Polyhydroxylated Alkaloids Isolated from Mulberry Trees (*Morus alba* L.) and Silkworms (*Bombyx mori* L.)

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New polyhydroxylated alkaloids, (2*R*,3*R*,4*R*)-2-hydroxymethyl-3,4-dihydroxypyrrolidine-*N*-propionamide from the root bark of *Morus alba* L., and 4-*O*-α-D-galactopyranosyl-calystegine B_2 and 3*β*,6*β*-dihydroxynortropane from the fruits, were isolated by column chromatography using a variety of ion-exchange resins. Fifteen other polyhydroxylated alkaloids were also isolated. 1-Deoxynojirimycin, a potent α-glucosidase inhibitor, was concentrated 2.7-fold by silkworms feeding on mulberry leaves. Some alkaloids contained in mulberry leaves were potent inhibitors of mammalian digestive glycosidases but not inhibitors of silkworm midgut glycosidases, suggesting that the silkworm has enzymes specially adapted to enable it to feed on mulberry leaves. The possibility of preventing the onset of diabetes and obesity using natural dietary supplements containing 1-deoxynojirimycin and other α-glucosidase inhibitors in high concentration is of great potential interest.

Keywords: *Morus alba; Bombyx mori; polyhydroxylated alkaloids; glycosidase inhibition; diabetes*

INTRODUCTION

Mulberry trees (*Morus alba* L. and other plants of the genus *Morus*) are cultivated in China, Korea, and Japan, and their leaves are used to feed silkworms (*Bombyx mori* L.). In recent years, the development of new uses for sericulture-related materials, such as silk, silkworms, and mulberry trees, has been of great interest in East Asia. In Japan, fibroin protein solution, gel, and powder obtained from cocoons have been processed into cosmetics, skin cream, face washing milk, shampoo, and bath agents. Production of biologically active compounds by the silkworm-vaculovirus expression system has also been attempted. Mulberry leaves have been used traditionally to cure and prevent "Xiaoke" (diabetes) in Chinese medicine. The root bark of mulberry trees has long been used for antiinflammatory, diuretic, antitussive, and antipyretic purposes in Oriental medicine, whereas mulberry fruits are used as a tonic and sedative.

There are many people who suffer from life-style related diseases such as diabetes, hyperlipidemia, and hypertension as they grow older. Among these diseases, diabetes continues to increase worldwide and is well recognized as a major global health problem. Current scientific evidence demonstrates that much of the morbidity and mortality of diabetes can be eliminated by aggressive treatment with diet, exercise, and new pharmacological approaches to achieve better control of blood glucose levels. Furthermore, the possibility of preventing the onset of diabetes using dietary supplements and/or herbal medicines has attracted considerable attention. Hikino et al. (*1*) discovered that the root bark extract of *M. alba* shows a significant antihyperglycemic effect in normal and alloxan-induced hyperglycemic mice, and Kimura et al. (*2*) found that mulberry leaf extract shows a potent antihyperglycemic effect in streptozotocin-induced diabetic mice. In 1976, Japanese agrochemists isolated 1-deoxynojirimycin (**1**, Figure 1) from the root bark of mulberry tree and called it moranoline (*3*). Its original isolation was prompted by the knowledge that mulberry extracts were able to suppress the rise in blood glucose that follows eating and that this component might be beneficial to diabetes. Later, we isolated 18 sugar-mimic alkaloids (*4, 5*), including 1-deoxynojirimycin, from the leaves and root bark of mulberry trees (*6, 7*), and found that some of them are potent inhibitors of mammalian α-glucosidases (*8, 9*). The crude drug "Sohaku-hi", the root bark of mulberry trees, is used in some formulas of Japanese-Oriental (Kampo) medicine. As a result of the general acceptance of the role of Kampo, the Ministry of Health and Welfare of Japan now covers Kampo prescriptions under the National Health Insurance Plan. About 120 Kampo formulations, which are preparations extracted from combinations of several herbs, have become available in Japan. Recently, sericulture-related materials such as silk powder, silkworm powder, and mulberry leaves and fruits have been developed as natural functional foods in Korea and Japan. A Korean group found that silkworm powder and extract showed a significant antihyperglycemic effect both in alloxan-induced diabetic mice (*10*) and in mice fed with a high carbohydrate-containing diet, respectively (*11*). The

