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Homonojirimycin analogues and their glucosides from *Lobelia sessilifolia* and *Adenophora* spp. (Campanulaceae)

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Abstract

2,6-Dideoxy-7-*O*-(β-D-glucopyranosyl) 2,6-imino-D-*glycero*-L-*gulo*-heptitol (7-*O*-β-D-glucopyranosyl-α-homonojirimycin, **1**) was isolated from the 50% methanol extract of the whole plant of *Lobelia sessilifolia* (Campanulaceae), which was found to potently inhibit rice α-glucosidase. Adenophorae radix, roots of *Adenophora* spp. (Campanulaceae), yielded new homonojirimycin derivatives, adenophorine (**2**), 1-deoxyadenophorine (**3**), 5-deoxyadenophorine (**4**), 1-*C*-(5-amino-5-deoxy-β-D-galactopyranosyl)butane (β-1-*C*-butyl-deoxygalactonojirimycin, **5**), and the 1-*O*-β-D-glucosides of **2** (**6**) and **4** (**7**), in addition to the recently discovered α-1-*C*-ethylfagomine (**8**) and the known 1-deoxymannojirimycin (**9**) and 2*R*,5*R*-bis(hydroxymethyl)-3*R*,4*R*-dihydroxypyrrolidine (DMDP, **10**). Compound **4** is a potent inhibitor of coffee bean α-galactosidase ($IC_{50} = 6.4$ μM) and a reasonably good inhibitor of bovine liver β-galactosidase ($IC_{50} = 34$ μM). Compound **5** is a very specific and potent inhibitor of coffee bean α-galactosidase ($IC_{50} = 0.71$ μM). The glucosides **1** and **7** were potent inhibitors of various α-glucosidases, with IC_{50} values ranging from 1 to 0.1 μM. Furthermore, **1** potently inhibited porcine kidney trehalase ($IC_{50} = 0.013$ μM) but failed to inhibit α-galactosidase, whereas **7** was a potent inhibitor of α-galactosidase ($IC_{50} = 1.7$ μM) without trehalase inhibitory activity.

Keywords: *Lobelia sessilifolia*; Adenophorae radix; Campanulaceae; α-Homonojirimycin derivative; 5-Deoxyadenophorine; 5-Deoxyadenophorine-1-*O*-β-D-glucoside; α-Glucosidase inhibitor; α-Galactosidase inhibitor

1. Introduction

In 1976, 2*R*,5*R*-bis(hydroxymethyl)-3*R*,4*R*-dihydroxypyrrolidine (DMDP), the β-D-fructofuranose mimic, was found in leaves of the legume *Derris elliptica* [1]. DMDP is now being reported from many disparate species of plants and microorganisms [2], which would indicate that this is a common metabolite. In a search for glycosidase inhibitors from plants, the 50% methanol extracts of some species in the Campanulaceae family showed potent inhibitory activities against rice α-glucosidase. Very recently, we have isolated 6-*C*-butyl-

