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N-Containing sugars from *Morus alba* and their glycosidase inhibitory activities

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Abstract

The reexamination of *N*-containing sugars from the roots of *Morus alba* by improved purification procedures led to the isolation of eighteen *N*-containing sugars, including seven that were isolated from the leaves of *Morus bombycis*. These *N*-containing sugars are 1-deoxynojirimycin (**1**), *N*-methyl-1-deoxynojirimycin (**2**), fagomine (**3**), 3-*epi*-fagomine (**4**), 1,4-dideoxy-1,4-imino-D-arabinitol (**5**), 1,4-dideoxy-1,4-imino-D-ribitol (**6**), calystegin B_2 (1α,2β,3α,4β-tetrahydroxy-*nor*-tropane, **7**), calystegin C_1 (1α,2β,3α,4β,6α-pentahydroxy-*nor*-tropane, **8**), 1,4-dideoxy-1,4-imino-(2-*O*-β-D-glucopyranosyl)-D-arabinitol (**9**), and nine glycosides of **1**. These glycosides consist of 2-*O*- and 6-*O*-α-D-galactopyranosyl-1-deoxynojirimycins (**10** and **11**, respectively), 2-*O*-, 3-*O*- and 4-*O*-α-D-glucopyranosyl-1-deoxynojirimycins (**12**, **13**, and **14**, respectively), and 2-*O*-, 3-*O*-, 4-*O*- and 6-*O*-β-D-glucopyranosyl-1-deoxynojirimycins (**15**, **16**, **17**, and **18**, respectively). Compound **4** is a new member of polyhydroxylated piperidine alkaloids, and the isolation of **6** is the first report of its natural occurrence. It has recently been found that the polyhydroxy-*nor*-tropane alkaloids possess potent glycosidase inhibitory activities. Calystegin A_3 is the trihydroxy-*nor*-tropane, and calystegins B_1 and B_2 are the tetrahydroxy-*nor*-tropane. Calystegin C_1, a new member of calystegins, is the first naturally occurring pentahydroxy-*nor*-tropane alkaloid. The inhibitory activities of these compounds were investigated against rat digestive glycosidases and various commercially available glycosidases.

Key words: Sugars with nitrogen in the ring; Glycosidase; Nojirimycin; Alkaloid

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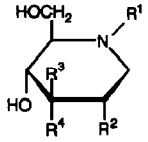
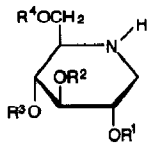
1. Introduction

1-Deoxynojirimycin (**1**), which is a potent inhibitor of intestinal α-glucosidase and α-glucosidases I and II involved in *N*-linked oligosaccharide processing, is known to be contained in the leaves and roots of *Morus* sp. [1,2]. We previously reported that we reexamined *N*-containing sugars in the leaves of *Morus bombycis*, and, by improving the purification procedures, we have isolated seven *N*-containing sugars including **1** (ref 3). These seven sugars are **1**, *N*-methyl-1-deoxynojirimycin (**2**), fagomine (**3**), 1,4-dideoxy-1,4-imino-D-arabinitol (**5**), nortropanoline (**7**), 1,4-dideoxy-1,4-imino-(2-*O*-β-D-glucopyranosyl)-D-arabinitol (**9**), and 2-*O*-α-D-galactopyranosyl-1-deoxynojirimycin (**10**).

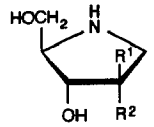
A new structural type of polyhydroxylated alkaloid has recently added to the known five structural types of polyhydroxylated piperidines, pyrrolidines, pyrrolines, indolizidines, and pyrrolizidines. This new type is the polyhydroxy-*nor*-tropane series. These alkaloids were first found in the underground organs and root exudates of *Calystegia sepium*, *Convolvulus arvensis* (Convolvulaceae), and *Atropa belladonna* (Solanaceae) [4]. Three of the six calystegins from *C. sepium* have been structurally characterized as polyhydroxy-*nor*-tropane alkaloids, and calystegin A_3 was identified as the trihydroxy-*nor*-tropane, while calystegins B_1 and B_2 were



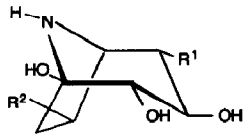
1 R^1=H R^2=OH R^3=OH R^4=H
2 R^1=CH_3 R^2=OH R^3=OH R^4=H
3 R^1=H R^2=H R^3=OH R^4=H
4 R^1=H R^2=H R^3=H R^4=OH



10 R^1= α-D-Gal R^2=R^3=R^4=H
11 R^4= α-D-Gal R^1=R^2=R^3=H
12 R^1= α-D-Glc R^2=R^3=R^4=H
13 R^2= α-D-Glc R^1=R^3=R^4=H
14 R^3= α-D-Glc R^1=R^2=R^4=H
15 R^1= β-D-Glc R^2=R^3=R^4=H
16 R^2= β-D-Glc R^1=R^3=R^4=H
17 R^3= β-D-Glc R^1=R^2=R^4=H
18 R^4= β-D-Glc R^1=R^2=R^3=H



5 R^1=OH R^2=H
6 R^1=H R^2=OH
9 R^1=O-β-D-Glc R^2=H



7 R^1=OH R^2=H
8 R^1=OH R^2=OH

Glc= Glucopyranosyl
Gal= Galactopyranosyl

established as tetrahydroxy-*nor*-tropanes [5,6]. Since calystegin B_2 and nortropanoline had the same relative structure, and nortropanoline exhibited the same biological activity as the calystegin B complex [3,7], we concluded that they are identical. Therefore, nortropanoline was renamed calystegin B_2.

