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ABUTILOSIDE A, A 26-ACYLAMINO-3β,16α-DIHYDROXY-5α-CHOLESTA-22-ONE GLYCOSIDE FROM *SOLANUM ABUTILOIDES**

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Key Word Index—*Solanum abutiloides*; Solanaceae; abutiloside A; 26-aminocholestanol derivative; steroid alkaloid glycoside.

Abstract—In addition to solamargine and proto-dioscin, three new steroid glycosides, abutilosides A–C, have been isolated from roots of the Solanaceae *Solanum abutiloides*. The structure of abutiloside A has been elucidated as 3β,16α-dihydroxy-26-isovalerylamino-5α,25ξ*H*-cholestan-22-one 3-*O*-[*O*-β-D-xylopyranosyl-(1 → 2)-*O*-α-L-rhamnopyranosyl-(1 → 4)-β-D-glucopyranoside]. De-*N*-acylation of its aglycone yielded solafloridine by 22,*N*-cyclization. Therefore, 26-aminocholestan-22-one derivatives are considered to be crucial intermediates in steroid biosynthesis. The co-occurrence of the (25*R*)-steroids soladulcidine, solafloridine and diosgenin together with abutiloside A in roots of *S. abutilosides* suggests that the configuration of abutiloside A at C-25 will also be *R*. Copyright © 1997 Elsevier Science Ltd

INTRODUCTION

Solanum abutiloides (Griseb.) Bitt. et Lillo is distributed in South America and grows up to 2–3 m high in summer with white flowers, which bear yellow-orange fruits. It has soft hairs on the leaves and no thorn on the stems of the plant. It is not invaded by harmful insects. We cultivated this plant in the Botanical Garden of Kumamoto University in order to survey its constituents. In former investigations, the spirostane alkaloid solasodine [1, 2], the 22,26-epiminocholestane alkaloids solafloridine and 20-iso-solafloridine [2] and the spirostane isonuatigenin [3] have been isolated after acid hydrolysis of the glucosides occurring in leaves, roots or fruits, respectively, of this plant. As part of our chemical studies on the compounds in *Solanum* plants, we have investigated the constituents of fresh roots of *Solanum abutiloides*. We reported the isolation of abutiloside A in a preliminary communication [4] and that of abutilosides B and C in a following publication [5]. This paper reports the isolation and structural elucidation of abutiloside A.

RESULTS AND DISCUSSION

Separation of the methanolic extracts of the roots of *S. abutiloides* (2.7 kg) by polystyrene gel, Chromatorex ODS and silica gel column chromatography resulted in the isolation of abutiloside A (**1**, 138 mg), together with abutiloside B (40 mg), abutiloside C (38 mg), the solasodine glucoside solamargine (337 mg) and the steroid saponin proto-dioscin-dioscin (11 mg).

Abutiloside A (**1**) was obtained as an amorphous powder, showing $[\alpha]_D - 49.2°$ (MeOH). The HR FAB-mass spectrum showed a quasi-molecular ion peak at *m/z* 980.5552 $[M+Na]^+$ (calcd for $C_{49}H_{83}NO_{17}Na$, 980.5558), indicating a molecular formula $C_{49}H_{83}NO_{17}$ and, moreover, it showed ions due to $[M+Na+H-\text{pentose}]^+$ at *m/z* 848, $[M+Na+H-\text{pentose}-\text{methyl pentose}]^+$ at *m/z* 702 and $[M+Na+H-\text{pentose}-\text{methyl pentose}-\text{hexose}]^+$ at *m/z* 540. In the IR spectrum, absorptions due to hydroxyl, carbonyl and amide groups at 3400, 1697 and 1643 cm^{-1}, respectively, were observed. The ^1H NMR spectrum suggested **1** to be a steroidal derivative, showing the signals due to two angular methyl groups (3H, each, *s*, at δ 0.68 and 0.69), two secondary methyl groups (3H, *d*, $J = 7.3$ Hz at δ 0.98, and 3H, *d*, $J = 6.7$ Hz at δ 1.19), a secondary methyl group (3H, *d*, $J = 6.11$ Hz, at δ 1.65) of a methyl pentosyl moiety, an amide proton (1H, *br t*, at δ 8.27), together with an isobutyl group (6H, *d*, $J = 7.3$ Hz, at δ 0.97, 2H, *d*, $J = 7.3$ Hz, at δ 2.23, and 1H, *m*, at δ 2.34), and three anomeric protons (1H, *d*, $J = 7.3$ Hz, at δ 4.97, 1H, *d*, $J = 7.3$ Hz, at δ 5.21 and 1H, *s*, at δ 6.00). The above evidence indicated that **1** was a triglycoside having a terminal pentosyl residue. A pair of fragment ions, such as *m/z* 114 due to $[C_6H_{12}NO]^+$ and *m/z* 138

