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Steroidal glycosides from the fruits of *Solanum abutiloides*

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Abstract

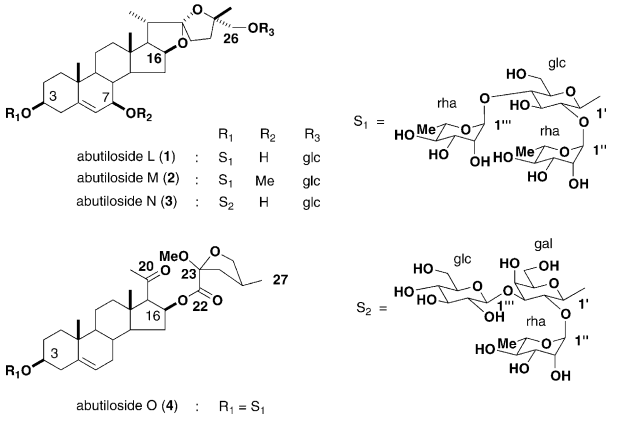
Four steroidal glycosides, named abutilosides L, M and N, these being 22*S*,25*S*-epoxy-furost-5-ene type glycosides, and abutiloside O, a 20-22 *seco*-type steroidal glycoside, were isolated from the fresh fruits of *Solanum abutiloides*. Their structures were determined by 2D NMR spectroscopic analysis and chemical evidence.

Keywords: *Solanum abutiloides*; Solanaceae; Steroidal glycoside; Abutiloside

1. Introduction

Solanum abutiloides grows wild in South America. From its roots, three steroidal alkaloids (solasodine, solafloridine and 20-isosolafloridine) (Ripperger, 1996) and abutilosides A (Ohmura et al., 1995), B, C (Tian et al., 1996), D, E, F, G (Yoshimitsu et al., 2000), H, I, J and K (Yoshimitsu et al., 2002) were isolated. The latter 26-hydroxy- and 26-amino-cholestane glycosides are important key intermediates in the biogenesis of steroidal alkaloids. A survey of the literature showed no chemical work being done on the fresh fruits of *S. abutiloides*.

Our studies on the constituents of more than 40 *Solanum* species resulted in the isolation of steroidal glycosides with potent cytotoxic (Nakamura et al., 1996), antifeedant (Nohara et al., 1998) and anti HSV-1 (Ikeda et al., 2000) properties. As part of this investigation, four new steroidal glycosides, abutilosides L (**1**), M (**2**) and N (**3**), being a 22*S*,25*S*-epoxy-furost-5-ene type glycosides, and abutiloside O (**4**), being a 20-22 *seco*-type steroidal glycoside, as well as three known ones, aculeatiosides A and B (Saijo et al., 1983), being a 22*S*,25*S*-epoxy-furost-5-ene type glycosides, and compound Pd (Nohara et al., 1973), being a pregnane-type glycoside, were isolated from the fresh fruits of *S. abutiloides*. This paper describes their structural elucidation.



2. Results and discussion

The methanolic extract of the fresh fruits of *S. abutiloides* was partitioned into a chloroform-water solvent system. The water-soluble portion was separated by MCI gel CHP20P, octadecyl silica gel (ODS) and silica gel column chromatographies and, finally, HPLC to give abutilosides L (**1**), M (**2**), N (**3**) and O (**4**).

Abutiloside L (**1**) was obtained as a white powder, $[\alpha]_D$ −107.1° (MeOH). In the negative-ion FAB MS of 1, a quasi-molecular ion peak was observed at *m*/*z* 1061 $[M-H]^-$, while its positive-ion FAB MS showed a quasi-molecular ion peak at *m*/*z* 1085 $[M+Na]^+$. The positive high-resolution (HR) FAB MS showed a clustered molecular ion at *m*/*z* 1085.5153 $[C_{51}H_{82}O_{23}Na]^+$. The