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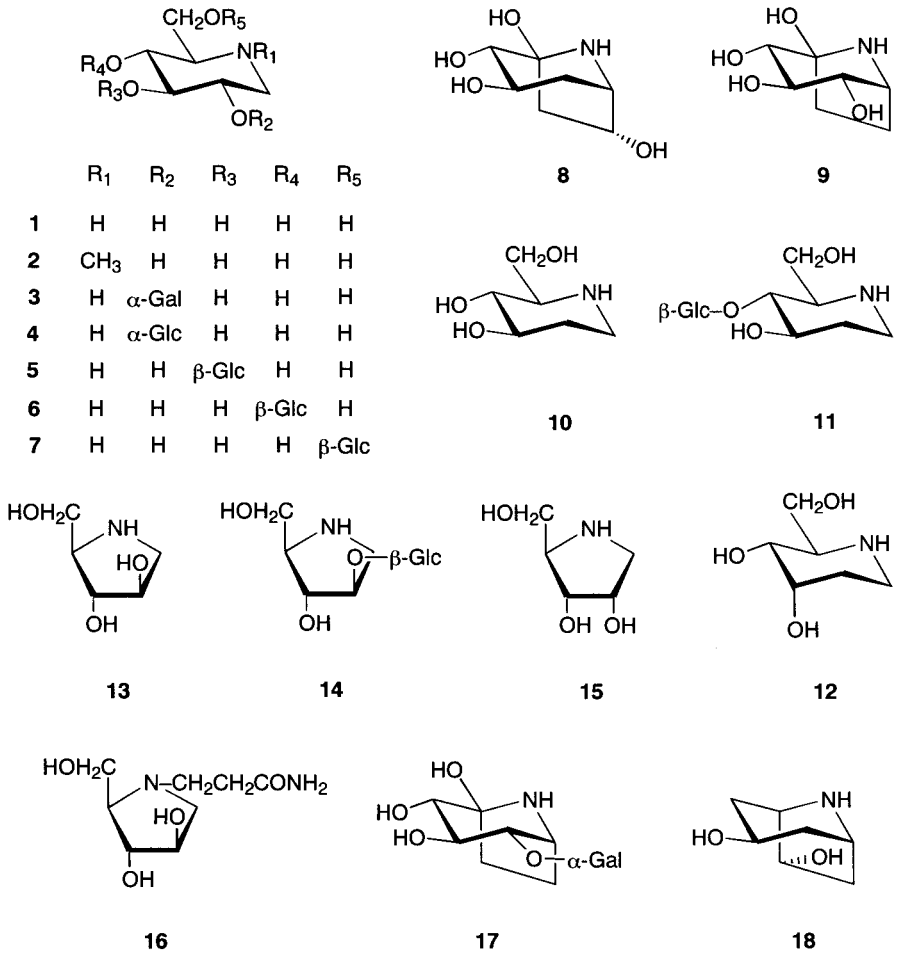

Figure 1. Structure of alkaloids isolated from sericulture related materials.

same group in Korea has developed ice-cream containing mulberry leaf powder with functionality and palatability, and has demonstrated that it decreases blood glucose levels after consumption (*12*). Mulberry fruits are broadly utilized as jam in Japan and other countries. In this paper we describe the yields of alkaloids in sericulture-related materials, the structural determination of several new alkaloids, and their glycosidase inhibitory activities.

MATERIALS AND METHODS

General Methods. The purity of samples was checked by HPTLC on silica gel $60F_{254}$ (E. Merck) using the solvent system PrOH−AcOH−H_2O (4:1:1), and a chlorine−*o*-tolidine reagent for detection. Optical rotations were measured with a JASCO DIP-370 digital polarimeter (Tokyo, Japan). ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were recorded on a JEOL JNM-GX 400 spectrometer (Tokyo, Japan). Chemical shifts are expressed in ppm downfield from sodium 3-(trimethylsilyl)propionate (TSP) as an internal standard in D_2O. MS were measured on a JEOL JMS-SX 102A spectrometer.

Sericulture-Related Materials. The root bark of *Morus alba* L. "Sohaku-hi", which was imported from An-Huei-Shong in China, was purchased from Daido Seiyaku Co. The fruits of *M. alba*, which were also imported from China, were purchased from a crude drug shop in Kanazawa, Japan. The leaves of *M. alba* cultivated at the National Sericulture and Entomology Institute, Korea, were blanched in 0.05−0.5% aqueous sodium bicarbonate solution, then dried and pulverized with a ball mill to produce powder. The fifth instar larvae of silkworm (*Bombyx mori* L.) grown in the same institute were freeze-dried and pulverized in the same way.