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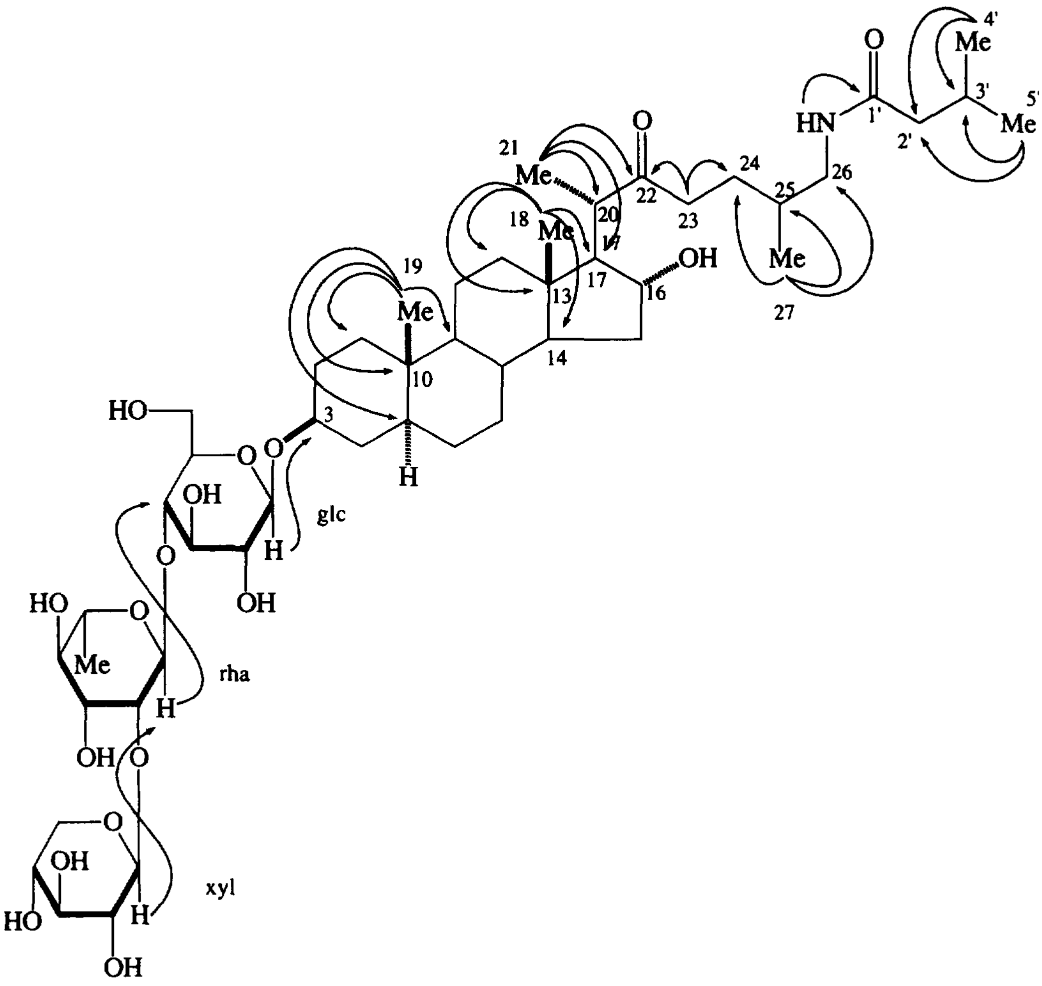