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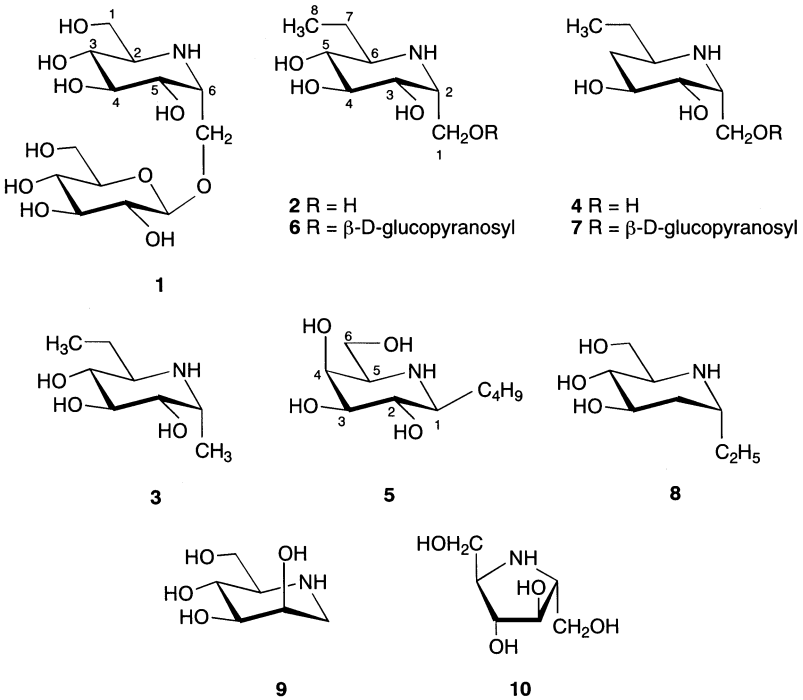
DMDP, α-1-*C*-ethylfagomine, 1,4-dideoxy-1,4-imino-D-arabinitol (D-AB1), 1-deoxynojirimycin (DNJ), and 1-deoxymannojirimycin (DMJ) from *Adenophora triphylla* var. *japonica* [3]. The potent inhibitory activity (IC_{50} = 0.1 μg/mL) of the 50% methanol extract of *A. triphylla* var. *japonica* against rice α-glucosidase was concluded to be mainly due to the presence of DNJ (IC_{50} = 0.05 μM). A 50% methanol extract of *Lobelia sessilifolia*, after preliminary purification by ion-exchange chromatography with Amberlite IR-120B (H^+ form) and Dowex 1-X2 (OH^- form), also showed potent inhibitory activity (IC_{50} = 0.1 μg/mL) against rice α-glucosidase, while the resin-treated hot-water extract of a traditional Chinese crude drug 'Sya-zin' (Adenophorae radix), which is the roots of *Adenophora* spp., exhibited good inhibitory activities against rice α-glucosidase (IC_{50} = 5.3 μg/mL), porcine kidney trehalase (IC_{50} = 5.4 μg/mL), coffee bean α-galactosidase (IC_{50} = 4.2 μg/mL), and bovine liver β-galactosidase (IC_{50} = 5.4 μg/mL). The aqueous methanol extract of *Campanula rotundifolia* has previously been reported to have a potent inhibitory activity against glucosidases, and this inhibition was shown to be due to a high concentration (up to 2% dry weight in leaves and stems) of DMDP, which is the major alkaloid in all parts of this plant [4]. In this paper, we describe the isolation and characterization of polyhydroxylated alkaloids from *L. sessilifolia* and Adenopharae radix, their structural determination, and their glycosidase inhibitory activities.

2. Results

Isolation and purification of alkaloids.—The alkaloid fraction was obtained by chromatography of the 50% methanol extract of the aerial parts (370 g) of *L. sessilifolia* on an Amberlite IR-120B (H^+ form) ion-exchange column. The alkaloid fraction was applied to a Dowex 1-X2 (OH^- form) column and eluted with water to give compound **1** (32 mg). On the other hand, the hot-water extract of Adenophorae radix (5 kg) was chromatographed with various ion-exchange resins to give compounds **2** (18 mg), **3** (25 mg), **4** (3 mg), **5** (13 mg), **6** (28 mg), **7** (18 mg), **8** (6 mg), **9** (63 mg), and **10** (380 mg). The HRFABMS, and ^1H and ^{13}C NMR spectral data of **1** were completely in accord with those of 7-*O*-β-D-glucopyranosyl-α-homonojirimycin (MDL 25637) isolated from *Aglaonema treubii* [5] and *Hyacinthus orientalis* [6]. MDL 25637 has been chemically synthesized as an oral antidiabetic agent [7]. The HRFABMS and ^1H and ^{13}C NMR spectral data of **8** were consistent with those of α-1-*C*-ethylfagomine isolated from *A. triphylla var. japonica* [3], and compounds **9** and **10** were identified as DMJ and DMDP, respectively, from their FABMS and NMR data (Scheme 1).

*Structural determination of compound **2**.*—The ^{13}C NMR spectral analysis of compound **2** revealed the presence of a single methyl, two methylene, and five methine carbon atoms (Table 1). This result and HRFABMS (*m/z* 192.1237 $[M+H]^+$) established that the molecular formula was $C_8H_{17}NO_4$. The complete connectivity of the carbon and hydrogen atoms was defined from extensive decoupling experiments and 2D ^1H–^{13}C COSY spectral data. From inspection of the ^{13}C NMR chemical shift data in D_2O, the methylene triplet at δ 59.2 (C-1) was assigned to the hydroxymethyl carbon and the methine doublets at δ 74.4, 77.2, and 77.6 to C-3, C-4, C-5 bearing the OH groups, respectively. The relatively high-field methine doublets at δ 56.5 (C-6) and 59.8 (C-2) must be bonded to the nitrogen of the piperidine ring. The large coupling constants ($J_{3,4}$ 10.0 Hz, $J_{4,5}$ 9.0 Hz, $J_{5,6}$ 9.8 Hz) seen in the H-3, H-4, and H-5 indicate an all-trans axial orientation of H-3, H-4, H-5, and H-6, and hence the six-membered ring is in a chair conformation. Irradiation of H-6 at δ 2.65 enhanced the NOE intensity of H-4 and the C-1 hydroxymethyl proton. This indicates the α orientation of the C-1 hydroxymethyl group. Thus, compound **2** was determined to be α-1-deoxy-1-*C*-methylhomonojirimycin or its enantiomer, and it was named adenophorine.