Extraction and Isolation. The root bark (10 kg) of *M. alba* was extracted three times with 50% aqueous MeOH (30 L). The extract was applied to a column of Amberlite IR-120B (66 cm × 5.2 cm i.d., H^+ form). The 0.5 M NH_4OH eluate was concentrated to give a brown oil (183 g), which was chromatographed over a Dowex 1-X2 (91 cm × 2.7 cm i.d., OH^- form) column with H_2O (pH 7.4) as eluant to remove brown pigments and anionic compounds. The H_2O eluate was concentrated to give a yellowish oil (124 g). Approximately 30-g portions of this yellowish oil were applied to an Amberlite CG-50 column (90 cm × 4 cm i.d., NH_4^+ form) with H_2O (pH 7.4) as eluant (fraction size 20 mL). The H_2O eluate was divided into three pools: A (fractions 30−50); B (fractions 51−80); and C (fractions 134−180). The 0.5 M NH_4OH eluate from the same column was designated pool D. Total yields of pools A, B, C, and D were 45, 30, 0.72, and 3.3 g, respectively. Repeated chromatography of pool A with Dowex 1-X2 (OH^- form) and/or Amberlite CG-50 (NH_4^+ form) with H_2O (pH 7.4) as eluant gave 1-deoxynojirimycin (0.3 g, **1**), *N*-methyl-1-deoxynojirimycin (64 mg, **2**), 2-*O*-α-D-galactopyranosyl-1-deoxynojirimycin (126 mg, **3**), 2-*O*-α-D-glucopyranosyl-1-deoxynojirimycin (10 mg, **4**), 3-*O*-*β*-D-glucopyranosyl-1-deoxynojirimycin (80 mg, **5**), 4-*O*-*β*-D-glucopyranosyl-1-deoxynojirimycin (11 mg, **6**), 6-*O*-*β*-D-glucopyranosyl-1-deoxynojirimycin (20 mg, **7**), and a new compound (6 mg, **16**). Pool B was similarly chromatographed with Dowex 1-X2 (OH^- form) and Amberlite CG-50 (NH_4^+ form) using H_2O (pH 7.4) as eluant to give **1** (16.2 g), **3** (40 mg), calystegines B_1 (16 mg, **8**) and B_2 (83 mg, **9**), and 4-*O*-*β*-D-glucopyranosyl-fagomine (18 mg, **11**). Pool C was chromatographed with a Dowex 1-X2 column (63 cm × 1.7 cm i.d., OH^- form) with H_2O (pH 7.4) as eluant to give 1,4-dideoxy 1,4-imino-(2-*O*-*β*-D-glucopyranosyl)-D-arabinitol (330 mg, **14**). Pool D was chromatographed on a CM-Sephadex C-25 column (60 cm × 2.2 cm i.d., NH_4^+ form) with 0.01 M NH_4OH as eluant

and separated into three fraction: D-1 (fractions 19−75, 620 mg), D-2 (fractions 76−118, 372 mg), and D-3 (fractions 120−140, 55 mg). The fraction size was 10 mL. Fraction D-1 was further chromatographed with a Dowex 1-X2 column (90 cm × 1.7 cm i.d., OH^- form) to give fagomine (210 mg, **10**) and 1,4-dideoxy-1,4-imino-D-arabinitol (330 mg, **13**). Fraction D-2 was chromatographed with a CM-Sephadex C-25 column (60 cm × 2.2 cm i.d., NH_4^+ form) with 0.01 M NH_4OH as eluant to give **13** (350 mg) and 1,4-dideoxy-1,4-imino-D-ribitol (10 mg, **15**). Fraction D-3 was chromatographed with a Dowex 1-X2 column (90 cm × 1.7 cm i.d., OH^- form) to give 3-*epi*-fagomine (33 mg, **12**).

A 50% aqueous MeOH (5 L) extract of the dry fruits (1 kg) of *M. alba* was applied to a column of Amberlite IR-120B (200 mL, H^+ form). The 0.5 M NH_4OH eluate was concentrated to give a brown oil (8.3 g), which was applied to an Amberlite CG-50 column (90 cm × 2.5 cm i.d., NH_4^+ form) and eluted with H_2O (pH 7.4) as eluant (fraction size 20 mL). The H_2O eluate was divided into three pools: A (fractions 16−36, 4.7 g); B (fractions 74−144, 110 mg); and C (fractions 145−200, 106 mg). The 0.5 M NH_4OH eluate from the same column was designated pool D (200 mg). Repeated chromatography of pool A with Dowex 1-X2 (OH^- form) and/or Amberlite CG-50 (NH_4^+ form) with H_2O (pH 7.4) as eluant gave **1** (840 mg), **2** (5 mg), **3** (140 mg), **7** (14 mg), **9** (12 mg), and a new compound **17** (10 mg). Pools B, C, and D were chromatographed on a CM-Sephadex C-25 column (60 cm × 2.2 cm i.d., NH_4^+ form) with 0.01 M NH_4OH as eluant and a Dowex 1-X2 column (63 cm × 1.7 cm i.d., OH^- form) with H_2O (pH 7.4) as eluant to give **14** (18 mg) from pool B, **10** (18 mg) from pool C, and **13** (12 mg) and a new compound **18** (8 mg) from pool D.