In this paper, we report the isolation of eighteen *N*-containing sugars from the roots of *Morus alba* by the combination of a variety of ion-exchange column chromatographies, their structure elucidation, and glycosidase inhibitory activities.

2. Results and discussion

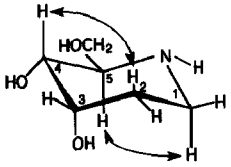
Isolation and purification of N-*containing sugars*.—The alkaloid fraction was obtained by chromatography of the hot water extracts of *Morus alba* roots from a commercial source on an Amberlite IR-120 (H^+ form) ion-exchange column. The alkaloid fraction was divided into four fractions of A, B, C, and D in order of elution from an Amberlite CG-50 (NH_4^+ form) column. Fractions A and D were further divided into four fractions of A-1 to A-4 and D-1 to D-4, respectively, in order of elution from a Dowex 1-X2 (OH^- form) column. By following ion-exchange chromatography of Dowex 50W-X8 (pyridine form), Dowex 1-X2 (OH^- form), or CM Sephadex C-25 (NH_4^+ form), the glycosides **10**, **11**, **12**, and *N*-methyl-1-deoxynojirimycin (**2**) were obtained from fraction A-1, the glycosides **13**, **15**, **17**, and **18** from fraction A-2, calystegin C_1 (**8**) and the glycoside **14** from fraction A-3, and the glycoside **16** from fraction A-4. Calystegin B_2 (**7**) and 1-deoxynojirimycin (**1**) were obtained from fractions B and C, respectively. Fagomine (**3**) and 3-*epi*-fagomine (**4**) were obtained from fraction D-1, and 1,4-dideoxy-1,4-imino-D-ribitol (**6**), 1,4-dideoxy-1,4-imino-D-arabinitol (**5**), and 1,4-dideoxy-1,4-imino-(2-*O*-β-D-glucopyranosyl)-D-arabinitol (**9**) from fractions D-2, D-3, and D-4, respectively.

The ^1H and ^{13}C NMR spectra of **1**, **2**, **3**, **5**, **7**, **9**, and **10** were completely in accord with those of corresponding authentic sample isolated from the leaves of *M. bombycis* [3]. The glycoside (**10**) is abundantly contained in the leaves of *M. bombycis* [3]. It was determined by the ^1H and ^{13}C NMR data that the glycosides **12**, **13**, **14**, **15** and **17** were 2-*O*-α-D-glucopyranosyl-1-deoxynojirimycin, 3-*O*-α-D-glucopyranosyl-1-deoxynojirimycin, 4-*O*-α-D-glucopyranosyl-1-deoxynojirimycin, 2-*O*-β-D-glucopyranosyl-1-deoxynojirimycin and 4-*O*-β-D-glucopyranosyl-1-deoxynojirimycin, respectively. Recently we have reported the enzymic synthesis and glycosidase inhibitory activities of these five glycosides [8].

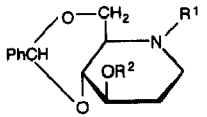
Both 1,4-dideoxy-1,4-imino-D-ribitol [9,10] and 1,4-dideoxy-1,4-imino-L-ribitol [11,12] have been enantiospecifically synthesized, and the specific rotation values in H_2O of their hydrochlorides are +57.6° (*c* 0.59) and −59.0° (*c* 0.59), respectively. Compound **6** was identified as 1,4-dideoxy-1,4-imino-D-ribitol by optical rotation (+52.7° in H_2O of its hydrochloride), MS, and NMR analyses (especially NOE effects).

3-Epi-*Fagomine (1,2,5-trideoxy-1,5-imino*-D-allo-*hexitol*) (**4**).—^{13}C NMR spectral analysis of **4** revealed the presence of three methine and three methylene groups,

and EIMS analysis of **4** showed the $[M]^+$ peak at m/z 147 (5%) and the $[M - CH_2OH]^+$ peak at m/z 116 (100%). These results, in combination with the chemical shifts in the ^{13}C NMR spectrum, indicate that **4** is an epimer of fagomine. The ^1H NMR spectral data, combined with extensive decoupling experiments and two-dimensional ^1H–^{13}C COSY spectral data, define the complete connectivity of carbon and hydrogen atoms. The splitting patterns of H-3 (δ 4.09, dt, $J_{2ax,3}$ 2.6, $J_{2eq,3} = J_{3,4} = 3.3$ Hz) and H-4 (δ 3.48, dd, $J_{3,4}$ 3.3, $J_{4,5}$ 9.9 Hz) indicate that H-3, H-4, and H-5 are equatorial, axial, and axial, respectively. The axial orientation of the hydroxyl group on C-3 produced 5.4-, 4.2-, and 5.1-ppm upfield shifts for C-3, C-1, and C-5, respectively, in the ^{13}C NMR spectrum. By NMR studies mentioned above and the NOE enhancements observed between H-1*ax* and H-5 and between H-2*ax* and H-4, **4** was established as 3-*epi*-fagomine.



4



19 R^1 = Cbz R^2 = H

20 R^1 = Cbz R^2 = Ms

⟷ NOE Effects

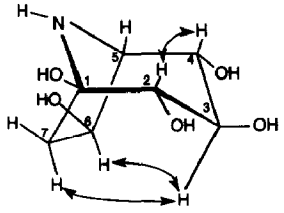
To determine the absolute structure of 3-*epi*-fagomine (**4**), the synthesis of **4** from fagomine (1,2,5-trideoxy-1,5-imino-D-*arabino*-hexitol) was performed. A solution of 4,6-*O*-benzylidene-*N*-benzyloxycarbonyl-(3-*O*-methylsulfonyl)fagomine (**20**) and sodium acetate in 2-methoxyethanol containing 5% H_2O was heated under reflux for 2 days. The removal of the protecting groups, followed by the chromatography on Dowex 1-X2 (OH^-), gave 3-*epi*-fagomine (50% yield from **20**).

