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Polyhydroxylated pyrrolidine and piperidine alkaloids from *Adenophora triphylla* var. *japonica* (Campanulaceae)

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Abstract

Adenophora triphylla var. *japonica* (Campanulaceae) yielded two new alkaloids, the 6-*C*-butyl derivative of 2*R*,5*R*-bis(hydroxymethyl)-3*R*,4*R*-dihydroxypyrrolidine (DMDP) and α-1-*C*-ethyl-fagomine, together with the known alkaloids 1,4-dideoxy-1,4-imino-D-arabinitol, 1-deoxynojirimycin, and 1-deoxymannojirimycin. 6-*C*-Butyl-DMDP showed inhibitory activity toward almond β-glucosidase (IC_{50} = 68 μM), whereas α-1-*C*-ethyl-fagomine inhibited bovine liver β-galactosidase (IC_{50} = 29 μM).

Keywords: *Adenophora triphylla* var. *japonica*; Campanulaceae; 1,4-Dideoxy-1,4-imino-D-arabinitol (D-AB1); 6-*C*-Butyl-DMDP; 1-Deoxynojirimycin; 1-Deoxymannojirimycin; α-1-*C*-Ethyl-fagomine; Glycosidase inhibitors

1. Introduction

It is now well recognized that glycosidase inhibitors have potential as antiviral, anticancer, and antidiabetic agents. Some α-glucosidase inhibitors have been already introduced into the market for the treatment of diabetes. The therapeutic application of α-glucosidase inhibitors as antiviral agents against human hepatitis viruses B (HBV) and C (HCV) is also under investigation (Mehta, Zitzmann, Rudd, Block & Dwek, 1998). In a search for a new type of α-glucosidase inhibitor from plants in the Campanulaceae using rice α-glucosidase as an assay enzyme, potent inhibitory activity (IC_{50} = 0.1 μg/ml) was found in the 50% aqueous MeOH extract of *Adenophora triphylla* var. *japonica* after preliminary purification by ion-exchange chromatography with Amberlite IR-120B (H^+ form) and Dowex 1-X2 (OH^- form). A similar aqueous MeOH extract of *Campanula rotundifolia* has been shown to have potent inhibitory activity toward glucosidases, and this inhibition was found to be due to a high concentration (up to 2% dry weight in leaves/stems) of DMDP (**1**) (2*R*,5*R*-bis-(hydroxymethyl)-3*R*,4*R*-dihydroxypyrrolidine, 2,5-dideoxy-2,5-imino-D-mannitol) which is the major alkaloid in all parts of this species (Nash et al., 1998). DMDP was not detected by GC–MS analysis of the ion-exchange resin-treated extract of *A. triphylla* var. *japonica*, indicating that the potent inhibition of rice α-glucosidase by this plant extract was due to constituents other than DMDP (**1**). In this paper, we describe the isolation of five polyhydroxylated alkaloids from *A. triphylla* var. *japonica*, as well as their structural determination and glycosidase inhibitory activities.

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2. Results and discussion

A 50% aqueous MeOH extract of the fresh whole plants (3 kg) of *A. triphylla* var. *japonica* was subjected to various ion-exchange resin chromatographic steps to give compounds **2** (27 mg), **3** (26 mg), **4** (13 mg), **5** (6 mg), and **6** (11 mg). The ^{1}H- and ^{13}C-NMR spectra of compounds **2**, **3**, and **4** were in accordance with those of the known compounds, 1,4-dideoxy-1,4-imino-D-arabinitol (D-AB1) and 1-deoxynojirimycin (DNJ) from *Morus bombycis* (Asano, Tomioka, Kizu & Matsui, 1994a), and 1-deoxymannojirimycin (DMJ) from *Hyacinthus orientalis* (Asano et al., 1998), respectively.