Powdered (1 kg) mulberry leaves and silkworms, respectively, were extracted with 50% aqueous EtOH (10 L). Each extract was chromatographed in a manner similar to that described above. Compounds **1** (690 mg), **3** (305 mg), **7** (6 mg), **9** (26 mg), **10** (185 mg), **11** (6 mg), **12** (21 mg), **13** (55 mg), **14** (65 mg), and **17** (9 mg) were obtained from the mulberry leaf extract, and compounds **1** (1.88 g), **10** (125 mg), **12** (21 mg), **13** (65 mg), and **14** (41 mg) were obtained from the silkworm extract.

*Compound **16***. $[\alpha]_D$ −53.7° (*c* 0.41, H_2O); HRFABMS: *m*/*z* 205.1189 $[M + H]^+$ ($C_8H_{17}N_2O_4$ requires 205.1188); ^1H NMR (400 MHz, D_2O) δ 2.49 (2H, m, -*N*-CH_2-**CH_2**-), 2.56 (1H, ddd, *J* = 5.1, 5.1, 5.3 Hz, H-2), 2.67 (1H, ddd, *J* = 7.0, 7.0, 12.5 Hz, -*N*-**CH_2**-), 2.78 (1H, dd, *J* = 5.8, 11.0 Hz, H-5), 3.02 (1H, dd, *J* = 2.2, 11.0 Hz, H-5′), 3.15 (1H, ddd, *J* = 8.0, 8.0, 12.5 Hz, -*N*-**CH_2**-), 3.72 (2H, d, *J* = 5.1 Hz, H-6, 6′), 3.94 (1H, dd, *J* = 2.8, 5.3 Hz, H-3), 4.12 (1H, ddd, *J* = 2.2, 2.8, 5.8 Hz, H-4); ^{13}C NMR (100 MHz, D_2O) δ 36.1 (-*N*-CH_2-**CH_2**-), 53.1 (-*N*-**CH_2**-), 60.8 (C-5), 63.6 (C-6), 74.2 (C-2), 78.2 (C-4), 81.8 (C-3), 180.8 (-*N*-CH_2-CH_2-**C**ONH_2).

*Compound **17***. $[\alpha]_D$ +114.5° (*c* 0.48, H_2O); HRFABMS: *m*/*z* 338.1456 $[M + H]^+$ ($C_{13}H_{24}NO_9$ requires 338.1451); ^1H NMR (400 MHz, D_2O) δ 1.55 (1H, m, H-7*exo*), 1.85 (1H, m, H-6*endo*), 1.94 (1H, m, H-6*exo*), 2.03 (1H, m, H-7*endo*), 3.45 (1H, dd, *J* = 1.5, 8.3 Hz, H-2), 3.48 (1H, dd, *J* = 8.3, 8.3 Hz, H-3), 3.67 (1H, dd, *J* = 4.0, 8.4 Hz, H-4), 3.74 (2H, d, *J* = 6.2 Hz, H-6′a, 6′b), 3.82 (1H, dd, *J* = 4.0, 10.3 Hz, H-2′), 3.92 (1H, dd, *J* = 3.3, 10.3 Hz, H-3′), 4.00 (1H, dd, *J* = ∼1, 3.3 Hz, H-4′), 4.15 (1H, ddd, *J* = ∼1, 6.2, 6.2 Hz, H-5′), 5.06 1H, d, *J* = 4.0 Hz, H-1′); ^{13}C NMR (100 MHz, D_2O) δ 24.6 (C-6), 31.6 (C-7), 54.6 (C-5), 63.8 (C-6′), 71.1 (C-2′), 72.1 (C-3′, C-4′), 73.6 (C-5′), 76.3 (C-3), 80.4 (C-2), 81.6 (C-4), 93.2 (C-1), 98.4 (C-1′).