The specific rotation value in H_2O of natural 3-*epi*-fagomine is +69° (*c* 0.5), which was very close to $[\alpha]_D$ + 72° (*c* 0.63) of the synthetic sample from fagomine. Therefore, the absolute structure of natural 3-*epi*-fagomine was determined to be 1,2,5-trideoxy-1,5-imino-D-*allo*-hexitol.

Eight polyhydroxylated piperidine alkaloids have been isolated from natural sources so far [13,14]. They include the analogues of D-glucose, D-mannose, D-galactose, and D-glucuronic acid. 3-*epi*-Fagomine (**4**) is the first analogue of D-allose to be isolated, and it is also an interesting compound from a biosynthetic standpoint.

*Calystegin C_1 (1α,2β,3α,4β,6α-pentahydroxy-*nor-*tropane)* (**8**).—The ^{13}C NMR spectral analysis of calystegin C_1 (**8**) revealed the presence of five methine groups, a methylene group, and a quaternary carbon. The chemical shift (93.6 ppm) of a quaternary carbon in **8** was similar to that (93.2 ppm) observed in calystegin B_2 (**7**). The FABMS analysis of **8** showed the $[M + H]^+$ peak at m/z 192, which was different from that (m/z 176) observed in **7**. The results of ^{13}C NMR and FABMS analyses indicated that **8** had one more hydroxyl group than **7**. The ^1H NMR spectral data, together with information from extensive homo-spin decoupling experiments and two-dimensional ^1H–^{13}C COSY spectral data, define the com-

plete connectivity of the carbon and hydrogen atoms. In the ^{1}H NMR spectrum, the large J values ($J_{2,3} = J_{3,4} = 8.8$ Hz) seen in the H-2, H-3, and H-4 signals indicate an all trans–axial orientation of H-2, H-3, and H-4, and hence the six-membered ring is in a chair conformation with all substituents in an equatorial orientation. The H-6 signal was observed as a broad doublet of doublets ($J_{6,7endo}$ 7.4, $J_{6,7exo}$ 2.9 Hz), due to a small coupling constant ($J_{5,6}$ 1.5 Hz) revealed by decoupling experiments and the H-5 signal pattern. The stereoconfigurations of **8** were corroborated by definite NOE effects between H-3 and H-6 or H-7endo and the presence of a W-shape long-range coupling ($J_{2,7exo}$ 1.8 Hz). Therefore the relative structure of calystegin C_1 (**8**) was shown to be 1α,2β,3α,4β,6α-pentahydroxy-*nor*-tropane. This new compound is the first naturally occurring pentahydroxy-*nor*-tropane alkaloid.



8

⟷ NOE Effects

Structure determination of the glycosides **11**, **16**, *and* **18**.—The structures of the glycosides **11**, **16**, and **18** were determined on the basis of ^{1}H and ^{13}C NMR data, including homo-spin decoupling experiments, and two-dimensional ^{1}H–^{1}H and ^{1}H–^{13}C COSY spectra. Consequently the complete connectivity of the carbon and hydrogen atoms of these three glycosides was defined.

From the chemical shift and the coupling constant of the anomeric proton (H-1′, δ 4.95, $J_{1',2'}$ 3.7 Hz) of the glycoside **11**, the type of glycosidic linkage was determined to be α. The splitting patterns of H-2′ (dd, $J_{1',2'}$ 3.7, $J_{2',3'}$ 10.2 Hz) and H-3′ (dd, $J_{2',3'}$ 10.2, $J_{3',4'}$ 2.8 Hz) indicated that the orientations of H-2′, H-3′, and H-4′ were axial, axial, and equatorial, respectively. These data and the signals of H-4′ (dd, $J_{3',4'}$ 2.8, $J_{4',5'}$ 1.0 Hz) and H-5′ (br t, $J_{4',5'}$ 1.0 (revealed by decoupling experiment), $J_{5',6'a} = J_{5',6'b} = 6.2$ Hz) were indicative of the galactopyranoside. In the ^{13}C NMR spectrum, glycoside formation for 1-deoxynojirimycin (**1**) produced a 5.9-ppm downfield shift for C-6 and a 1.7-ppm upfield shift for C-5, while the chemical shifts of C-1 to C-4 remained unchanged. Therefore the structure of the glycoside **11** was shown to be 6-*O*-α-D-galactopyranosyl-1-deoxynojirimycin.

The glycosidic linkages of the glycosides **16** and **18** were both indicated to be β from the chemical shifts (δ 4.73 and 4.45, respectively) and the coupling constants ($J_{1',2'}$ 8.1 and $J_{1',2'}$ 8.0 Hz, respectively) of the anomeric protons. The large vicinal J values seen in H-2′, H-3′, H-4′ and H-5′ of both glycosides indicate that the glycosyl residues of **16** and **18** are both D-glucopyranose. The β-glucoside formation of **16** produced a 9.3-ppm downfield shift for C-3, and both 1.0-ppm upfield shifts for C-2 and C-4 in ^{13}C NMR spectrum. These data indicate that the position of the glucosidic linkage is at C-3. On the other hand, the β-glucoside formation of

Table 1
Concentration of *N*-containing sugars (molar) giving 50% inhibition of rat digestive glycosidase activities