*Structural determination of compound **3**.*—The ^{13}C NMR spectral data (Table 1) of compound **3** revealed the presence of two methyl, a single methylene, and five methine carbon



Scheme 1.

atoms. This result and HRFABMS (*m*/*z* 176.1280 $[M+H]^+$) established that the molecular formula was $C_8H_{17}NO_3$. From inspection of the ^{13}C NMR chemical shift data in D_2O, the relatively high-field methine doublets at δ 53.6 (C-2) and 56.1 (C-6) must be bonded to the nitrogen of the piperidine ring. Decoupling experiments and 2D $^1H-^{13}C$ COSY revealed that the methyl group at δ 14.2 and the ethyl group (δ 11.7 and 26.5) were bonded to C-2 and C-6, respectively. The large coupling constants ($J_{3,4}$ 10.0 Hz, $J_{4,5}$ 8.8 Hz, $J_{5,6}$ 10.0 Hz) and an NOE between H-6 and H-4 or the C-1 methyl proton indicate an all-trans axial orientation of H-3, H-4, H-5, and H-6, and an α orientation of the C-1 methyl group. Thus, compound **3** was determined to be 1-deoxyadenophorine.

Structural determination of compound ***4***.— The ^{13}C NMR spectral data (Table 1) of compound **4** revealed the presence of a single methyl, three methylene, and four methine carbon. This result and HRFABMS (*m*/*z* 176.1280 $[M+H]^+$) established that the molecular formula was $C_8H_{17}NO_3$. Decoupling experiments and 2D COSY defined a linear CH_2–$(CH)_3$–CH_2–CH–CH_2–CH_3, showing a linear sequence from C-1H/H′ to the C-8 methyl group. From inspection of the ^{13}C NMR chemical shift data in D_2O (Table 1), the relatively high-field methine doublets at δ 51.9 (C-6) and 60.3 (C-2) must be bonded to the nitrogen of the piperidine ring, and the methylene triplet at δ 59.4 (C-1) was at-tributed to the hydroxymethyl carbon. From the MS and NMR data described above, com-

Table 1
^{13}C NMR chemical shifts [a] for homonojirimycin analogues and their glucosides

Carbon	2	3	4	5	6	7
C-1	59.2	14.2	59.4	61.8	68.4	68.5
C-2	59.8	53.6	60.3	74.9	58.3	58.7
C-3	74.4	75.6	76.0	77.9	74.2	75.7
C-4	77.2	76.5	71.4	71.8	77.3	71.4
C-5	77.6	78.0	40.2	61.1	77.6	40.0
C-6	56.5	56.1	51.9	64.2	56.7	52.2
C-7	26.8	26.5	30.7	33.5	26.8	30.5
C-8	11.7	11.7	12.4	29.6	11.8	12.4
C-9				25.0		
C-10				16.1		
C-1′					105.9	106.0
C-2′					76.0	76.0
C-3′					78.5	78.5
C-4′					72.5	72.5
C-5′					78.8	78.8
C-6′					63.6	63.6

[a] Chemical shifts are expressed in ppm downfield from TSP in D_2O.

pound **4** was presumed to be the 5-deoxy derivative of adenophorine or its isomer. NOEs between H-3 and H-5ax and between H-6 and H-4 or the C-1 hydroxymethyl proton established that **4** is 5-deoxyadenophorine.