*Compound **18***. $[\alpha]_D$ −8.2° (*c* 0.34, H_2O); HRFABMS: *m*/*z* 144.1023 $[M + H]^+$ ($C_7H_{14}NO_2$ requires 144.1025); ^1H NMR (400 MHz, D_2O) δ 1.55 (2H, m, H-2*ax*, H-4*ax*), 1.80 (1H, m, H-7*exo*), 2.00 (1H, m, H-2*eq*), 2.13 (1H, m, H-4*eq*), 2.35 (1H, dd, *J* = 7.3, 14.3 Hz, H-7*endo*), 3.62 (1H, m, H-5), 3.83 (1H, m, H-3), 3.95 (1H, m, H-1), 4.39 (1H, m, H-6); ^{13}C NMR (100 MHz, D_2O) δ 38.4 (C-4), 39.9 (C-2), 41.4 (C-7), 57.4 (C-1), 65.2 (C-3), 65.5 (C-5), 75.5 (C-6).

Glycosidase Inhibitory Activities. The enzymes α-glucosidase (from yeast), β-glucosidase (from *Caldocellum saccharolyticum*), α-galactosidase (from coffee beans), β-galactosidase (from bovine liver), trehalase (from porcine kidney), *p*-nitrophenyl glycosides, and disaccharides were purchased from Sigma Chemical Co. The rat epididymal fluid was purified from epididymis according to the literature (*13*). Brush border membranes were prepared from rat small intestine (*14*) and midguts of last instar larvae of silkworms (*15*) according to the literature, and used as the source of digestive glycosidases of rat and silkworms, respectively. The activity of trehalase and isomaltase was determined using trehalose and isomaltose as substrates at pH 5.8, respectively. The released D-glucose was determined colorimetrically using Glucose B-test Wako (Wako Pure Chemical Ind.). 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.

Table 1. Yields of Alkaloids from 1 kg of Sericulture-Related Materials

	yield (mg)/1 kg of dry material			
alkaloid	root bark	fruits	leaves	silkworms
1	1650	840	690	1880
2	6.4	5	–	–
3	16.6	140	305	–
4	1	–[a]	–	–
5	8	–	–	–
6	1.1	–	–	–
7	2	14	6	–
8	1.6	–	–	–
9	8.3	12	26	–
10	21	18	185	125
11	1.8	–	6	–
12	3.3	–	21	21
13	68	12	55	65
14	33	18	65	41
15	1	–	–	–
16	0.6	–	–	–
17	–	10	9	–
18	–	8	–	–

[a] – indicates not detected.

RESULTS AND DISCUSSION

Isolation of Alkaloids. The structures of the polyhydroxylated alkaloids isolated from sericulture-related materials were determined by a variety of 1D and 2D NMR spectral data and HRFABMS data. Sixteen polyhydroxylated alkaloids, including a new compound, were isolated from the root bark of *M. alba*. These alkaloids are 1-deoxynojirimycin (**1**), *N*-methyl-1-deoxynojirimycin (**2**), 2-*O*-α-D-galactopyranosyl-1-deoxynojirimycin (**3**), 2-*O*-α-D-glucopyranosyl-1-deoxynojirimycin (**4**), 3-*O*-β-D-glucopyranosyl-1-deoxynojirimycin (**5**), 4-*O*-β-D-glucopyranosyl-1-deoxynojirimycin (**6**), 6-*O*-β-D-glucopyranosyl-1-deoxynojirimycin (**7**), calystegine B_1 (**8**), calystegine B_2 (**9**), fagomine (**10**), 4-*O*-β-D-glucopyranosyl-fagomine (**11**), 3-*epi*-fagomine (**12**), 1,4-dideoxy-1,4-imino-D-arabinitol (**13**), 1,4-dideoxy-1,4-imino-(2-*O*-β-D-glucopyranosyl)-D-arabinitol (**14**), 1,4-dideoxy-1,4-imino-D-ribitol (**15**), and a new compound **16** (Figure 1). The fruits of *M. alba* contained two new compounds (**17** and **18**) in addition to the known compounds **1**, **2**, **3**, **7**, **9**, **10**, **13**, and **14**. The yields of the polyhydroxylated alkaloids isolated from sericulture-related materials are summarized in Table 1.

We have previously reported the isolation of compounds **1**, **2**, **3**, **4**, **5**, **6**, **7**, **9**, **10**, **12**, **13**, **14**, and **15** from the root bark of *M. alba* (*7*). Polyhydroxy-*nor*tropanes, calystegines, are widely distributed in many species of the Solanaceae and Convolvulaceae (*5*). Although we have isolated two calystegines B_2 and C_1 from the root bark of *M. alba* (*7*), this is the first report on the

occurrence of calystegine B_1 (**8**) in the Moraceae. 4-*O*-β-D-Glucopyranosyl-fagomine (**11**) has also been isolated from *Xanthocercis zambesiaca* (Leguminosae) (*16, 17*), but this is the first report of **11** in plants other than *X. zambesiaca*.