Substrate	IC_{50} (M)							
	4	**7**	**8**	**9**	**10**	**11**	**16**	**18**
Maltose	5.0×10^{-4}	4.4×10^{-4}	2.0×10^{-4}	NI	4.4×10^{-6}	4.0×10^{-5}	1.7×10^{-6}	NI
Sucrose	1.8×10^{-4}	1.6×10^{-4}	7.0×10^{-5}	NI	7.8×10^{-7}	3.5×10^{-5}	3.1×10^{-7}	9.4×10^{-4}
Palatinose	6.4×10^{-6}	2.7×10^{-4}	2.3×10^{-4}	NI	1.8×10^{-5}	1.2×10^{-4}	2.3×10^{-7}	3.1×10^{-5}
Trehalose	NI[a]	9.6×10^{-6}	7.5×10^{-4}	NI	4.6×10^{-5}	NI	3.2×10^{-4}	NI
Cellobiose	1.0×10^{-4}	2.0×10^{-4}	1.6×10^{-5}	NI	NI	NI	NI	NI
Lactose	6.0×10^{-6}	7.8×10^{-6}	3.8×10^{-7}	NI	2.5×10^{-4}	NI	2.5×10^{-4}	NI

[a] Less than 50% inhibition at 1.0×10^{-3} (M). The enzyme activities were measured by D-glucose oxidase–peroxidase method.

18 produced a 8.7-ppm downfield shift for C-6 and a 1.3-ppm upfield shift for C-5. This indicates that the position of a glucosidic linkage is at C-6. Therefore, the structures of the glycosides **16** and **18** were shown to be 3-*O*-β-D-glucopyranosyl-1-deoxynojirimycin and 6-*O*-β-D-glucopyranosyl-1-deoxynojirimycin, respectively.

Glycosidase inhibitory activities.—The IC_{50} values of *N*-containing sugars against rat digestive glycosidases are shown in Table 1. 3-*epi*-Fagomine (**4**), calystegins B_2 (**7**), and C_1 (**8**) were more potent inhibitors than 1-deoxynojirimycin ($IC_{50} = 3.4\times10^{-5}$ M, **1**) of lactase. Particularly, calystegin C_1 was a powerful inhibitor of lactase ($IC_{50} = 3.4\times10^{-7}$ M), and its inhibitory activity was comparable to that of castanospermine, which is also a powerful inhibitor of mouse intestinal α- and β-glucosidases [15]. 3-*epi*-Fagomine and calystegin B_2 exhibited a potent inhibitory activity against isomaltase and trehalase, respectively. The β-D-glucoside (**9**) of 1,4-dideoxy-1,4-imino-D-arabinitol (**5**), which was known to be a potent inhibitor of yeast α-glucosidase [16] and mouse intestinal isomaltase [15], completely lost inhibitory activity against rat digestive glycosidases. We have reported that the enzymically synthesized glycosides **13** and **14** retained the potent inhibitory activity of **1** for sucrase [8]. Glycoside **16** also retained a potency for sucrase and isomaltase. From the inhibitory activity of the nine glycosides of **1** against rat digestive glycosidases, it was found that the glycosylation at C-3 of **1** retains a potency for sucrase and isomaltase, while the glycosylation at C-6 of **1** causes a great loss of inhibitory activity.

The IC_{50} values of *N*-containing sugars against a variety of commercially available glycosidases are shown in Table 2. We have reported that 3-*O*-α-D-glucopyranosyl-1-deoxynojirimycin (**13**) is a more effective inhibitor than the parent compound **1** of rice α-glucosidase [8]. Generally speaking, a loss of inhibitory activity was seen in the glycosides of **1**, especially in the β-D-glucosides. Although fagomine (**3**) exhibited no inhibition for β-glucosidase, 3-*epi*-fagomine (**4**) was a moderately good inhibitor of *Caldocellum saccharoliticum* β-glucosidase. In contrast, fagomine showed an IC_{50} value of 5.6×10^{-5} M against green coffee bean α-galactosidase, while no inhibition was seen for 3-*epi*-fagomine. Recently Molyneux et al. [7] have reported that the calystegin B complex exhibits potent inhibitory

Table 2
Concentration of *N*-containing sugars (molar) giving 50% inhibition of commercially available glycosidase activities

Enzyme	IC_{50} (M)							
	4	**7**	**8**	**9**	**10**	**11**	**16**	**18**
α-Glucosidase								
Baker's yeast	NI	NI	NI	4.6×10^{-4}	NI	2.3×10^{-4}	NI	NI
Rice	1.2×10^{-4}	7.5×10^{-5}	4.2×10^{-4}	7.3×10^{-4}	9.5×10^{-7}	6.0×10^{-6}	3.0×10^{-5}	5.4×10^{-4}
β-Glucosidase								
Almond	1.2×10^{-4}	2.6×10^{-6}	8.2×10^{-7}	NI	NI	NI	NI	NI
C. saccharolyticum [a]	6.8×10^{-5}	2.4×10^{-6}	8.6×10^{-7}	9.0×10^{-4}	NI	NI	NI	NI
Trehalase								
Porcine kidney	NI	1.0×10^{-5}	2.7×10^{-4}	NI	5.2×10^{-5}	NI	NI	NI
α-Galactosidase								
Green coffee bean	NI	1.9×10^{-6}	3.6×10^{-4}	NI	NI	NI	NI	NI
Aspergillus niger	NI	3.9×10^{-6}	4.4×10^{-4}	NI	NI	NI	NI	NI
β-Galactosidase								
Aspergillus niger	NI	NI	NI	NI	NI	NI	NI	NI