Structural determination of compound ***5***.—The HRFABMS (m/z 220.1543 $[M+H]^+$) and ten resonances (Table 1) in the ^{13}C NMR spectrum of **5** established that the molecular formula was $C_{10}H_{21}NO_4$, which was the same as that of 6-*C*-butyl-DMDP isolated from *A. triphylla* var. *japonica* [3]. The complete connectivity of the carbon and hydrogen atoms was defined from extensive decoupling experiments and 2D $^1H-^{13}C$ COSY and HMBC spectral data. These data indicated the methine doublets at δ 61.1 (C-5) and 61.8 (C-1) were bonded to the nitrogen of the piperidine ring, bearing the hydroxymethyl (δ 64.2) and butyl groups (δ 16.1, 25.0, 29.6, 33.5), respectively. The strong NOE between H-1 and H-3 or H-5 indicates that these three protons are 1,3-trans-diaxial. The coupling patterns of H-2 (δ 3.40, dd, $J_{1,2}$ 9.2 Hz, $J_{2,3}$ 9.9 Hz) and H-3 (δ 3.50, dd, $J_{2,3}$ 9.9 Hz, $J_{3,4}$ 3.3 Hz) imply that H-2, H-3 and H-4 are axial, axial, and equatorial, respectively. Thus, compound **5** was determined to be 'β-1-*C*-butyl-deoxygalactonojirimycin' or its enantiomer. Very recently, Krülle et al. have enantiospecifically synthesized β-1-*C*-butyl-deoxygalactonojirimycin from D-galacto-1,4-lactone [10]. From the NMR data and specific rotation values (natural sample: $[\alpha]_D$ −12.8° (*c* 0.36, water); synthetic sample: $[\alpha]_D$ −14.8° (*c* 0.39, water)), both compounds were determined to be identical.

Structural determination of compound ***6***.—The HRFABMS (m/z 354.1758 $[M+H]^+$) and 14 resonances (Table 1) in the ^{13}C NMR spectrum of **6** established that the molecular formula was $C_{14}H_{27}NO_9$. The response to the naphthoresorcinol–sulfuric acid reagent and the characteristic anomeric proton (H-1′, δ 4.48, $J_{1',2'}$ 8.1 Hz) and carbon (C-1′, δ 105.9) signals in the NMR suggested that **6** was a glycoside of an alkaloid. After acid hydrolysis of this glycoside using Dowex 50W-X2 (H^+) resin, D-glucose was detected in the filtrate by the D-glucose oxidase peroxidase method, while the aglycone part was displaced from the resin with 0.5 M NH_4OH, concentrated to dryness, and confirmed as adenophorine by direct comparison of its ^{13}C NMR spectrum with that of **2**. The complete connectivity of the carbon and hydrogen atoms was defined from extensive decoupling experiments and 2D $^1H-^{13}C$ COSY spectral data. From the coupling constant of the anomeric proton of this D-glucoside, the type of glucosidic linkage was determined to be β. In the ^{13}C NMR spectrum, glucoside formation for adenophorine produced a 9.2-ppm downfield shift for C-1 and a 1.5-ppm upfield shift for C-2, while the chemical shifts of C-3 to C-8 remained unchanged (Table 1). Therefore, the structure of **6** was shown to be 1-*O*-β-D-glucopyranosyladenophorine.

Structural determination of compound ***7***.—The HRFABMS (m/z 338.1817 $[M+H]^+$) and 14 resonances (Table 1) in the ^{13}C NMR spectrum of **7** established that the molecular formula was $C_{14}H_{27}NO_8$. From the response to the naphthoresorcinol–sulfuric acid reagent and the characteristic anomeric proton (H-1′, δ 4.47, $J_{1',2'}$ 8.1 Hz) and carbon (C-1′, δ 106.0) signals in the NMR, compound **7** was also found to be a glycoside of an alkaloid. Acid hydrolysis of **7** in the same manner as described above gave D-glucose and 5-deoxyadenophorine. The linkage site was determined from the observation of strong NOEs from the anomeric proton to the C-1 hydroxymethyl protons (H-1a and H-1b) of the aglycone, obtained from 2D NOESY. Thus, compound **7** was determined to be 1-*O*-β-D-glucopyranosyl-5-deoxyadenophorine.