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^{1}H NMR spectrum displayed signals for three quaternary methyls at δ 0.90, 1.06 and 1.40, three secondary methyls at δ 1.08 (*d*, $J=6.7$ Hz), 1.63 (*d*, $J=6.1$ Hz) and 1.76 (*d*, $J=6.1$ Hz) and an olefinic proton at δ 5.79 (1H, *d*, $J=4.9$ Hz), along with four anomeric protons at δ 4.90 (1H, *d*, $J=7.6$ Hz), 4.95 (1H, *d*, $J=7.8$ Hz), 5.85 (1H, *br s*) and 6.39 (1H, *br s*). The ^{1}H NMR spectral data of **1** was similar to those of (22*S*,25*S*) 26-*O*-β-D-glucopyranosyl 16β,22;22,25-diepoxy-furost-5-ene-3β,26-diol 3-*O*-β-chacotrioside (aculeatioside A). On acid hydrolysis, **1** afforded D-glucose and L-rhamnose the structures of which were confirmed by ^{1}H NMR coupling pattern and optical rotations using chiral detection in HPLC analysis, together with two unidentified sapogenols. The ^{13}C NMR spectrum of **1** displayed 27 carbon signals due to the aglycone, along with carbon signals of a β-chacotriosyl moiety [δ_C 100.3 (δ_H 4.90), δ_C 102.0 (δ_H 6.93) and δ_C 102.9 (δ_H 5.85)] linked to the C-3 hydroxyl group and a β-D-glucopyranosyl moiety [δ_C 105.4 (δ_H 4.95)] linked to the C-26 hydroxyl group. Meanwhile, the molecular weight of **1** was higher by 16 mass units than that of aculeatioside A. The foregoing evidence indicated that **1** has an additional hydroxyl group in the aglycone. Moreover, a sequence of connectivities through an olefinic proton at δ 5.79 (*d*, $J=4.9$ Hz), an oxygen-bearing methine proton at δ 3.96 (*m*) and a methine proton at δ 1.61, in turn, was observed in the ^{1}H-^{1}H COSY and their signals could be assigned to H-6, H-7 and H-8, respectively. The above data determined the presence of an additional C-7 hydroxyl group. Besides, full assignment of the NMR spectral data was established by ^{1}H–^{1}H COSY, HMQC and HMBC experiments. The configuration of the C-3 hydroxyl group was determined to be β from the multiplicity of the H-3 proton ($W_{1/2}=22$ Hz). The NOE correlations, H3-18/H-15β and H-20, H-20/H-23β, H-23β/H-24β, H3-27/H-24β, H-21/H-17, H-17/H-16α, H-16α/H-15α and H-14, H-15α/H-7α, H-14/H-7α, H-9/H-7α and H3-19/H-8 in the NOESY and NOE difference spectrum (NOEDS), suggested 7*R*, 16*S*, 22*S* and 25*S* configurations (Fig. 1). Furthermore, the anomeric proton signals at δ 4.90 (H-1′), 6.39 (H-1″), 5.85 (H-1‴) and 4.95 (H-1⁗) showed long-range correlations with the carbon signals at δ 78.0 (C-3), 77.7 (C-2′), 78.6 (C-4′) and 77.5 (C-26), respectively. These long-range correlations proved that the β-chacotriosyl moiety was attached to the C-3 hydroxyl group and the β-D-glucopyranosyl moiety was linked to the C-26 hydroxyl group. Therefore, the structure of **1** was elucidated as (22*S*,25*S*) 26-*O*-β-D-glucopyranosyl-22,25-epoxy-furost-5-ene-3β,7β,26-triol 3-*O*-β-chacotrioside.

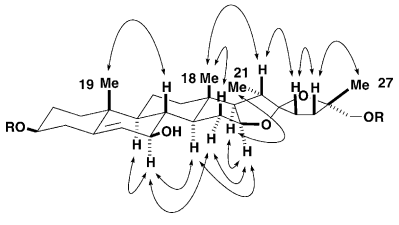

Fig. 1. NOE correlations of the aglycone moiety of **1**.