The ^{13}C-NMR spectrum of compound **5** revealed the presence of a single methyl, four methylene, and five methine carbon atoms (Table 1). The HR-FABMS of **5** gave an $[M + H]^+$ ion at m/z 220.1545 ($C_{10}H_{22}O_4N$ requires 220.1549). The complete connectivity of the carbon and hydrogen atoms was defined from analysis of ^{1}H–^{1}H COSY, ^{1}H–^{13}C COSY, and HMBC spectral data. In the coupled ^{13}C-NMR spectra (CD_3OD), the methylene triplet at δ 64.0 (C-1) was attributed to the hydroxymethyl carbon, and the methine doublets at δ 75.2, 75.8, and 76.1 were assigned to C-6, C-4, and C-3 bearing OH groups. The relatively high-field chemical shifts of the methine doublets at δ 64.4 (C-2) and 68.7 (C-5) indicated that they must be bonded to the nitrogen of the heterocyclic ring. The ^{1}H–^{1}H COSY and HMBC spectral data indicated that the butyl group (δ 16.2, 25.6, 31.2, and 36.7) was bonded to C-6. Since the stereogenic centers of the pyrrolidine ring protons could not be determined from their coupling constants ($J_{2,3} = 4.2$ Hz, $J_{3,4} = 4.9$ Hz, $J_{4,5} = 6.8$ Hz), we performed extensive NOE experiments. Irradiation of H-4 enhanced the NOE signal intensity of H-2 and H-6, and NOE effects between H-3 and the C-1 (CH_2OH) protons were also observed. These results suggest that H-2, H-3, H-4, and H-5 are in the β, α, β, and α orientations, respectively. Thus, compound **5** was determined to be 6-*C*-butyl-DMDP or its enantiomer. The relative configuration at C-6 cannot be determined from the NMR spectral data.

The ^{13}C-NMR spectrum of compound **6** revealed the presence of a single methyl, three methylene, and four methine carbon atoms (Table 1). The HR-FABMS of **6** gave $[M + H]^+$ ion at m/z 176.1286 ($C_8H_{18}O_3N$ requires 176.1287). The complete connectivity of the carbon and hydrogen atoms was defined from extensive decoupling experiments, and from analysis of ^{1}H–^{13}C COSY and HMBC spectral data. From inspection of the ^{13}C-NMR chemical shift data in D_2O, the methylene triplet at δ 64.5 was assigned to the C-6 hydroxymethyl carbon, and the methine doublets at δ 72.2 and 76.2 were assigned to C-3 and C-4 bearing OH groups. The relatively high-field methine doublets at δ 55.4 (C-1) and 57.8 (C-5) must be bonded to the nitrogen of the piperidine ring. The large J values ($J_{2ax,3} = 11.5$ Hz, $J_{3,4} = 9.0$ Hz, $J_{4,5} = 10.0$ Hz) seen in the H-3 and H-4 signals indicate all *trans*-axial orientations of H-3, H-4, and H-5, and, hence, the six-membered ring is in a chair conformation. The strong NOE effects between H-5 and the proton of the methylene group bonded to C-1 indicate an α orientation of the ethyl side chain at C-1. Additional NOEs were observed between H-5 and H-3, between H-4 and one of the C-2 protons, and between the C-7 methylene protons and the equatiorial C-2 proton. Thus, compound **6** was determined to be α-1-*C*-ethyl-fagomine or its enantiomer.

The IC_{50} values of compounds **5** and **6** toward various glycosidases are shown in Table 2. DMDP is a potent inhibitor of yeast α-glucosidase, almond β-glu-

Table 1
^{13}C-NMR chemical shifts[a] of compounds **5** and **6**, and their related compounds (**5**: CD_3OD, **6**: D_2O, homoDMDP: D_2O, fagomine: D_2O, 100 MHz)[a]

C	homoDMDP	**5**	Fagomine	**6**
1	64.5 *t*	64.0 *t*	45.4 *t*	55.4 *d*
2	64.7 *d*	64.4 *d*	35.6 *t*	37.1 *t*
3	80.8 *d*	76.1 *d*	76.1 *d*	72.2 *d*
4	80.6 *d*	75.8 *d*	76.1 *d*	76.2 *d*
5	64.3 *d*	68.7 *d*	63.7 *d*	57.8 *d*
6	75.7 *d*	75.2 *d*	64.5 *t*	64.5 *t*
7	66.2 *t*	36.7 *t*		26.1 *t*
8		31.2 *t*		13.1 *q*
9		25.6 *t*		
10		16.2 *q*		