A very high content (0.165% of dry weight) of 1-deoxynojirimycin (**1**) was found in the root bark of *M. alba* imported from An-Huei-Shong in China. 2-*O*-α-D-Galactopyranosyl-1-deoxynojirimycin (**3**) is much more abundant in the leaves than in the other parts. 1-Deoxynojirimycin was concentrated 2.7-fold by silkworms fed on mulberry leaves.

Structural Determinations. *Structural determination of compound **16***. Compound **16** showed the molecular formula $C_8H_{16}N_2O_4$ by HRFABMS and ^{13}C NMR spectral data. The ^{13}C NMR spectra (100 MHz in D_2O) of **16** revealed the presence of a single carbonyl (δ 180.8), four methylene (δ 36.1, 53.1, 60.8, 63.6), and three methine (δ 74.2, 78.2, 81.8) carbon atoms. The complete connectivity of the carbon and hydrogen atoms was determined from analysis of decoupling experiments and two-dimensional HMQC spectral data. These experiments elucidated a linear $C^5H_2-C^4H-C^3H-C^2H-C^6H_2$-OH. The C-2 (δ 74.2) methine carbon bearing a hydroxymethyl group (δ 63.6) and the C-5 (δ 60.8) methylene carbon must be bonded to the nitrogen of the heterocyclic ring. The methine carbons at δ 78.2 and 81.8 were assigned to C-4 and C-3, respectively, bearing OH groups, and the remaining two methylenes were assigned at δ 36.1 and 53.1 to *N*-CH_2-CH_2-. From these results, and an odd-numbered $[M + H]^+$ ion in the FABMS, a carbonyl at δ 180.8 was attributed to the amide ($CONH_2$) carbon. The stereogenic centers of the pyrrolidine ring protons were determined by extensive NOE experiments. The definite NOE effects between the C-6 (CH_2OH) proton and H-3, and between H-2 and H-4, suggest that H-2, H-3, and H-4 are in the α, β, and α orientations, respectively (Figure 2). Thus, compound **16** was determined to be (2*R*,3*R*,4*R*)-2-hydroxymethyl-3,4-dihydroxypyrrolidine-*N*-propionamide or its enantiomer.

*Structural determination of compound **17***. The HR-FABMS of **17** gave an $[M + H]^+$ ion at *m/z* 338.1456 corresponding to $C_{13}H_{24}NO_9$. The response to the naphthoresorcinol−sulfuric acid reagent and the characteristic anomeric proton (H-1′, δ 5.06, $J_{1',2'} = 4.0$ Hz) and carbon (C-1′, δ 98.4) signals in the NMR suggested that **17** was a glycoside of an alkaloid. After acid hydrolysis of this glycoside using Dowex 50W-X2 (H^+ form), the aglycon part was eluted with 0.5 M aqueous ammonia solution from the resin, concentrated to dryness, and confirmed as calystegine B_2 (**9**) by direct comparison of its ^{13}C NMR chemical shifts with those of an authentic sample. The coupling constant ($J_{1',2'} = 4.0$ Hz) of the anomeric proton and the characteristic coupling pattern of H-4′ (δ 4.00, dd, $J_{3',4'} = 3.3$, $J_{4',5'} = 1.0$ Hz) suggested that this glycoside was the α-D-galactoside of **9**. The ^{13}C NMR chemical shifts of the aglycon part of **17** were consistent with those of 2-*O*-α-D-galactopyranosyl-1-deoxynojirimycin (*6*) and 5-*O*-α-D-galactopyranosyl-α-homonojirimycin (*18*). The HMBC spectrum showed a correlation peak between the anomeric proton and the C-4 carbon of the calystegine B_2 part, defining the linkage site (Figure 2). Thus, the structure of **17** was determined to be 4-*O*-α-D-galactopyranosyl-calystegine B_2.