Abutiloside M (**2**) was obtained as a white powder, $[\alpha]_D$ −110.9° (MeOH), and showed a quasi-molecular ion peak due to [M–H]$^-$ at *m*/*z* 1075, which was higher by 14 mass units than that of **1**, in the negative-ion FAB MS. Comparison of the ^{1}H NMR spectrum of **2** with that of **1** showed them to be identical except for the appearance of the methoxyl proton signal at δ 3.26 (*s*). In the ^{13}C NMR spectrum of **2**, signals due to the aglycone moiety, except for the signals of the B-ring, and the sugar moiety were in good agreement with those of **1**. In the HMBC, the methoxyl proton signal at δ 3.26 showed long-range correlation with the carbon signal at δ 73.7 (C-7). The above data indicated the presence of a methoxyl group at C-7 in **2**. An NOE was observed between H_3-19 and the methoxyl proton signal at δ 3.26 in the NOESY and NOEDS spectra. From the data presented above, the structure of **2** was elucidated as (22*S*,25*S*) 26-*O*-β-D-glucopyranosyl-22,25-epoxy-7β-methoxy-furost-5-ene-3β,26-diol 3-*O*-β-chacotrioside.

Abutiloside N (**3**) was obtained as a white powder, $[\alpha]_D$ −84.8° (MeOH). In the negative-ion FAB MS of **3**, a quasi-molecular ion peak was observed at *m*/*z* 1077 [M–H]$^-$. The ^{1}H NMR spectral data of **3** was similar to those of (22*S*,25*S*) 26-*O*-β-D-glucopyranosyl 16β,22;22,25-diepoxy-furost-5-ene-3β,26-diol 3-*O*-β-solatrioside (aculeatioside B). On acid hydrolysis, **3** afforded D-glucose, D-galactose and L-rhamnose. Comparison of the ^{13}C NMR spectrum of **3** with that of **1** proved the same aglycone moiety as that of **1**. Furthermore, the anomeric proton signals at δ 4.88 (H-1′), 6.32 (H-1″), 5.22 (H-1‴) and 4.96 (H-1⁗) showed long-range correlations with the carbon signals at δ 78.6 (C-3), 75.0 (C-2′), 84.8 (C-3′) and 77.6 (C-26), respectively. Therefore, the structure of **1** was elucidated as (22*S*,25*S*) 26-*O*-β-D-glucopyranosyl-22,25-epoxy-furost-5-ene-3β,7β, 26-triol 3-*O*-β-solatrioside.

Abutiloside O (**4**) was obtained as a white powder, $[\alpha]_D$ −46.5° (MeOH). In the negative-ion FAB MS of **4**, a quasi-molecular ion peak was observed at *m*/*z* 927 [M–H]$^-$. The ^{1}H NMR spectrum displayed signals for two quaternary methyls at δ 1.05 and 1.41, a quaternary methyl on olefinic linkage at δ 2.30, three secondary methyls at δ 0.76 (*d*, $J=6.4$ Hz), 1.65 (*d*, $J=6.1$ Hz) and 1.77 (*d*, $J=6.1$ Hz), a methoxyl proton at δ 3.42 and an olefinic proton at δ 5.32 (1H, *d*, $J=4.4$ Hz), along with three anomeric protons at δ 4.95 (1H, *d*, $J=7.6$ Hz), 5.88 (1H, *d*, $J=7.8$ Hz) and 6.42 (1H, *br s*). The above data indicated that **4** was a steroidal triglycoside derivative. The ^{13}C NMR spectrum of **4** displayed 28 carbon signals due to the aglycone, along with carbon signals of a β-chacotriosyl moiety [δ_C 100.3, (δ_H 4.95), δ_C 102.1 (δ_H 6.42) and δ_C 103.0 (δ_H 5.88)] due to the

sugar part. Moreover, in the ^{13}C NMR spectrum of **4**, signals due to the aglycone moiety, except for the signals of the D-ring and side chain, and the sugar moiety were in good agreement with those of 3β-hydroxy-pregn-5,16-dien-20-one 3-*O*-β-chacotrioside (compound Pd). With regard to the signals from around the D-ring,

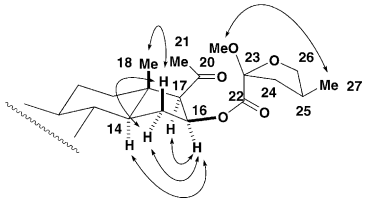

Fig. 2. NOE correlations of the aglycone moiety of **4**.