[a] *Caldocellum saccharolyticum* (Recombinant). The rice α-Glucosidase and trehalase activities were measured with maltose and trehalose, respectively, by the D-glucose oxidase-peroxidase method. Other enzyme activities were measured using corresponding *p*-nitrophenyl glycosides. The *p*-nitrophenol released was measured at 400 nm.

activity against almond β-glucosidase ($K_i = 3 \times 10^{-6}$ M) and *Aspergillus niger* α-galactosidase ($K_i = 7 \times 10^{-6}$ M). We also reported that calystegin B_2 showed K_i values of 1.2×10^{-6} M for almond β-glucosidase and 2.3×10^{-6} M for *A. niger* α-galactosidase. In addition, calystegin B_2 potently inhibited *C. saccharoliticum* β-glucosidase and green coffee bean α-galactosidase in a competitive manner, with K_i values of 5.5×10^{-7} M and 8.6×10^{-7} M, respectively. Calystegin C_1, which is a new member of calystegins and the first naturally occurring pentahydroxy-*nor*-tropane alkaloid, was a more powerful competitive inhibitor ($K_i = 4.5 \times 10^{-7}$ M for almonds, 2.9×10^{-7} M for *C. saccharolyticum*) than calystegin B_2 of β-glucosidases. However, calystegin C_1 was a much weaker inhibitor than calystegin B_2 of α-galactosidases. It has been found that an increasing degree of hydroxylation is associated with enhanced inhibitory potential, as seen in the deoxycastanospermines [17–20], deoxyswainsonine [21], and calystegins [7]. The present work proved that the theory mentioned above is applicable to β-glucosidase but not to α-galactosidase.

3. Experimental

General.—The alkaloids were monitored by HPTLC Silica Gel-60F_{254} (E. Merck) using the solvent system 4:1:1 PrOH–AcOH–H_2O, unless otherwise specified, with detection by spraying with chlorine–*o*-tolidine reagent. 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 as indicated in D_2O using sodium 3-(trimethylsilyl)propionate (TSP) as the internal standard. MS data were measured on a Jeol JMX-DX 300 JMA-DA spectrometer.

Materials.—Baker's yeast and rice α-glucosidases (EC 3.2.1.20), almonds and *Caldocellum saccharolyticum* (recombinant) β-glucosidases (EC 3.2.1.21), porcine kidney trehalase (EC 3.2.1.28), green coffee bean, and *Aspergillus niger* α-galactosidases (EC 3.2.1.22) and *A. niger* β-galactosidase (EC 3.2.1.23) were purchased from Sigma Chemical Co. *p*-Nitrophenyl glycosides and palatinose were purchased from Sigma Chemical Co. Other disaccharides were purchased from Wako Pure Chemical Industries.

Enzyme assays.—The activities of rice α-glucosidase, rat digestive glycosidases, and porcine kidney trehalase were determined using the appropriate disaccharides as substrates at the optimum pH of each enzyme. The D-glucose released was determined colorimetrically using the commercially available Glucose B-test Wako (Wako Pure Chemical Industries). Other enzyme activities were determined using the appropriate *p*-nitrophenyl glycoside as the substrate at the optimum pH of each enzyme. The *p*-nitrophenol released was measured at 400 nm.

Preparation of rat digestive glycosidases.—Brush border membranes, prepared from the intestine of male Wister rats by the method of Kessler et al. [22], were used as the source of rat digestive glycosidases.

Isolation and purification of alkaloids.—The root bark (10 kg) of *Morus alba* (Mori Cortex) from a commercial source was extracted three times with hot water

(20 L) for 2 h. After cooling, an equivalent volume of MeOH was added to this solution. After filtration through Celite, the filtrate was applied to a column of Amberlite IR-120B (H^+ form, 1.5/L) prepared in 50% MeOH. A 0.5 N NH_4OH eluate was concentrated to give a brown oil (82 g). Approximately 20 g portions of the brown oil were applied to an Amberlite CG-50 column (3.8 × 90 cm, NH_4^+ form) and eluted with water. The fraction size was 20 mL. The water eluate was separated into three fractions, A (fractions 31–48), B (fractions 49–60), and C (fractions 61–110). The 0.5 N NH_4OH eluate from the same column was designated fraction D. Concentration of the pooled fractions A, B, C, and D gave brown solids of 32, 4.3, 9.6, and 4 g, respectively.

After decolorization and removal of the anionic compounds from fraction A with a Dowex 1-X2 column (200 mL), the concentrated water eluate was chromatographed on a Dowex 1-X2 (2.5 × 90 cm, OH^- form) and further separated into four fractions, A-1 (fractions 22–30, 850 mg, fraction size; 20 mL), A-2 (fractions 31–60, 800 mg), A-3 (fractions 61–84, 300 mg), and A-4 (fractions 100–124, 220 mg). Fraction A-1 was chromatographed on a Dowex 50W-X8 (1.2 × 65 cm, pyridine form) with 0.1 M pyridinium acetate buffer (pH 6.0) as an eluant. The fraction size was 10 mL. Fractions 41–47, 49–58, and 61–66 were concentrated and lyophilized to give colorless powders of the glycosides **12** (50 mg), **10** (50 mg), and **11** (7 mg), respectively. The M pyridine eluate from this column was concentrated and lyophilized to give *N*-methyl-1-deoxynojirimycin (55 mg, **2**). Fraction A-2 was chromatographed on a Dowex 1-X2 column (1.5 × 95 cm, OH^- form) with water as an eluant. The fraction size was 10 mL. Fractions 29–33, 37–42, 44–49, and 52–60 were concentrated to give the glycosides **18** (10 mg), **17** (37 mg), **15** (10 mg), and **13** (15 mg), respectively. Fraction A-3 was chromatographed on a Dowex 50W-X8 (1 × 46 cm, pyridine form) with 0.1 M pyridinium acetate buffer (pH 6.0) as eluant. The fraction size was 5 mL. Fractions 43–58 and 65–79 were concentrated and lyophilized to give calystegin C_1 (44 mg, **8**) and the glycoside **14** (10 mg), respectively. Fraction A-4 was chromatographed on the same column and eluted with the same buffer to give the glycoside **16** (95 mg).