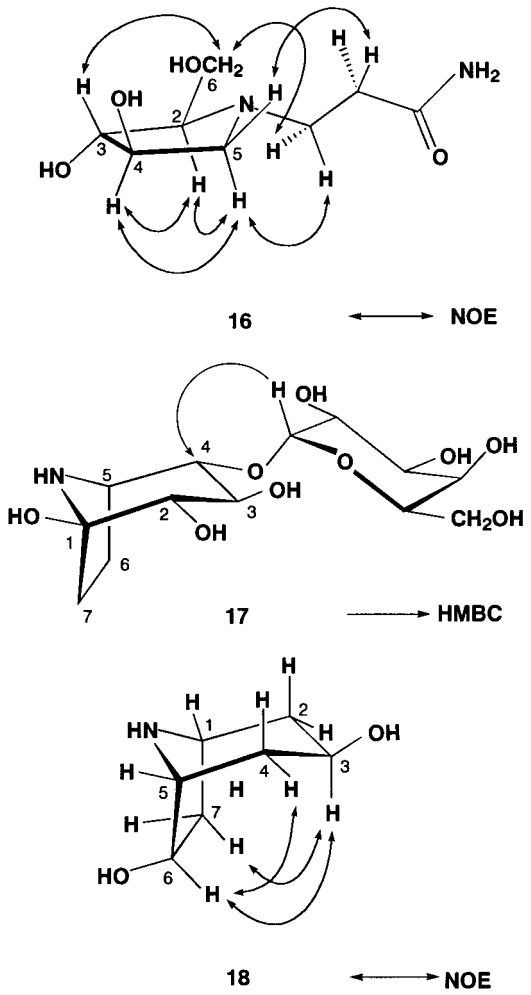

Figure 2. NOE correlations for (2*R*,3*R*,4*R*)-2-hydroxymethyl-3,4-dihydroxypyrrolidine-*N*-propionamide (**16**); Selected HMBC correlation for 4-*O*-α-D-galactopyranosyl-calystegine B_2 (**17**); and NOE correlations for 2β,7β-dihydroxynortropane (**18**).

*Structural determination of compound **18***. The ^{13}C NMR spectrum of **18** revealed the presence of three methylene and four methine carbon atoms. The HR-FABMS of **18** gave an $[M + H]^+$ ion at *m/z* 144.1023 $[M + H]^+$ corresponding to $C_7H_{14}NO_2$. We recently reported the occurrence of three new dihydroxynortropanes, namely, 2α,7β-dihydroxynortropane, 2α,3β-dihydroxynortropane, and 3α,7β-dihydroxynortropane, in the families Convolvulaceae and Solanaceae (*19*). The ^{13}C NMR and HRFABMS data suggest that compound **18** is also a dihydroxynortropane alkaloid. The complete carbon and hydrogen atom connectivity was defined from extensive decoupling experiments and 2D $^1H-^{13}C$ COSY spectral data. The methine carbons at δ 57.4 and 65.5 were identified as the bridgeheads C-1 and C-5, respectively. The H-6 in the ^1H NMR spectrum was observed as a doublet of doublets with no resolvable coupling between H-5 and H-6 at δ 4.39, indicating a dihedral angle between them close to 90°. This establishes the *endo* orientation of H-6. The methine carbon at δ 65.2, with a mutiplet at δ 3.83 in the ^1H NMR spectrum, was assigned to C-3 bearing an OH group. The strong NOE effect between H-3 and H-6 indicates the *axial* orientation of H-3 (Figure 2). Thus, compound **18** was determined to be 3β,6β-dihydroxynortropane or its enantiomer. Chinese researchers have reported the isolation of two dihydroxynortropanes, baogongten C

Table 2. Concentration of New Alkaloids and Related Compounds Giving 50% Inhibition of Glycosidase Activities

	IC_{50} (μM)			
enzyme[a]	**13**	**16**	**9**	**17**
α-glucosidase				
yeast	0.15	12.5	–[b]	–
rat intestinal isomaltase	5.8	70	n.d.[c]	n.d.
β-glucosidase				
caldocellum saccharolyticum	280	–	1.7	190
α-galactosidase				
coffee beans	–	–	2.5	150
β-galactosidase				
bovine liver	1000	–	240	–
α-mannosidase				
rat epididymis	84	850	–	–
β-mannosidase				
rat epididymis	290	–	–	–
trehalase				
porcine kidney	4.8	130	10	1000

[a] Activities of rat intestinal isomaltase and porcine kidney trehalase were determined using isomaltose or trehalose as substrate at pH 5.8. Other glycosidase activities were determined using an appropriate *p*-nitrophenyl glycoside as substrate at the optimum pH of each enzyme. [b] – indicates no inhibition (less than 50% inhibition at 1000 mM). [c] n.d. = not determined.

(2β,6β-dihydroxynortropane) (*20*) and erycibelline (2β,7β-dihydroxynortropane) (*21*) from *Erycibe* species (Convolvulaceae), other than the four dihydroxynortropanes we reported.

Biological Activities. The IC_{50} values of new compounds **16** and **17** and related compounds against various glycosidases are shown in Table 2. Compound **18** showed no inhibitory activity against any glycosidases tested.