the ^1H–^1H COSY showed a sequence of connectivities through a methine proton at δ 0.70 (*m*, H-14), methylene protons at δ 1.62 (1H, *m*, H-15) and 2.43 (1H, *m*, H-15), an oxygen-bearing methine proton at δ 5.70 (*m*, H-16), a methine proton at δ 2.46 (*d*, $J = 8.3$ Hz, H-17), methylene protons at δ 1.60 (1H, *m*, H-24) and 2.10 (1H, *m*, H-24), a methine proton at δ 2.41 (*m*, H-25), a secondary methyl at δ 0.76 (*d*, $J = 6.4$ Hz, H-27), methylene protons at δ 2.79 (1H, *m*, H-26) and 3.26 (1H, *m*, H-26). The long-range correlations between δ 2.30 (H-21) and δ 206.4 (C-20), 67.8 (C-17); δ 5.70 (H-16) and δ 167.4 (C-22); δ 2.10 (H-24) and δ 97.2 (C-23), 167.4 (C-22); a methoxyl proton at δ 3.42 (*s*) and δ 97.2 (C-23) indicated the ester group linked to the C-16 hydroxyl group and the five-membered acetal ring between C-23 and C-26.

Table 1
^{1}HNMR chemical shift of **1**–**4** (pyridine-d_5)

	1	**2**	**3**	**4**
H-3	3.78 *m*	3.87 *m*	3.90 *m*	3.89 *m*
6	5.79 *d* (4.9)	5.78 *d* (3.7)	5.82 *d* (5.2)	5.32 *d* (4.4)
7	3.96 *m*	3.25 *m*	3.99 *m*	1.47, 1.92
14	2.01	1.82	2.03	0.70
15	1.51, 2.51	1.50, 2.15	1.52, 2.52	1.62, 2.43
16	4.80 *m*	4.75 *m*	4.81 *m*	5.70 *m*
17	1.83	1.76	1.84	2.46 *d* (8.3)
18	0.90 *s*	0.84 *s*	0.89 *s*	1.41 *s*
19	1.06 *s*	1.02 *s*	1.07 *s*	0.13 *d* (3.7)
21	1.08 *d* (6.7)	1.07 *d* (6.7)	1.08 *d* (6.7), 2.30 *s*	
25				2.41 *m*
26	3.88 *d* (9.8)	3.94 *d* (9.8)	3.89 *d* (9.8)	2.79 *m*
	4.18 *d* (9.8)	4.21 *d* (9.8)	4.19 *d* (9.8)	3.26 *m*
27	1.40 *s*	1.42 *s*	1.41 *s*	0.76 *d* (6.4)
Ome		3.26 *s*		3.42 *s*
	glc	glc	gal	Glc
H-1′	4.90 *d* (7.6)	4.96 *d* (7.7)	4.88 *d* (7.8)	4.95 *d* (7.8)