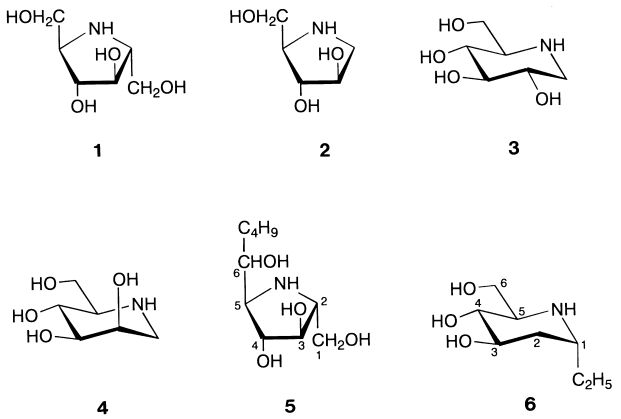
[a] Chemical shifts are expressed in ppm downfield from sodium 3-(trimethylsilyl)propionate (TSP) in D_2O and TMS in CD_3OD as internal standards.

Table 2
Concentration of alkaloids giving 50% inhibition of glycosidase activities

Enzyme	IC_{50} (μM)				
	DMDP	homoDMDP	**5**	Fagomine	**6**
α-Glucosidase					
Rice	300	130	NI[a]	240	490
Yeast	3.6	270	NI	NI	NI
Rat intestinal maltase	290	400	NI	820	NI
β-Glucosidase					
Almond	13	23	68	NI	NI
β-Galactosidase					
Bovine liver	2.2	4.4	390	38	29
Trehalase					
Porcine kidney	200	5.0	NI	NI	NI
Amyloglucosidase					
Aspergillus niger	19	180	40	NI	NI

[a] No inhibition (less than 50% inhibition at 1000 μM).

cosidase, and bovine liver β-galactosidase (Fleet, Nicolas, Smith, Evans, Fellows & Nash, 1985; Asano, Oseki, Kizu & Matsui, 1994b). The 6-*C*-hydroxymethyl derivative of DMDP (homoDMDP), which has been isolated from *H. orientalis*, is also a potent inhibitor of β-glucosidase and β-galactosidase (Asano et al., 1998). The *C*-butylation of DMDP at C-6 significantly lowered or abolished its inhibition toward bovine liver β-galactosidase and α-glucosidases, although inhibitory activity toward almond β-glucosidase was reasonably retained. The introduction of an ethyl group to the C-1 α position of fagomine, as in **6**, caused no significant change in its inhibitory spectrum. Although the resin-treated extract of *A. triphylla* var. *japonica* was found to show potent inhibitory activity (IC_{50} = 0.1 μg/ml) toward rice α-glucosidase, this inhibition was concluded to be mainly due to the inhibitory activity (IC_{50} = 0.05 μM) of 1-deoxynojirimycin toward the enzyme.



3. Experimental

3.1. General

The purity of samples was checked by HPTLC on Silica Gel 60F_{254} (Merck) using the solvent system 4:1:1 PrOH–AcOH–H_2O, and a chlorine-*O*-toluidine reagent (Pataki, 1963) was used for detection. Optical rotations were measured with a Jasco DIP-370 digital polarimeter. ^{1}H-NMR (400 MHz) and ^{13}C-NMR (100 MHz) spectra were recorded on a Jeol JNM-GX 400 spectrometer. Chemical shifts are expressed in ppm downfield from sodium 3-(trimethylsilyl)propionate (TSP) in D_2O and TMS in CD_3OD as internal standards. MS were measured on a Jeol JMS-SX 102A spectrometer.

3.2. GLC–MS analysis

Samples were dried and silylated at 20°C for 60 min using 100 μl of Sigma Sil-A (Sigma) per milligram of material (Nash, Goldstein, Evans & Fellows, 1986). The column was a 25 m × 0.22 mm BPX5 (film thickness, 0.25 μm) capillary column (SGE), and the 25-min temperature program ran from 180 to 300°C with an initial rate of increase of 10°C/min and then held at 300°C. The mass spectrometer was a Perkin Elmer QMASS 910 set at 70 eV and a mass range of 100–650 amu.

3.3. Plant material

Adenophora triphylla var. *japonica* was grown at the Medicinal Plants Garden, Hokuriku University, Japan and collected in July 1996. A voucher specimen was deposited in the Herbarium of the Medicinal Plants Garden.