1,4-Dideoxy-1,4-imino-D-arabinitol (**13**) is a potent inhibitor of yeast α-glucosidase (*22*) and mammalian isomaltase (*23*), and is also an inhibitor of mammalian α-mannosidase and porcine kidney trehalase (*8*). Its *N*-propionamide derivative (**16**) significantly decreased its inhibition against all glycosidases described above. It is known that the *N*-alkylation of **13** markedly lowers or abolishes its inhibition toward all glycosidases tested (*9*). Calystegine B_2 is a potent inhibitor of β-glucosidases and α-galactosidases (*24*). The first glycoside of a calystegine was isolated from *Nicandra physalodes* (Solanaceae) fruits and the structure was determined to be 3-*O*-β-D-glucopyranosyl-calystegine B_1 (*25*). This β-glucoside was a potent inhibitor (IC_{50} = 1.9 μM) of rice α-glucosidase, although calystegine B_1 showed no inhibitory activity toward the enzyme (*26*). The introduction of the α-glucosyl residue to calystegine B_1 and of the β-glucosyl or β-galactosyl residue to calystegine B_2 resulted in the significant decrease of glycosidase inhibitory activity (*26*). 4-*O*-α-D-Galactopyranosyl-calystegine B_2 (**17**) showed a similar result. We have found that potatoes synthesize this glycoside on cold storage, and recently isolated it from the roots of *Atropa belladonna* L. (Solanaceae) (unpublished data).

α-Glucosidase inhibitors have a potential for the treatment of diabetes because they reduce diet-induced hyperglycemia and endogenous insulin secretion by inhibiting intestinal α-glucosidases. A pseudo tetrasaccharide, acarbose (Glucobay) was introduced onto the market in Germany in 1990, and voglibose (Basen) is also now on the market in Japan. More recently, the *N*-hydroxyethyl derivative of 1-deoxynojirimycin, miglitol (Glyset), has been introduced onto the market as an antidiabetic drug with a long-lasting effect in vivo.

Table 3. Concentration of Alkaloids in Mulberry Leaves Giving 50% Inhibition of Digestive Glycosidase Activities from Rat and Silkworm

	IC_{50} (μM)									
enzyme[a]	**1**	**3**	**7**	**9**	**10**	**11**	**12**	**13**	**14**	**17**
rat										
maltase	0.4	4.4	–	440	820	–	500	55	–	–
sucrase	0.2	0.8	940	160	90	–	180	16	–	–
trehalase	42	46	–	9.4	–	–	–	25	–	1000
cellobiase	520	–	–	200	–	–	100	–	–	–
silkworm										
maltase	160	–	–	–	–	–	–	50	530	–
sucrase	–[b]	–	–	–	–	–	–	–	–	–
trehalase	250	–	–	90	–	–	–	46	–	–
cellobiase	–	–	–	–	–	–	–	–	–	–

[a] Brush border membrane vesicles prepared from rat small intestine and silkworm midgut were assayed at pH 5.8 for rat glycosidases and at pH 7.4 for silkworm enzymes using the appropriate disaccharide as substrate. The released D-glucose was determined colorimetrically using Glucose B-test Wako (Wako Pure Chemical Ind.). [b] No inhibition (less than 50% inhibition at 1000 μM).

1-Deoxynojirimycin (**1**) is present in high concentrations in all parts of the mulberry tree (Table 1). Silkworms feed exclusively on its leaves and appear to accumulate it in their bodies, as the alkaloid content in silkworms is 2.7-fold more than that in the mulberry leaves. As shown in Table 3, silkworm digestive glycosidases were much less sensitive to the inhibitors isolated from mulberry leaves than were the mammalian digestive enzymes. The IC_{50} value of **1** for silkworm midgut maltase was 400-fold higher than that for rat intestinal maltase. Furthermore, although **1** was a potent inhibitor (IC_{50} = 0.2 μM) of rat intestinal sucrase, it showed no inhibition toward the silkworm enzyme. This suggests that the silkworm has developed enzymes which are resistant to the inhibitors present in *M. alba*, and, so, has become a specialized herbivore little affected by any anti-feedant or growth inhibitory effects of these compounds. The mulberry leaves cultivated at the National Sericulture and Entomology Institute in Korea and the silkworms fed them contained a considerable amount of fagomine (**10**). Fagomine shows an antihyperglycemic effect in streptozotocin-diabetic mice by intraperitoneal (ip) administration (*2*). As **1** does not show its effect in the same manner, it is concluded that α-glucosidase inhibition does not play a key role in the antihyperglycemic effect of fagomine. Fagomine potentiates glucose-induced insulin secretion in the perfused pancreas of normal rats (*2*, *27*). The antihyperglycemic effect by the sericulture-related materials is concluded to be mainly due to α-glucosidase inhibition by **1** and its derivatives, but also partly due to different mechanisms other than α-glucosidase inhibition shown by fagomine, and moran A (*1*).

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