2′	4.23	4.25	4.71 *dd* (7.8,8.5)	4.25 *dd* (7.8,8.5)
3′	4.23	4.25	4.34 *dd* (2.9,8.5)	4.23
4′	4.40 *dd* (8.5,8.5)	4.42 *dd* (8.5,8.5)	4.83 *d* (2.9)	4.42 *dd* (8.5,8.5)
5′	3.62 *br d* (9.2)	3.62 *br d* (9.2)	4.02 *br* t (6.0)	3.67 *br d* (9.3)
6′	4.12 *br d* (12.2)	4.10 *br d* (12.5)	4.26	4.11 *br d* (11.2)
	4.23 *dd* (3.8,11.6)	4.19 *br d* (10.6)	4.40 *dd* (6.8,11.5)	4.23
	rha	rha	rha	Rha1
H-1″	6.39 *br s*	6.40 *br s*	6.32 *br s*	6.42 *br s*
2″	4.85 *br s*	4.88 *br s*	4.92 *br s*	4.85 *br s*
3″	4.64 *dd* (2.7,8.5)	4.67 *dd* (3.0,8.5)	4.62 *dd* (3.2,8.5)	4.65 *dd* (3.3,8.5)
4″	4.38 *dd* (8.5,8.5)	4.40 *dd* (8.5,8.5)	4.30 *dd* (8.5,8.5)	4.38 *dd* (8.5,8.5)
5″	4.98 *m*	4.98 *m*	4.94 *m*	4.98 *m*
6″	1.76 *d* (6.1)	1.76 *d* (6.1)	1.70 *d* (6.1)	1.77 *d* (6.1)
	rha	rha	glc	Rha
H-1‴	5.85 *br s*	5.86 *br s*	5.22 *d* (7.8)	5.88 *br s*
2‴	4.71 *br s*	4.71 *br s*	3.97 *dd* (7.8,8.5)	4.70 *br s*
3‴	4.56 *dd* (3.0,8.5)	4.57 *dd* (3.0,8.5)	4.22 *dd* (8.5,8.5)	4.57 *dd* (3.2,8.5)
4‴	4.36 *dd* (8.5,8.5)	4.37 *dd* (8.5,8.5)	4.22 *dd* (8.5,8.5)	4.35 *dd* (8.5,8.5)
5‴	4.95 *m*	4.98	3.95	4.94 *m*
6‴	1.63 *d* (6.1)	1.63 *d* (6.1)	4.33	1.65 *d* (6.1)
			4.49 *br d* (10.4)	
	glc	glc	glc1	
H-1⁗	4.95 *d* (7.8)	5.00 *d* (7.6)	4.96 *d* (7.6)	
2⁗	4.04 *dd* (7.8,8.5)	4.06 *dd* (7.6,8.5)	4.04 *dd* (7.6,8.5)	
3⁗	4.28 *dd* (8.5,8.5)	4.32 *dd* (8.5,8.5)	4.28 *dd* (8.5,8.5)	
4⁗	4.27 *dd* (8.5,8.5)	4.29 *dd* (8.5,8.5)	4.27 *dd* (8.5,8.5)	
5⁗	3.95 *m*	3.96 *m*	3.97	
6⁗	4.43 *dd* (5.1,11.6)	4.44 *dd* (5.1,10.6)	4.43 *dd* (5.1,11.4)	
	4.57 *dd* (3.6,11.6)	4.55 *br d* (10.6)	4.56 *br d* (10.3)	

