40.2	39.9	40.2	39.9	40.1
C-13	44.4	44.1	44.4	44.1	44.4
C-14	53.7	53.9	53.7	53.9	53.7
C-15	37.0	37.0	37.0	37.0	36.9
C-16	75.9	75.9	75.9	75.9	75.9
C-17	63.4	63.3	63.4	63.3	63.4
C-18	13.7	13.4	13.7	13.4	13.7
C-19	12.3	19.4	12.3	19.4	12.3
C-20	49.3	49.3	49.3	49.3	49.3
C-21	16.8	16.8	16.8	16.8	16.8
C-22	214.9	214.8	214.9	214.8	215.0
C-23	39.0	39.0	39.0	39.0	39.1
C-24	28.0	28.0	28.0	28.0	27.9
C-25	33.4	33.4	33.4	33.4	36.1
C-26	75.1	75.1	75.1	75.1	67.3
C-27	17.6	17.6	17.5	17.6	17.3
3-O-					
Glc-1	102.0	102.4	102.8	102.7	102.0
Glc-2	75.6	75.6	75.5	75.5	75.5
Glc-3	76.6	76.6	76.8	76.7	76.6
Glc-4	77.8	77.7	78.5	78.2	77.8
Glc-5	77.4	77.3	77.2	77.2	77.3
Glc-6	61.7	61.7	61.7	61.6	61.7
Rha-1	101.2	101.2	102.1	101.5	101.2
Rha-2	81.9	81.9	72.6	72.6	81.9
Rha-3	72.9	72.9	72.8	72.8	72.8
Rha-4	74.4	74.4	74.0	74.0	74.4
Rha-5	70.0	70.0	70.4	70.4	69.9
Rha-6	18.4	18.4	18.5	18.5	18.4
Xyl-1	107.6	107.6			107.5
Xyl-2	75.2	75.2			75.5
Xyl-3	78.5	78.5			78.4
Xyl-4	71.0	71.0			71.0
Xyl-5	67.4	67.4			67.4
26-O-					
Glc-1	104.9	104.9	104.9	104.9	
Glc-2	75.2	75.2	75.2	75.3	
Glc-3	78.5	78.5	78.5	78.5	
Glc-4	71.7	71.7	71.7	71.7	
Glc-5	78.6	78.6	78.6	78.6	
Glc-6	62.8	62.8	62.8	62.8	

culture, Forestry and Fisheries, Ano, Mie in Japan, were cultivated at the Botanical Garden of Kumamoto University. The harvested roots (2.24 kg) of this plant were extracted with MeOH, and the extract was partitioned in AcOEt and water (1:1, v/v). The water soluble portion (87.6 g) was subjected to MCI gel CHP-20P column chromatography with MeOH–H₂O (20→30→40→50→60→70→80→90→100%) to afford ten fractions (fr.1–fr.10). Fraction 3 (1.04 g) was further separated by ODS column chromatography with MeOH–H₂O (40→50→60→70%) and silica gel column chromatography with CHCl₃–MeOH–H₂O (6:4:0.5, v/v), followed by HPLC with MeOH–H₂O (3:2), to furnish abutilosides D (1) (423 mg) and E (2) (27 mg). Fraction 4 (1.67 g) was subjected to ODS column chromatography with MeOH–H₂O (40→50→60→70→80%) to afford two fractions (fr.11–

fr.12). Fraction 11 was further separated by Sephadex LH-20 column chromatography with MeOH and silica gel column chromatography with CHCl₃–MeOH–H₂O (6:4:0.5, v/v), followed by HPLC with MeOH–H₂O (3:2), to furnish abutiloside G (4) (72 mg). Fraction 12 was further separated by Sephadex LH-20 column chromatography with MeOH and silica gel column chromatography with CHCl₃–MeOH–H₂O (6:4:0.5, v/v), followed by HPLC with MeOH–H₂O (3:2), to furnish abutiloside F (3) (229 mg).

Abutiloside D (1) A white powder, $[\alpha]_D^{25}$ –56.6° ($c=0.80$, MeOH). Neg. FAB-MS (m/z): 1035 $[\text{M}-\text{H}]^-$. ^1H -NMR (pyridine- d_5) δ : 0.67 (3H, s, H-19), 0.68 (3H, s, H-18), 0.98 (3H, d, $J=6.1$ Hz, H-27), 1.19 (3H, d, $J=6.7$ Hz, H-21), 3.61 (1H, dd, $J=6.7$, 8.7 Hz, H-26a), 3.87 (1H, m, H-3), 3.95 (1H, dd, $J=6.7$, 9.1 Hz, H-26b), 4.24 (1H, H-16). Signals for the saccharide moiety are shown in Table 1. ^{13}C -NMR (pyridine- d_5): Table 2.