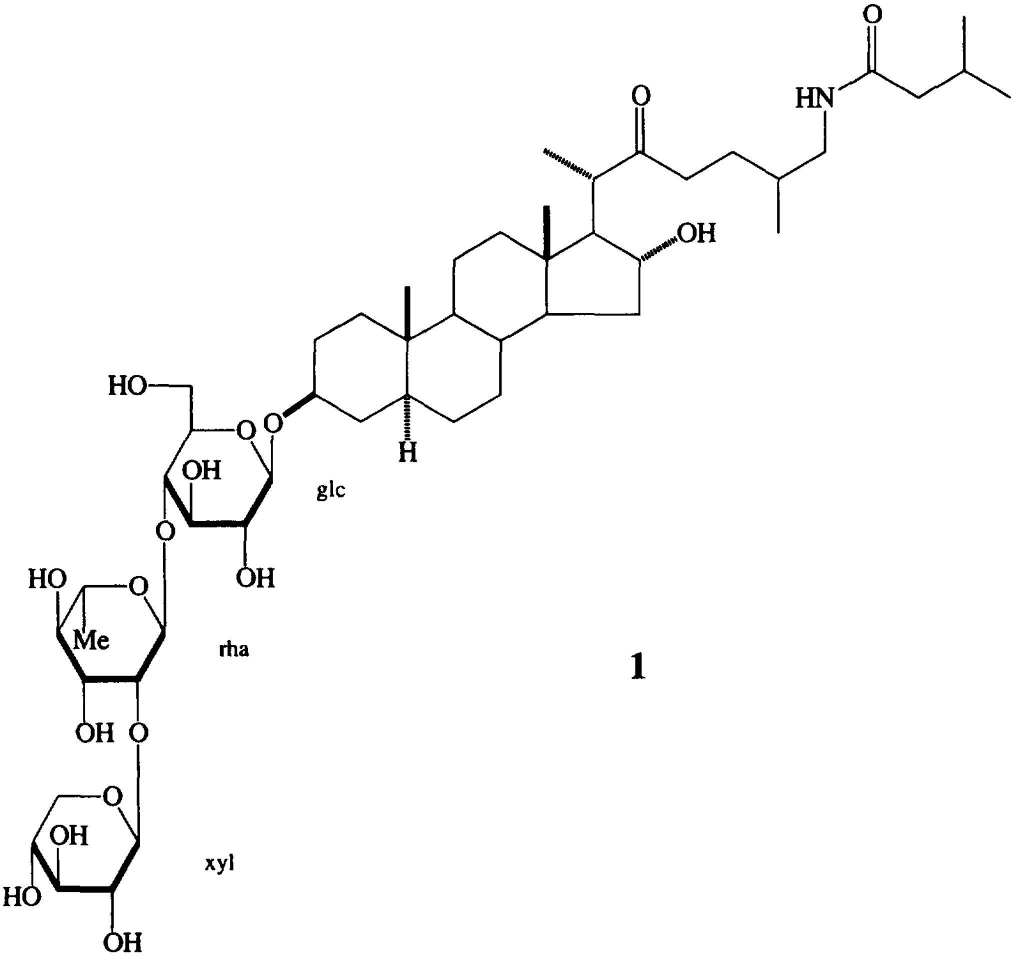
Fig. 1. HMBC correlations in compound 1.

due to $[C_9H_{16}N]^+$ [6], is usually observed in the EI-mass spectrum of a spirosolane derivative. However, only a prominent fragment ion (base peak) at m/z 114 was observed, suggesting 1 not to be a spirosolane derivative. Acid hydrolysis of 1 afforded D-xylose, L-rhamnose and D-glucose [7] but an aglycone could not be obtained owing to labile decomposition during the reaction. In addition to the signals of 27 carbons due to the steroidal skeleton [8], the signals of five carbons at δ 22.7, 26.4, 46.0, 22.7 × 2 due to the isobutyl group were observed in the ^{13}C NMR spectrum of 1. Therefore, the structure of 1 was deduced to be a triglycosyl 26-aminocholestane derivative.

In the ^{1}H detected heteronuclear multiple bond connectivity (HMBC) spectrum of 1, correlations were observed (Fig. 1) between protons at C-18 and carbons at C-12, C-13, C-14, C-17, between protons at C-19 and carbons at C-1, C-5, C-9, C-10, between protons at C-21 and carbons at C-17, C-20, C-22, between protons at C-27 and carbons at C-24, C-25, C-26, between protons at C-4′ and C-5′ and carbons at C-2′, C-3′, between protons C-23 and carbons at C-22, C-24, and between proton at NH and carbon at C-1′. The C-16 carrying a hydroxy group could be thus assigned by HMBC. Further, a NOE experiment between H-16 and H_3-18 disclosed the substituent at C-16 to be an α-hydroxyl group. That is, when the proton at C-18 in 1-peracetate was irradiated, the peak at δ 4.90 due to the H-16 increased, indicating the protons at C-18 and C-16 are located in the same side of the rings, and to both have the β-configuration. The ^{1}H–^{1}H, ^{1}H–^{13}C COSY and HMBC enabled the assignment of the ^{1}H and ^{13}C signals of 1 as listed in Table 1.