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

The NOE correlations, H3-18/H-15β, H-15β/H-15α, H-17/H-16α, H-16α/H-15α and H-14/H-16α in the NOESY and NOEDS spectrum, suggested a 16*S* configuration (Fig. 2). Moreover, an NOE was observed between H_3-27 and the methoxyl proton signal at δ 3.42, but their configurations at C-23 and C-25 were uncertain. Furthermore, the anomeric proton signals at δ 4.95 (H-1′), 6.42 (H-1″) and 5.88 (H-1‴) showed long-range correlations with the carbon signals at δ 78.1 (C-3), 77.8 (C-2′) and 78.7 (C-4′), respectively. Consequently, the structure of **4** was determined 3-*O*-β-D-chacotriosyl-3β,16β-dihydroxy-pregn-5-en-20-one 16-*O*-(2,5-epoxy-2-methoxy-4-methyl-pentanolic acid)-ester.

Table 2
^{13}CNMR spectral data for **1**–**4** (pyridine-d_5)

	1	2	3	4
C-1	37.3	37.2	37.3	37.6
2	30.1	30.1	30.1	30.3
3	78.0	78.1	78.6	78.1
4	39.0	39.2	38.8	39.1
5	143.7	146.0	143.9	140.8
6	125.9	121.3	125.9	121.8
7	64.5	73.7	64.6	32.2
8	37.9	37.1	38.0	31.2
9	42.5	43.1	42.5	50.6
10	38.0	38.1	38.0	37.2
11	21.0	21.0	21.0	20.9
12	39.8	39.5	39.8	38.4
13	40.4	40.4	40.4	42.5
14	49.7	49.0	49.7	54.4
15	32.4	32.3	32.4	37.7
16	81.2	81.1	81.2	74.6
17	62.6	62.6	62.7	67.8
18	16.3	16.0	16.3	15.0
19	18.4	18.3	18.4	19.6
20	38.7	38.7	38.7	206.4
21	15.3	15.3	15.3	31.5
22	120.1	120.2	120.2	167.4
23	33.2	33.2	33.2	97.2
24	34.1	34.1	34.1	40.8
25	83.8	83.9	83.8	26.1
26	77.5	77.6	77.6	49.1
27	24.5	24.5	24.6	18.7
OMe		56.4		50.0
	glc	glc	gal	glc
C-1′	100.3	100.6	100.4	100.3
2′	77.7	77.8	75.0	77.8
3′	77.8	78.0	84.8	78.1
4′	78.6	78.5	70.6	78.7
5′	77.0	76.9	76.6	77.0
6′	61.4	61.2	61.4	61.4
	rha	rha	rha	rha
C-1″	102.0	102.0	102.2	102.1
2″	72.5	72.6	72.7	72.6
3″	72.8	72.9	72.9	72.9
4″	74.1	74.2	74.2	74.2
5″	69.6	69.6	69.5	69.6
6″	18.7	18.7	18.7	18.8
	rha	rha	glc	rha
C-1‴	102.9	102.9	105.9	103.0
2‴	72.6	72.6	75.4	72.8
3‴	72.8	72.8	78.5	72.9
4‴	73.9	74.0	71.7	74.0
5‴	70.5	70.4	77.2	70.5
6‴	18.6	18.6	62.7	18.7
	glc	glc	glc	
C-1⁗	105.4	105.3	105.5	
2⁗	75.4	75.4	75.4	
3⁗	78.4	78.5	78.5	
4⁗	71.6	71.6	71.6	
5⁗	78.6	78.5	78.6	
6⁗	62.7	62.7	62.7	

Previous phytochemical investigations have resulted in the isolation of three 22*S*,25*S*-epoxy-furost-5-ene type sapognols [nuatigenin (Tschesche et al., 1964), (22*S*,25*S*)-22,25-epoxy-furost-5-ene-2α,3β,26-triol (Brunengo et al., 1988) and taccagenin (Aziz et al., 1990)] from the plant kingdom. Abutilosides L (**1**), M (**2**) and N (**3**) are very rare steroidal glycosides possessing a 22*S*,25*S*-epoxy-furost-5-ene type sapogenol having the β-hydroxyl group at C-7. Abutiloside O (**4**), being a 20-22 *seco*-type steroidal glycoside, would be produced from furostanol glycoside through biogenetic route B in the plant body (Zhu et al., 2001).

3. Experimental

3.1. General

Optical rotations were taken with a JASCO DIP-1000 automatic digital polarimeter. NMR spectra were measured with a JEOL AL 400 NMR spectrometer and chemical shifts are given on a δ (ppm) scale with TMS as an internal standard. FAB MS were recorded with a JEOL DX-303 HF spectrometer. HR FAB MS was recorded with a JEOL HX-110 spectrometer. HPLC was carried out using a TSK gel-120A (7.8 mm i.d.×30 cm) column with a Tosoh CCPM pump, Tosoh RI-8010 detector and JASCO OR-2090 detector. TLC was performed on pre-coated Kieselgel 60 F_{254} (Merck), and detection was achieved by spraying with 10% H_2SO_4 followed by heating. CC was carried out on Kieselgel (230–400 mesh, Merck), ODS (PrePAK-500/C_{18}, Waters) and MCI gel CHP20P (Mitsubishi Chemical Ind.)

3.2. Plant material

Solanum abutiloides were cultivated at the Botanical Garden of Kumamoto University. The fresh fruits (271 g) 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.