Fraction B was chromatographed on a Dowex 1-X2 column (1.5 × 95 cm, OH^- form) with water as an eluant to give calystegin B_2 (176 mg, **7**) and 1-deoxynojirimycin (2.7 g, **1**). Chromatography of fraction C with the same column gave **1** (7.5 g).

Fraction D was chromatographed on a Dowex 1-X2 column (1.5 × 95 cm, OH^- form) with water as an eluant and further separated into four fractions, D-1 (fractions 21–25, 660 mg, fraction size; 20 mL), D-2 (fractions 31–34, 220 mg), D-3 (fractions 35–42, 780 mg), and D-4 (fractions 53–68, 160 mg). Fraction D-1 was chromatographed on a CM Sephadex C-25 (2.2 × 65 cm, NH_4^+ form) and eluted with 0.01 N NH_4OH. The fraction size was 10 mL. Fractions 11–47 and 64–78 were concentrated to give fagomine (590 mg, **3**) and 3-*epi*-fagomine (40 mg, **4**), respectively. Chromatography of fraction D-2 with the same column gave 1,4-dideoxy-1,4-imino-D-ribitol (20 mg, **6**). Chromatography of fractions D-3 and D-4 with a Dowex 1-X2 column (1.5 × 95 cm, OH^- form) gave 1,4-dideoxy-1,4-imino-D-

arabinitol (590 mg, **5**) and 1,4-dideoxy-1,4-imino-(2-*O*-β-D-glucopyranosyl)-D-arabinitol (135 mg, **9**), respectively.

3-epi-*Fagomine (1,2,5-trideoxy-1,5-imino*-D-allo-*hexitol)* (**4**).—Compound **4** was isolated as a colorless powder with R_f 0.37; $[\alpha]_D$ + 69.0° (*c* 0.5, H_2O); EIMS *m/z* 147 (5%) $[M]^+$. 116 (100%) $[M - CH_2OH]^+$; ^1H NMR (D_2O): δ 1.73 (dddd, 1 H, $J_{1eq,2ax}$ 5.9, $J_{1ax,2ax}$ 11.7, $J_{2ax,2eq}$ 14.3, $J_{2ax,3}$ 2.6 Hz, H-2*ax*), 1.85 (ddt, 1 H, $J_{1eq,2eq} = J_{1ax,2eq} = 2.6$, $J_{2ax,2eq}$ 14.3, $J_{2eq,3}$ 3.3 Hz, H-2*eq*), 2.77 (ddd, 1 H, $J_{1ax,1eq}$ 11.7, $J_{1eq,2ax}$ 5.9, $J_{1eq,2eq}$ 2.6 Hz, H-1*eq*), 2.79 (dt, 1 H, $J_{1ax,1eq} = J_{1ax,2ax} = 11.7$, $J_{1ax,2eq}$ 2.6 Hz, H-1*ax*), 2.87 (ddd, 1 H, $J_{4,5}$ 9.9, $J_{5,6a}$ 6.6, $J_{5,6b}$ 3.3 Hz, H-5), 3.48 (dd, 1 H, $J_{3,4}$ 3.3, $J_{4,5}$ 9.9 Hz, H-4), 3.63 (dd, 1 H, $J_{5,6a}$ 6.6, $J_{6a,6b}$ 11.7 Hz, H-6a), 3.82 (dd, 1 H, $J_{5,6b}$ 3.3, $J_{6a,6b}$ 11.7 Hz, H-6b), and 4.09 (dt, 1 H, $J_{2ax,3}$ 2.6, $J_{2eq,3} = J_{3,4} \approx 3.3$ Hz, H-3); ^{13}C NMR (D_2O): δ 33.8 (C-2), 41.2 (C-1), 58.6 (C-5), 64.9 (C-6), 70.7 (C-3), and 72.4 (C-4).

Synthesis of 3-epi-*fagomine* (**4**) *from fagomine* (**3**).—Fagomine (**3**, 100 mg) and $NaHCO_3$ (100 mg) were dissolved in water (2 mL), and a solution of benzyloxycarbonylchloride (0.2 mL) in toluene (1 mL) was added dropwise to the above solution under ice cooling, followed by stirring at the same temperature for 1 h and then at room temperature for 3 h. The mixture was adjusted to pH 5 and extracted with toluene. The water layer was applied to a Amberlite XAD-4 column (5 mL) and eluted with MeOH. A mixture of the concentrated eluate, benzaldehyde dimethyl acetal (0.2 mL), and *p*-toluenesulfonic acid (5 mg) in DMF (2 mL) was stirred at 60°C for 1 h at 60 mmHg and then concentrated. A solution of the residue in $CHCl_3$ was washed with aq $NaHCO_3$, dried ($NaSO_4$), and concentrated to give a syrup. The syrup was chromatographed on a silica gel column (50 mL) and developed with 10:1 toluene–acetone to give 4,6-*O*-benzylidene-*N*-(benzyloxycarbonyl)fagomine(180 mg, 72% yield from **3**, **19**).