The linkage position of the sugar in 1 was determined by the HMBC spectrum. Correlations between glc H-1 and aglycone C-3, between rha H-1 and glc C-4, between xyl H-1 and rha C-2, were observed. The ^{1}H–^{1}H 2D COSY spectrum of 1-acetate led to the assignments as listed in Table 2. By comparing this spectrum with that of 1, the glc H-4 and rha H-2 in the acetate remained at a higher magnetic field, and the other protons attached to the carbons having acetoxy groups were shifted to a lower field.

Therefore, the structure of 1 could be determined to be 3β,16α-dihydroxy-26-isovalerylamino-5α,25ξ*H*-cholestan-22-one 3-*O*-[*O*-β-D-xylopyranosyl-(1 → 2)-*O*-α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranoside].



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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 ^1H and ^{13}C NMR spectra were measured with a JEOL JUM-GX 270, 400 and 500 NMR spectrometer, and chemical shifts are given as δ (ppm) values with TMS as an internal standard. The FAB-MS was recorded with a JEOL DX-300 spectrometer and HR FAB-MS was measured with a JEOL HX-110. TLC was performed on precoated Kieselgel 60 F_{254} plates (Merck), and detection was achieved by spraying it with 10% H_2SO_4 followed by heating. Column chromatography was carried out with MCI gel CHP 20P (Mitsubishi Chemical Ind. Co. Ltd), and Kieselgel 60 (70–230 mesh and 230–400 mesh, Merck) and Chromatorex ODS-DU 3050MT (Fuji silysia).

Extraction and separation. The seeds were identified as *Solanum abutiloides* by the National Research Institute of Vegetables, Ornamental Plants and Tea, Ano, Mie in Japan and they were cultivated at the Botanical Garden of Kumamoto University in 1993. The fresh roots (2.72 kg) of this plant were extracted with refluxing MeOH and the resulting extract was then evaporated to dryness to give a residue (106.2 g), which was subsequently partitioned between benzene and H_2O. The aq. layer was evaporated to dryness to give a residue (93.8 g) which was subjected to CHP-20P CC eluting with H_2O to MeOH and 2.8% NH_4OH to provide six fractions. These fractions were further separated and purified by using silica gel with $CHCl_3$–MeOH–water solvent system and Chromatorex ODS with a 40% MeOH to MeOH gradient to afford abutiloside A (138 mg), abutiloside B (40 mg), abutiloside C (38 mg) together with solamargine (337 mg) and proto-dioscin (11 mg).

Abutiloside A 1. An amorphous powder, $[\alpha]_D^{22}-49.2°$ (MeOH). IR ν_{max}^{KBr} cm^{-1}: 3400 (−OH), 1697 (−CO), 1643 (−NH). HR FAB-MS *m/z*: 980.5552 $[M+Na]^+$ (calcd for $C_{49}H_{83}NO_{17}Na$, 980.5558), molecular formula $C_{49}H_{83}NO_{17}$, 848 $[M+Na+H-xyl]^+$, 702 $[M+Na+H-xyl-rha]^+$, 540 $[M+Na+H-xyl-rha-glu]^+$. ^1H and ^{13}C NMR (pyridine-d_5): Table 1.

Peracetate of 1. A soln of 1 (15 mg) in 3 ml Ac_2O–pyridine (2:1) was kept at room temp. for 1 hr. The reaction mixture was evap. under a stream of N_2 to remove solvent and the residue was chromatographed on silica gel ($CHCl_3$–MeOH, 20:1) to afford the acetate of 1. ^1H NMR of the sugar moiety (in $CDCl_3$): Table 2.