Glycosidase inhibitory activities of the alkaloids.—The IC_{50} values of homonojirimycin analogues and their glucosides against various glycosidases are shown in Table 2. We have reported that α-homonojirimycin (HNJ) is a potent inhibitor of rice α-glucosidase and all types of mammalian α-glucosidases [8]. Adenophorine (**2**), which is the 1-deoxy-1-*C*-methyl derivative of HNJ, was approximately a 100–1000-fold weaker inhibitor of α-glucosidases than HNJ, but it was a reasonably good inhibitor of coffee bean α-galactosidase (IC_{50} = 11 μM). Deoxygenation at C-1 of **2** to give **3** retained inhibitory potential toward α-glucosidases and α-galactosidase, while de-

Table 2
Concentration of homonojirimycin analogues and their glucosides giving 50% inhibition of glycosidase activities

Enzyme	IC_{50} (μM)						
	1	**2**	**3**	**4**	**5**	**6**	**7**
α-Glucosidase							
Rice	0.25	34	21	NI	NI	610	0.49
Rat intestinal sucrase	3.3	20	110	NI	NI	1000	2.4
Rat intestinal maltase	2.3	150	280	NI	NI	NI	6.1
Rat intestinal isomaltase	0.27	330	110	NI	NI	880	2.1
β-Glucosidase							
Almond	NI [a]	NI	NI	NI	NI	NI	NI
Trehalase							
Porcine kidney	0.013	NI	NI	1000	NI	53	NI
α-Galactosidase							
Coffee bean	NI	11	23	6.4	0.71	NI	1.7
β-Galactosidase							
Bovine liver	NI	NI	NI	34	NI	NI	NI

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

oxygenation at C-5 of **2** to give **4** enhanced its inhibitory activity toward α-galactosidase ($IC_{50} = 6.4$ μM) and further generated an inhibitory activity toward β-galactosidase ($IC_{50} = 34$ μM), but abolished its inhibition of α-glucosidases. Although 1-deoxygalactonojirimycin is known as an extremely powerful inhibitor ($K_i = 0.0016$ μM) of coffee bean α-galactosidase [9], its β-1-*C*-butyl derivative (**5**) was also a very specific inhibitor of this enzyme, with an IC_{50} value of 0.71 μM. β-1-*C*-Butyl-deoxygalactonojirimycin has been chemically synthesized [10] and its IC_{50} value toward coffee bean α-galactosidase was 0.69 μM. Thus, the natural compound **5** was identical with the synthetic sample in both the physicochemical aspects and biological activity. 7-*O*-β-D-Glucopyranosyl-α-homonojirimycin (MDL 25637) (**1**) is a potent inhibitor of α-glucosidases and trehalase [7,11]. 7-*O*-β-D-Glucopyranosyladenophorine (**6**), which can be regarded as the 1-deoxy-1-*C*-methyl derivative of **1**, significantly reduced inhibitory potential toward α-glucosidases and trehalase. Very interestingly, the deoxygenation at C-5 of **6** to give **7** remarkably enhanced its inhibitory activity toward α-glucosidases and α-galactosidase.

3. Discussion

Some of the polyhydroxylated alkaloids, such as DMDP, are being reported from many disparate species of plants, and more recently from microorganisms [2], which would indicate that this is clearly a common metabolite. Others appear to be restricted in distribution. For instance, until 1995 α-homonojirimycin had only been found in the neotropical liana *Omphalea diandra* (Euphorbiaceae) [12]. However, our recent work and this study elucidated that α-homonojirimycin or its glucoside also occurs in species of the Araceae (*A. treubii* [5] and *Nephthytis poissoni* [2]), *H. orientalis* (Hyacinthaceae) [6], and *L. sessilifolia* (Lobeliaceae). Similarly, until recently the polyhydroxylated pyrrolizidine alkaloids such as alexine had only been found in two closely related legume genera, *Alexa* and *Castanospermum* [2], but since 1994 they have been reported from the families Hyacinthaceae [13], Casuarinaceae [14], and Myrtaceae [15]. So, it is probably too early to discuss the significance of the distribution of polyhydroxylated alkaloids until broader surveys have been conducted.

We recently isolated the novel polyhydroxylated piperidine alkaloid with a linear eight-carbon backbone, α-1-*C*-ethylfagomine (**8**), from *A. triphylla* var. *japonica* [3]. Interestingly, compound **8** has the same carbon backbone structure as 5-deoxyadenophorine (**4**). Compounds **4** and **8** could be synthesized from a common precursor in the biosynthetic pathway. Also 6-*C*-butyl-DMDP from *A. triphylla* var. *japonica* [3] and β-1-*C*-butyl-deoxy-

galactonojirimycin (**5**) from Adenophorae radix could both be biosynthesized by the five- or six-membered ring closure from a common precursor.