Abutiloside E (2) A white powder, $[\alpha]_D^{25}$ –60.5° ($c=0.35$, MeOH). Neg. FAB-MS (m/z): 1033 $[\text{M}-\text{H}]^-$. ^1H -NMR (pyridine- d_5) δ : 0.70 (3H, s, H-18), 0.91 (3H, s, H-19), 0.98 (3H, d, $J=6.7$ Hz, H-27), 1.20 (3H, d, $J=6.7$ Hz, H-21), 3.60 (1H, dd, $J=5.9$, 9.2 Hz, H-26a), 3.82 (1H, m, H-3), 3.96 (1H, H-26b), 4.23 (1H, H-16), 5.30 (1H, brs, H-6). Signals for the saccharide moiety are shown in Table 1. ^{13}C -NMR (pyridine- d_5): Table 2.

Abutiloside F (3) A white powder, $[\alpha]_D^{25}$ –51.8° ($c=0.75$, MeOH). Neg. FAB-MS (m/z): 903 $[\text{M}-\text{H}]^-$. ^1H -NMR (pyridine- d_5) δ : 0.68 (3H, s, H-19), 0.69 (3H, s, H-18), 0.98 (3H, d, $J=6.1$ Hz, H-27), 1.19 (3H, d, $J=6.7$ Hz, H-21), 3.61 (1H, dd, $J=6.1$, 9.2 Hz, H-26a), 3.81 (1H, m, H-3), 3.95 (1H, dd, $J=6.1$, 9.2 Hz, H-26b), 4.24 (1H, H-16). Signals for the saccharide moiety are shown in Table 1. ^{13}C -NMR (pyridine- d_5): Table 2.

Abutiloside G (4) A white powder, $[\alpha]_D^{25}$ –81.3° ($c=0.75$, MeOH). Neg. FAB-MS (m/z): 901 $[\text{M}-\text{H}]^-$. ^1H -NMR (pyridine- d_5) δ : 0.71 (3H, s, H-18), 0.92 (3H, s, H-19), 0.98 (3H, d, $J=6.1$ Hz, H-27), 1.20 (3H, d, $J=6.1$ Hz, H-21), 3.61 (1H, dd, $J=6.7$, 9.8 Hz, H-26a), 3.74 (1H, m, H-3), 3.95 (1H, dd, $J=6.1$, 9.8 Hz, H-26b), 4.24 (1H, H-16), 5.31 (1H, brs, H-6). Signals for the saccharide moiety are shown in Table 1. ^{13}C -NMR (pyridine- d_5): Table 2.

Sugars Analysis of 1 and 3 A solution of each of 1 and 3 (2 mg) in 2 N HCl–dioxane (1:1, 2 ml) was heated at 100 °C for 1 h. The reaction mixture was diluted with H₂O and evaporated to remove dioxane. The solution was neutralized with Amberlite MB-3 and passed through a SEP-PAK C₁₈ cartridge to give a sugar fraction. A solution of sugar fraction analyzed by TLC [CH_3CN –MeOH–H₂O (6:4:1), 1: rhamnose, R_f 0.54; xylose, R_f 0.40; glucose, R_f 0.29; 3: rhamnose, R_f 0.54; glucose, R_f 0.29]. A solution of the sugar fraction in 1 ml of H₂O was treated with a solution of L-(–)- α -methylbenzylamine (150 μl) and NaBH₃CN (8 mg) in 1 ml of EtOH, and the mixture was kept at 40 °C for 3 h. Then, several drops of acetic acid were added and the mixture was concentrated to dryness. The residue dissolved in Ac₂O–C₂H₅N (1:1, 2 ml) was treated with 4-(dimethylamino)pyridine (DMAP) (20 mg), and the whole mixture was left at room temperature overnight. After the removal of excess Ac₂O and C₂H₅N, the residue dissolved in 20% CH₃CN was loaded into a SEP-PAK C₁₈ cartridge and eluted with 20% CH₃CN (total 7 ml) and 100% CH₃CN. The fraction eluted with 100% CH₃CN was analyzed by normal-phase HPLC. Conditions of HPLC: column, Develosil 60-3, 3 μm (4.6 mm i.d. $\times$ 150 mm); solvent, *n*-hexane–EtOH (19:1); flow rate, 1.20 ml/min; detection, UV (230 nm). t_R (min) of 1-(*N*-acetyl-L- α -methylbenzylamino)-1-deoxyalditol acetates were as follows. 1: L-rhamnose 17.2, D-xylose 30.8, D-glucose 30.8 (reference: L-rhamnose 17.0, D-xylose 30.7, D-glucose 30.8). 3: L-rhamnose 17.2, D-glucose 30.7.

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