3.3. Extraction and isolation

The fresh fruits of *S. abutiloides* (271 g) were extracted with MeOH (1 l) at room temperature for three months,

and the extract (22 g) was partitioned in chloroform and water (1:1). The water-soluble portion (20 g) was subjected to MCI gel CHP20P CC with MeOH–H_2O (40→50→60→70→80→90%) to afford nine fractions (fr.1–fr.9). Fraction 3 (1.1 g) was further separated by ODS CC with MeOH–H_2O (40→50→60→70%) to afford three fractions (fr.10–fr.12). Fraction 12 was subjected to silica gel CC with $CHCl_3$–MeOH–H_2O (6:4:1), followed by HPLC with MeOH–H_2O (13:7), to furnish abutilosides L (**1**) (23 mg), M (**2**) (8 mg) and N (**3**) (5 mg). Fraction 5 (2.6 g) was further separated by ODS CC with MeOH–H_2O (50→60→70→80%) to afford two fractions (fr.13 and fr.14). Fraction 13 was subjected to silica gel CC with $CHCl_3$–MeOH–H_2O (6:4:0.5), followed by HPLC with MeOH–H_2O (7:3), to furnish aculeatiosides A (1.4 g) and B (424 mg). Fraction 6 (315 mg) was subjected to silica gel CC with $CHCl_3$–MeOH–H_2O (6:4:0.5), followed by HPLC with MeOH–H_2O (13:7), to furnish abutiloside O (**4**) (7 mg). Fraction 8 (206 mg) was subjected to silica gel CC with $CHCl_3$–MeOH–H_2O (6:4:0.5), followed by HPLC with MeOH–H_2O (7:3), to furnish compound Pd (71 mg).

3.4. Abutiloside L (*1*)

White powder; $[\alpha]_D^{25}$ −107.1° (*c* 1.15, MeOH); Neg. FAB MS (*m*/*z*): 1061 $[M–H]^-$; Pos. FAB-MS (*m*/*z*): 1085 $[M+Na]^+$; HR FAB MS (*m*/*z*): 1085.5153 $[M+Na]^+$ (Calcd for $C_{49}H_{80}O_{20}Na$ 1085.5145); for ^1H and ^{13}C NMR (pyridine-d_5) spectra, see Tables 1 and 2.

3.5. Abutiloside M (*2*)

White powder; $[\alpha]_D^{25}$ −110.9° (*c* 0.37, MeOH); Neg. FAB MS (*m*/*z*): 1075 $[M–H]^-$; for ^1H and ^{13}C NMR (pyridine-d_5) spectra, see Tables 1 and 2.

3.6. Abutiloside N (*3*)

White powder; $[\alpha]_D^{25}$ −84.8° (*c* 0.24, MeOH); Neg. FAB MS (*m*/*z*): 1077 $[M–H]^-$; for ^1H and ^{13}C NMR (pyridine-d_5) spectra, see Tables 1 and 2.

3.7. Abutiloside O (*4*)

White powder; $[\alpha]_D^{25}$ −46.5° (*c* 0.34, MeOH); Neg. FAB MS (*m*/*z*): 927 $[M–H]^-$; for ^1H and ^{13}C NMR (pyridine-d_5) spectra, see Tables 1 and 2.

3.8. Sugar analysis of *1* and *3*

A solution each of compound (**1** or **3**) (1 mg) in 2 N HCl–dioxane (1:1, 2 ml) was heated at 100 °C for 1 h. The reaction mixture was diluted with H_2O and evaporated to remove dioxane. The solution was neutralized with Amberlite MB-3 and passed through a SEP-PAK C_{18} cartridge to give a sugar fraction. The sugar fraction was concentrated to dryness *in vacuo* to give a residue, which was dissolved in CH_3CN–H_2O (3:1, 250 μl). The sugar fraction was analyzed by HPLC under the following conditions: column, Shodex RS-Pac DC-613 (6.0 mm i.d.×150 mm, Showa-Denko, Tokyo, Japan); solvent, CH_3CN-H_2O (3:1); flow rate, 1.0 ml/min; column temperature, 70 °C; detection, refractive index (RI) and optical rotation (OR). *tR* (min) of sugars were as follow. **1**: L-rhamnose 4.8 (−), D-glucose 7.4 (+). **3**: L-rhamnose 4.8 (−), D-glucose 7.4 (+), D-galactose 8.0 (+). [reference: L-rhamnose 4.8 (negative optical rotation: -), D-glucose 7.4 (positive optical rotation: +), D-galactose 8.0 (positive optical rotation: +)].

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