Compound **19**: R_f 0.27 10:1 toluene–acetone; $[\alpha]_D$ + 11.5° (*c* 0.83, $CHCl_3$); FABMS *m/z* 370 $[M + H]^+$. Anal. Calcd for $C_{21}H_{23}NO_5$: C, 68.28; H, 6.28; N, 3.79. Found: C, 68.12; H, 6.41; N, 3.88.

To a solution of **19** (160 mg) in pyridine (6 mL) at 0°C was added dropwise methanesulfonyl chloride (0.23 mL), followed by stirring at the same temperature for 1 h and then at room temperature for 24 h. The mixture was filtered and concentrated, and a solution of the residue in $CHCl_3$ was washed with aq $NaHCO_3$, dried ($NaSO_4$), and concentrated. The residue was chromatographed on a silica gel column (20 mL) and developed with 2:1 hexane–acetone to give 4,6-*O*-benzylidene-*N*-(benzyloxycarbonyl)-3-*O*-(methylsulfonyl)fagomine (159 mg, 82%, **20**).

Compound **20**: R_f 0.55 10:1 toluene–acetone; $[\alpha]_D$ + 3.8° (c 0.71, $CHCl_3$); FABMS *m/z* 448 $[M + H]^+$. Anal. Calcd for $C_{22}H_{25}NO_7S$: C, 59.05; H, 5.63; N, 3.13; S, 7.16. Found: C, 59.16; H, 5.66; N, 3.21; S, 7.02.

A solution of **20** (150 mg) and NaOAc (150 mg) in 5 mL of 2-methoxyethanol containing 5% water was heated under reflux for 2 days. The mixture was filtered and concentrated. A solution of the residue in $CHCl_3$ was washed with $NaHCO_3$, dried ($NaSO_4$), and concentrated. To a solution of the residue in 3:1 MeOH–acetone (5 mL) was added 0.5 N HCl (1.5 mL), followed by heating under reflux

for 30 min. The mixture was cooled to room temperature, adjusted to pH 5 with satd aq $NaHCO_3$, and concentrated. The residue was dissolved in water, applied to a Amberlite XAD-4 column (5 mL), and eluted with MeOH. The eluate was concentrated, and a solution of the residue in 50% EtOH (5 mL) and AcOH (1 mL) was hydrogenated in the presence of 5% Pd–C (0.5 g) for 5 h. The mixture was processed conventionally and purified by a Dowex 1-X2 column (1 × 60 cm, OH^- form) with water as eluant to give 3-*epi*-fagomine (24.7 mg, 50% yield from **20**); $[\alpha]_D$ + 72.0° (*c* 0.63, H_2O). The synthetic sample was identical by ^1H and ^{13}C NMR spectra with the natural product.

1,4-Dideoxy-1,4-imino-D-*ribitol* (**6**).—Compound **6** was isolated as a colorless solid with R_f 0.36; $[\alpha]_D$ + 42.0° (*c* 0.53, H_2O); FABMS *m/z* 134 $[M + H]^+$; ^1H NMR (D_2O): δ 2.88 (dd, 1 H, $J_{1a,1b}$ 12.4, $J_{1a,2}$ 3.7 Hz, H-1a), 3.13 (ddd, 1 H, $J_{3,4}$ 7.4, $J_{4,5a}$ 6.2, $J_{4,5b}$ 4.4 Hz, H-4), 3.21 (dd, 1 H, $J_{1a,1b}$ 12.4, $J_{1b,2}$ 5.1 Hz, H-1b), 3.65 (dd, 1 H, $J_{4,5a}$ 6.2, $J_{5a,5b}$ 11.8 Hz, H-5a), 3.77 (dd, 1 H, $J_{4,5b}$ 4.4, $J_{5a,5b}$ 11.8 Hz, H-5b), 3.91 (dd, 1 H, $J_{2,3}$ 5.1, $J_{3,4}$ 7.4 Hz, H-3), and 4.17 (dt, 1 H, $J_{1a,2}$ 3.7, $J_{1b,2} = J_{2,3} = 5.1$ Hz, H-2); ^{13}C NMR (D_2O): δ 52.8 (C-1), 64.4 (C-5), 65.2 (C-4), 73.9 (C-2), and 75.8 (C-3).

1,4-Dideoxy-1,4-imino-D-*ribitol hydrochloride*.—The free base **6** was dissolved in water and acidified to pH 4 with dil aq HCl. The solution was then freeze-dried to give 1,4-dideoxy-1,4-imino-D-ribitol hydrochloride as a solid; $[\alpha]_D$ + 52.7° (*c* 0.72, H_2O) {lit. [9] $[\alpha]_D$ + 57.6° (*c* 0.59, H_2O)}; EIMS *m/z* 133 (3%) $[M]^+$, 102 (100%) $[M - CH_2OH]^+$; ^{13}C NMR (D_2O): δ 52.5 (C-1), 60.8 (C-5), 64.6 (C-4), 72.3 (C-2), and 74.0 (C-3).