The resin-treated extract of Adenophora radix exhibited a good inhibitory activity against rice α-glucosidase, coffee bean α-galactosidase, bovine liver β-galactosidase, and porcine kidney trehalase. This α-glucosidase inhibitory activity is concluded to be due to a combination of effects of **2** ($IC_{50} = 34$ μM), **3** ($IC_{50} = 21$ μM), **6** ($IC_{50} = 610$ μM), **7** ($IC_{50} = 0.49$ μM), **8** ($IC_{50} = 490$ μM), and **10** ($IC_{50} = 300$ μM) on rice α-glucosidase. The α- and β-galactosidase inhibitory activities are attributed to a combination of effects of **2** ($IC_{50} = 11$ μM), **3** ($IC_{50} = 23$ μM), **4** ($IC_{50} = 6.4$ μM), **5** ($IC_{50} = 0.71$ μM), and **7** ($IC_{50} = 1.7$ μM) on coffee bean α-galactosidase and that of **4** ($IC_{50} = 34$ μM), **8** ($IC_{50} = 29$ μM), and **10** ($IC_{50} = 2.2$ μM) on bovine liver β-galactosidase, respectively. With regard to the trehalase inhibition by the resin-treated extract, it would not be sufficient to explain this inhibitory activity only by that of DMDP ($IC_{50} = 500$ μM) and **6** ($IC_{50} = 53$ μM). We previously proved the presence of the two subsites, one for catalysis and one for recognition, on the active center of pig kidney trehalase from a kinetic analysis of the multiple inhibition by two competitive-type monosaccharide analogue inhibitors [16]. DMDP and DMJ are competitive inhibitors, with K_i values of 150 and 390 μM, respectively, and are classified into catalytic-site and recognition-site targeting inhibitors (Fig. 1). An equimolar mixture of DMDP and DMJ may produce the synergistic interactions with the two subsites, that is, the potent inhibition by the extract may be due to the synergistic effect obtained by multiplying the inhibitory activity for DMDP on the catalytic site times that for DMJ on the recognition site (data not shown).

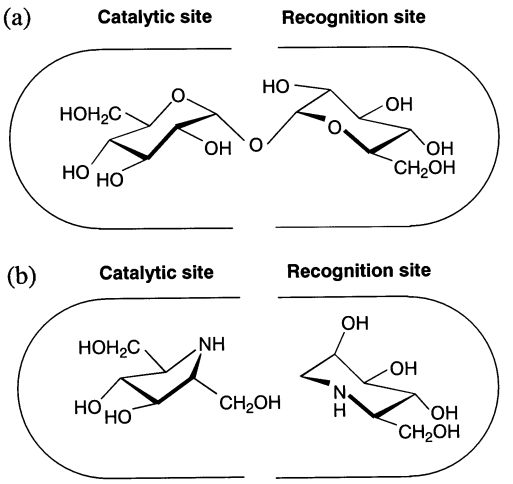


Fig. 1. (a) Trehalose and (b) DMDP and DMJ in the two subsites on the active center of trehalase.

4. Experimental

General methods.—The purity of samples was checked by HPTLC on Silica Gel $60F_{254}$ (E. Merck) using the solvent system 4:1:1 PrOH–AcOH–water, and a chlorine–*o*-tolidine reagent was used for detection. Optical rotations were measured with a Jasco DIP-370 digital polarimeter. ^1H 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. Mass spectra were measured on a Jeol JMS-SX 102A spectrometer.

Extraction and isolation.—A 50% MeOH extract of the fresh aerial parts (370 g) of *L. sessilifolia* was applied to a column of Amberlite IR-120B (200 mL, H^+ form). The 0.5 M NH_4OH eluate was concd to give a brown oil (650 mg), which was chromatographed over Dowex 1-X2 (1.5×63 cm, OH^- form) with water as eluant (fraction size 10 mL). Concentration of Fractions 18–23 gave compound **1** (32 mg). A hot-water extract of Adenophorae radix (5 kg) was applied to a column of Amberlite IR-120B (1 L, H^+ form) and eluted with 0.5 M NH_4OH. The NH_4OH eluate was concd to give a brown oil (59 g), which was applied to a column of Dowex 1-X2 (300 mL, OH^- form) and eluted with water (3 L). The water eluate was concd to give a colorless oil (37 g). This oil was chromatographed over a Dowex 1-X2 column (2.5×95 cm, OH^-form) with water as eluant (fraction size 20 mL). The water eluate was divided into two pools A (Fractions 23–45, 25 g) and B (Fractions 46–60, 0.85 g). Each pool was further chromatographed on an Amberlite CG-50 (2.5×95 cm, NH_4^+ form) column with water

as eluant to give **5** (13 mg), **2** (18 mg), **3** (25 mg), **6** (28 mg), **7** (18 mg), **8** (6 mg), **4** (3 mg), and DMJ (63 mg) from pool A in order of elution, and DMDP (380 mg) from pool B.