Table 1. ^1H and ^{13}C NMR data of abutiloside A in pyridine-d_5

Position	^1H NMR	^{13}C NMR
1		37.1
2		29.9
3	3.90 (*m*)	77.3
4		34.8
5	0.87 (*m*)	44.6
6		28.9
7		32.3
8		35.1
9		54.4
10		35.7
11	1.19 (*m*), 1.84 (*m*)	21.1
12		40.2
13		44.4
14	1.45 (*m*)	53.7
15		37.0
16	4.27 (*m*)	76.0
17	1.82 (*m*)	63.4
18	0.69 (*s*)	13.7
19	0.68 (*s*)	12.3
20	2.80 (*m*)	49.3
21	1.19 (*d*, J = 6.7 Hz)	16.8
22		214.7
23	2.80 (*m*), 2.82 (*m*)	38.8
24		28.4
25	1.93 (*m*)	33.4
26	3.31 (*m*), 3.48 (*m*)	45.0
27	0.98 (*d*, J = 7.3 Hz)	18.3
1′		172.6
2′	2.23 (*d*, J = 7.3 Hz)	46.0
3′	2.34 (*m*)	26.4
4′	0.97 (*d*, J = 7.3 Hz)	22.7
5′	0.97 (*d*, J = 7.3 Hz)	22.7
glc-1	4.97 (*d*, J = 7.3 Hz)	102.0
2	3.96 (*br t*, J = 8.5 Hz)	75.5
3	4.24 (*m*)	76.6
4	4.44 (*br t*, J = 9.1 Hz)	77.8
5	3.88 (*m*)	77.3
6	4.25 (*m*), 4.42 (*br t*, J = 9.1 Hz)	61.7
rha-1	6.00 (*s*)	101.2
2	4.67 (*br s*)	81.9
3	4.57 (*dd*, J = 3.6, 9.2 Hz)	72.9
4	4.22 (*m*)	74.4
5	5.00 (*m*)	69.9
6	1.65 (*d*, J = 6.1 Hz)	18.4
xyl-1	5.21 (*d*, J = 7.3 Hz)	107.6
2	4.05 (*br t*, J = 8.0 Hz)	75.5
3	4.12 (*m*)	78.4
4	4.11	71.0
5	3.65, 4.27	67.4

Table 2. ^1H NMR of sugar of abutiloside A and its peracetate in $CDCl_3$

H	Abutiloside A	Acetate of abutiloside A
glc-1	4.97	4.45
-2	3.96	4.98
-3	4.24	5.18
-4	4.44	4.92
-5	3.88	3.32
-6	4.25, 4.42	4.11, 4.88
rha-1	6.00	4.84
-2	4.67	3.91
-3	4.57	5.03
-4	4.22	4.90
-5	5.00	3.76
-6	1.65	1.12
xyl-1	5.21	4.58
-2	4.05	4.80
-3	4.12	5.16
-4	4.11	3.78
-5	3.65, 4.27	3.57, 4.24
H-16	4.27	4.90

GLC analysis of sugar in **1**. Compound **1** was heated in 1 M HCl H_2O–dioxane (1:1) at 90°C for 2 hr. The ppt. was removed by filtration and the supernatant was treated with Amberlite IRA-400 to give a sugar fraction (*ca* 1 mg). The pyridine soln of the sugar (1 mg/100 μl) and a pyridine soln of L-cysteine methyl ester hydrochloride (2 mg/100 μl) were mixed and warmed at 60°C for 1 hr. The solvent was removed and the product dried *in vacuo*. The *N*-trimethylsilyl (TMS)–imidazole (100 μl) was added and the mixture was heated at 60° for another 30 min. The reaction mixture was then partitioned between *n*-hexane and water. The *n*-hexane soln was subjected to GLC. The 3 peaks were observed at R_t (min): 8.825′ (D-xyl), 10.709′ (L-rha), 15.538′ (D-glc). The standard monosaccharides were subjected to reaction and GLC under the same conditions.

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REFERENCES

1. Schreiber, K., *Alkaloids* (*New York*), 1968, **10**, 1, and references cited therein.
2. Ripperger, H., *Phytochemistry*, 1996, **41**, 1629.
3. Evans, W. C., Grout, R. J. and Rowland, J. P., *Planta Medica*, 1981, **41**, 166.
4. Ohmura, E., Nakamura, T., Tian, R. H., Yahara, S., Yoshimitsu, H. and Nohara, T., *Tetrahedron Letters*, 1995, **36**, 8443.
5. Tian, R. H., Ohmura, E., Yoshimitsu, H., Nohara T. and Matsui, M., *Chemical Pharmaceutical Bulletin*, 1996, **44**, 1119.
6. Budzikiewicz, H., Djerassi, C. and Williams, H. D., *Structure Elucidation of Natural Products by Mass Spectrometry*, 1962, **2**, 21.
7. Hara, S., Okabe, H. and Mihashi, K., *Chemical Pharmaceutical Bulletin*, 1987, **35**, 501.
8. Eggert, H., Vanantwerp, L. C., Bhacca, S. N. and Djerassi, C., *Journal of Organic Chemistry*, 1976, **41**, 71.