3.4. Extraction and isolation

A 50% aqueous MeOH extract of the fresh whole plants (3 kg) of *A. triphylla* var. *japonica* was applied to a column of Amberlite IR-120B (300 ml, H^+ form). The 0.5 M NH_4OH eluate was concentrated to give a brown oil (3.8 g), which was chromatographed over a Dowex 1-X2 (2 × 97 cm, OH^- form) with H_2O as eluent (fraction size 9 ml). The H_2O eluent was divided into two pools A (fractions 48–62, 130 mg) and B (fractions 73–115, 580 mg). Pool A was further chromatographed over an Amberlite CG-50 (2 × 95 cm, NH_4^+ form) column with H_2O as eluent to give **3** (26 mg) and then the column was eluted with 0.1 M NH_4OH to give **4** (13 mg) and **6** (11 mg) in order of elution. Pool B was chromatographed over a Dowex 1-X2 (2 × 97 cm, OH^- form) with H_2O as eluent to give **2** (27 mg) and **5** (6 mg) in order of elution.

3.5. Glycosidase inhibitory activities

The α-glucosidases (from rice and yeast), β-glucosidase (from almond), β-galactosidase (from bovine liver), amyloglucosidase (*Aspergillus niger*), trehalase (from porcine kidney), *p*-nitrophenyl glycosides, and disaccharides were purchased from Sigma. Brush border membranes, prepared from the intestine of male Wister rats according to the literature (Kessler, Acuto, Strelli, Murer & Semenza, 1978), were used as the source of rat intestinal glycosidases.

The activities of rice α-glucosidase, rat intestinal maltase, amyloglucosidase, and trehalase were determined using maltose or trehalose as a substrate at the optimum pH of each enzyme. The released D-glucose was determined calorimetrically using Glucose B-test Wako (Wako). Other glycosidase activities were determined using an appropriate *p*-nitrophenyl glycoside as a substrate at the optimum pH of each enzyme. The

reaction was stopped by adding 400 mM Na_2CO_3. The released *p*-nitrophenol was measured spectrometrically at 400 nm.

3.6. 6-C-Butyl-DMDP (**5**)

$[\alpha]_D$ +174.3° (*c* 0.32, H_2O); HR-FABMS: *m/z* 220.1545 $[M + H]^+$ ($C_{10}H_{22}O_4N$ requires 220.1549); ^{1}H-NMR spectral data (400 MHz, CD_3OD); δ 0.94 (3H, *t*, $J = 7.3$ Hz, CH_3), 1.30–1.43 (3H, *m*, H-8a, H-9a, H-9b), 1.48–1.66 (3H, *m*, H-7a, H-7b, H-8b), 3.05 (1H, *dd*, $J = 4.2$, 6.8 Hz, H-5), 3.25 (1H, *ddd*, $J = 4.2$, 5.9, 6.4 Hz, H-2), 3.63 (1H, *dt*, $J = 4.2$, 8.3 Hz, H-6), 3.68 (1H, *dd*, $J = 6.4$, 11.0 Hz, H-1a), 3.78 (1H, *dd*, $J = 5.9$, 11.0 Hz, H-1b), 4.07 (1H, *dd*, $J = 4.2$, 4.9 Hz, H-3), 4.13 (1H, *dd*, $J = 4.9$, 6.8 Hz, H-4); ^{13}C-NMR spectral data: Table 1.

3.7. α-1-C-Ethyl-fagomine (**6**)

$[\alpha]_D$ +45.7° (*c* 0.71, H_2O); HR-FABMS: *m/z* 176.1286 $[M + H]^+$ ($C_8H_{18}O_3N$ requires 176.1287); ^{1}H-NMR spectral data (400 MHz, D_2O) δ 0.91 (3H, *t*, $J = 7.3$ Hz, CH_3), 1.55–1.67 (3H, *m*, H-2*ax*, H-7a, H-7b), 2.02 (1H, *ddd*, $J = 2.2$, 4.9, 13.4 Hz, H-2*eq*), 2.85 (1H, *ddd*, $J = 2.9$, 6.8, 10.0 Hz, H-5), 3.05 (1H, *m*, H-1), 3.20 (1H, *dd*, $J = 9.0$, 10.0 Hz, H-4), 3.63 (1H, *dd*, $J = 6.8$, 11.5 Hz, H-6a), 3.78 (1H, *ddd*, $J = 4.9$, 9.0, 11.5 Hz, H-3), 3.89 (1H, *dd*, $J = 2.9$, 11.5 Hz, H-6b); ^{13}C-NMR spectral data: Table 1.

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