Glycosidase inhibitory activities.—The enzymes α-glucosidase (from rice), β-glucosidase (from almond), β-galactosidase (from bovine liver), trehalase (from porcine kidney), α-galactosidase (from coffee bean), *p*-nitrophenyl glycosides, and disaccharides were purchased from Sigma Chemical Co. Brush border membranes, prepared from the intestine of male Wister rats by the method of Kessler et al. [17], were used as the source of rat intestinal glycosidases.

The activities of rice α-glucosidase, rat intestinal glycosidases, and trehalase were determined using the appropriate disaccharides as substrates at the optimum pH of each enzyme. The released D-glucose was determined colorimetrically using the Glucose B-test (Wako Pure Chemical Industries). 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 specrometrically at 400 nm.

Adenophorine (**2**).—$[\alpha]_D$ +59.7° (*c* 1.0, water); HRFABMS: *m/z* 192.1237 $[M+H]^+$ ($C_8H_{18}NO_4$ requires 192.1236); ^{1}H NMR (400 MHz, D_2O) δ 0.94 (t, 3 H, *J* 7.6 Hz, CH_3), 1.35 (m, 1 H, H-7a), 1.84 (m, 1 H, H-7b), 2.65 (ddd, 1 H, *J* 3.4, 8.0, 9.8 Hz, H-6), 3.11 (dd, 1 H, *J* 9.0, 9.8 Hz, H-5), 3.26 (m, 1 H, H-2), 3.46 (dd, 1 H, *J* 9.0, 10.0 Hz, H-4), 3.75 (dd, 1 H, *J* 6.1, 10.0 Hz, H-3), 3.76–3.90 (2 H, H-1a, H-1b); ^{13}C NMR data, see Table 1.

1-Deoxyadenophorine (**3**).—$[\alpha]_D$ +67.8° (*c* 0.83, water); HRFABMS: *m/z* 176.1280 $[M+H]^+$ ($C_8H_{18}NO_3$ requires 176.1287); ^{1}H NMR (400 MHz, D_2O) δ 0.92 (t, 3 H, *J* 7.6 Hz, CH_3), 1.13 (d, 3 H, *J* 7.1 Hz, CH_3), 1.36 (m, 1 H, H-7a), 1.80 (m, 1 H, H-7b), 2.68 (ddd, 1 H, *J* 3.4, 7.6, 10.0 Hz, H-6), 3.09 (dd, 1 H, *J* 8.8, 10.0 Hz, H-5), 3.28 (m, 1 H, H-2), 3.53 (dd, 1 H, *J* 8.8, 10.0 Hz, H-4), 3.60 (dd, 1 H, *J* 5.6, 10.0 Hz, H-3); ^{13}C NMR data, see Table 1.

5-Deoxyadenophorine (**4**).—$[\alpha]_D$ +50.1° (*c* 0.32, water); HRFABMS: *m/z* 176.1285 $[M+H]^+$ ($C_8H_{18}NO_3$ requires 176.1287); ^{1}H NMR (400 MHz, D_2O) δ 0.91 (t, 3 H, *J* 7.6 Hz, CH_3), 1.16 (m, 1 H, H-5ax), 1.45 (m, 2 H, H-7a, H-7b), 2.09 (m, 1 H, H-5eq), 2.88 (m, 1 H, H-6), 3.32 (m, 1 H, H-2), 3.69–3.74 (2 H, H-3, H-4), 3.75–3.85 (2 H, H-1a, H-1b); ^{13}C NMR data, see Table 1.

1-C-(5-amino-5-deoxy-β-D-galactopyranosyl)-butane (β-1-C-butyl-deoxygalactonojirimycin, **5**).—$[\alpha]_D$ −12.8° (*c* 0.36, water); HR-FABMS: *m/z* 220.1543 $[M+H]^+$ ($C_{10}H_{22}NO_4$ requires 220.1549); ^{1}H NMR (400 MHz, D_2O) δ 0.91 (t, 3 H, CH_3), 1.25–1.47 (5 H, H-7a, H-8a, H-8b, H-9a, H-9b), 1.80 (m, 1 H, H-7b), 2.47 (ddd, 1 H, *J* 3.3, 7.7, 9.2 Hz, H-1), 2.80 (ddd, 1 H, J 1.5, 6.6, 7.0 Hz, H-5), 3.40 (dd, 1 H, *J* 9.2, 9.9 Hz, H-2), 3.50 (dd, 1 H, *J* 3.3, 9.9 Hz, H-3), 3.63 (dd, 1 H, *J* 6.6, 11.0 Hz, H-6a), 3.67 (dd, 1 H, *J* 7.0, 11.0 Hz, H-6b), 3.99 (dd, 1 H, *J* 1.5, 3.3 Hz, H-4); ^{13}C NMR data, see Table 1.

1-O-β-D-Glucopyranosyladenophorine (**6**).—$[\alpha]_D$ +24.9° (*c* 0.92, water); HRFABMS: *m/z* 354.1758 $[M+H]^+$ ($C_{14}H_{28}NO_9$ requires 354.1764); ^{1}H NMR (400 MHz, D_2O) δ 0.94 (t, 3 H, *J* 7.6 Hz, CH_3), 1.18 (m, 1 H, H-7a), 1.83 (m, 1 H, H-7a), 2.71 (ddd, 1 H, *J* 3.2, 7.8, 9.8 Hz, H-6), 3.12 (dd, 1 H, *J* 9.3, 9.8 Hz, H-5), 3.31 (dd, 1 H, *J* 8.1, 9.3 Hz, H-2′), 3.39 (dd, 1 H, *J* 9.0, 9.8 Hz, H-4′), 3.41–3.50 (3 H, H-2, H-4, H-5′), 3.51 (dd, 1 H, *J* 9.0, 9.3 Hz, H-3′), 3.73 (dd, 1 H, *J* 5.9, 12.5 Hz, H-6′a), 3.74 (dd, 1 H, *J* 6.1, 10.0 Hz, H-3), 3.92 (t, 1 H, *J* 10.7 Hz, H-1a), 3.92 (dd, 1 H, *J* 2.2, 12.5 Hz, H-6′b), 4.11 (dd, 1 H, *J* 3.7, 10.7 Hz, H-1b), 4.48 (d, 1 H, *J* 8.1 Hz, H-1′); ^{13}C NMR data, see Table 1.

1-O-β-D-Glucopyranosyl-5-deoxyadenophorine (**7**).—$[\alpha]_D$ +11.4° (*c* 0.33, water); HRFABMS: *m/z* 338.1817 $[M+H]^+$ ($C_{14}H_{28}NO_8$ requires 338.1815); ^{1}H NMR (400 MHz, D_2O) δ 0.91 (t, 3 H, *J* 7.6 Hz, CH_3), 1.17 (m, 1 H, H-5ax), 1.46 (m, 2 H, H-7a, H-7b), 2.10 (ddd, 1 H, *J* 2.9, 4.4, 12.9 Hz, H-5eq), 2.88 (m, 1 H, H-6), 3.31 (dd, 1 H, *J* 8.1, 9.3 Hz, H-2′), 3.40 (dd, 1 H, J 9.0, 9.8 Hz, H-4′), 3.47 (ddd, 1 H, J 2.2, 5.9, 9.8 Hz, H-5′), 3.49–3.54 (m, 1 H, H-2), 3.51 (dd, 1 H, *J* 9.0, 9.3 Hz, H-3′), 3.68–3.73 (m, 2 H, H-3, H-4), 3.73 (dd, 1 H, *J* 5.9, 12.4 Hz, H-6′a), 3.92 (dd, 1 H, *J* 12.4 Hz, H-6′b), 3.92 (t, 1 H, *J* 10.7 Hz, H-1a), 4.12 (dd, 1 H, *J* 3.7, 10.7 Hz, H-1b),

4.47 (d, 1 H, *J* 8.1 Hz, H-1′); ^{13}C NMR data, see Table 1.

Acid hydrolysis of compounds **6** *and* **7**.—A small amount (6 mg) of compound **6** or **7** was heated at 100° C with Dowex 50W-X2 (0.5 g, H^+ form) in water for 8 h. The resin was filtered off and packed into a short column. The alkaloid moiety was eluted with 0.5 M NH_4OH, concd and lyophilized. On the other hand, after neutalizing the filtrate, the amount of the released D-glucose was determined by the D-glucose oxidase peroxidase method using the commercially available Glucose B-test (Wako).

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