Calystegin C_1 *(1α,2β,3α,4β,6α-pentahydroxy*-nor-*tropane)* (**8**).—Compound **8** was as a colorless powder with R_f 0.44; $[\alpha]_D$ + 23.1° (*c* 0.8, H_2O); FABMS *m/z* 192 $[M + H]^+$; ^1H NMR (D_2O): δ 1.47 (ddd, 1 H, $J_{2,7exo}$ 1.8, $J_{6,7exo}$ 3.1, $J_{7endo,7exo}$ 14.3 Hz, H-7exo), 2.55 (dd, 1 H, $J_{6,7endo}$ 7.4, $J_{7endo,7exo}$ 14.3 Hz, H-7endo), 3.15 (t, 1 H, $J_{2,3} = J_{3,4} = 8.8$ Hz, H-3), 3.20 (dd, 1 H, $J_{5,6}$ 1.5, $J_{4,5}$ 4.7 Hz, H-5), 3.35 (dd, 1 H, $J_{2,7exo}$ 1.8, $J_{2,3}$ 8.8 Hz, H-2), 3.54 (dd, 1 H, $J_{3,4}$ 8.8, $J_{4,5}$ 4.7 Hz, H-4), and 4.29 [br dd, 1 H, $J_{5,6}$ 1.5 (revealed by decoupling experiments), $J_{6,7exo}$ 2.9, $J_{6,7endo}$ 7.4 Hz, H-6]; ^{13}C NMR (D_2O) δ 43.6 (C-7), 67.4 (C-5), 71.7 (C-6), 75.4 (C-4), 77.7 (C-3), 79.3 (C-2), and 93.6 (C-1).

*6-O-(α-*D-*galactopyranosyl)-1-deoxynojirimycin* (**11**).—The glycoside **11** was isolated as a colorless powder with R_f 0.26; $[\alpha]_D$ + 107.0° (*c* 0.1, H_2O); FABMS *m/z* 326 $[M + H]^+$; ^1H NMR (D_2O): δ 2.48 (dd, 1 H, $J_{1ax,1eq}$ 12.5, $J_{1ax,2}$ 10.6 Hz, H-1*ax*), 2.75 (ddd, 1 H, $J_{4,5}$ 9.0, $J_{5,6a}$ 2.6, $J_{5,6b}$ 5.1 Hz, H-5), 3.12 (dd, 1 H, $J_{1ax,1eq}$ 12.5, $J_{1eq,2}$ 5.1 Hz, H-1*eq*), 3.32 (2 H, H-3,4), 3.50 (m, 1 H, H-2), 3.64 (dd, 1 H, $J_{5,6a}$ 2.6, $J_{6a,6b}$ 10.3 Hz, H-6a), 3.75 (d, 2 H, H-6′a,6′b), 3.82 (dd, 1 H, $J_{1',2'}$ 3.7, $J_{2',3'}$ 10.2 Hz, H-2′), 3.878 (dd, 1 H, $J_{2',3'}$ 10.2, $J_{3',4'}$ 2.8 Hz, H-3′), 3.884 (dd, 1 H, $J_{5,6b}$ 5.1, $J_{6a,6b}$ 10.3 Hz, H-6b), 3.94 (br t, 1 H, H-5′), 3.99 (dd, 1 H, $J_{3',4'}$ 2.8, $J_{4',5'}$ 1.0 Hz, H-4′), and 4.95 (d, 1 H, $J_{1',2'}$ 3.7 Hz, H-1′); ^{13}C NMR (D_2O): δ 51.5 (C-1), 61.6 (C-5), 64.0 (C-6′), 70.1 (C-6), 71.4 (C-2′), 72.1 (C-4′), 72.4 (C-3′), 73.6 (C-2), 73.8 (C-5′), 74.1 (C-4), 81.3 (C-3), 101.3 (C-1′).

*3-O-(β-*D-*Glucopyranosyl)-1-deoxynojirimycin* (**16**).—The glycoside **16** was isolated as a colorless powder with R_f 0.32; $[\alpha]_D$ + 18.1° (*c* 0.74, H_2O); FABMS

m/z 326 $[M + H]^+$; ^1H NMR (D_2O): δ 2.50 (dd, 1 H, $J_{1ax,1eq}$ 12.4, $J_{1ax,2}$ 10.7 Hz, H-1*ax*), 2.58 (ddd, 1 H, $J_{4,5}$ 9.9, $J_{5,6a}$ 5.9, $J_{5,6b}$ 2.9 Hz, H-5), 3.14 (dd, 1 H, $J_{1ax,1eq}$ 12.4, $J_{1eq,2}$ 5.1 Hz, H-1eq), 3.36 (ddd, 1 H, $J_{1ax,2}$ 10.7, $J_{1eq,2}$ 5.1, $J_{2,3}$ 10.7 Hz, H-2), 3.37 (dd, 1 H, $J_{1',2'}$ 8.1, $J_{2',3'}$ 9.2 Hz, H-2'), 3.42 (dd, 1 H, $J_{3',4'}$ 9.2, $J_{4',5'}$ 9.5 Hz, H-4'), 3.49 (ddd, 1 H, $J_{4',5'}$ 9.5, $J_{5',6'a}$ 5.9, $J_{5',6'b}$ 2.2 Hz, H-5'), 3.53 (t, 1 H, $J_{2',3'} = J_{3',4'} = 9.2$ Hz, H-3'), 3.57 (t, 1 H, $J_{2,3} = J_{3,4} = 9.0$ Hz, H-3), 3.67 (dd, 1 H, $J_{5,6a}$ 5.9, $J_{6a,6b}$ 11.8 Hz, H-6a), 3.68 (dd, 1 H, $J_{3,4}$ 9.0, $J_{4,5}$ 9.9 Hz, H-4), 3.73 (dd, 1 H, $J_{5',6'a}$ 5.9, $J_{6'a,6'b}$ 12.4 Hz, H-6'a), 3.83 (dd, 1 H, $J_{5,6b}$ 2.9, $J_{6a,6b}$ 11.8 Hz, H-6b), 3.92 (dd, 1 H, $J_{5',6'b}$ 2.2, $J_{6'a,6'b}$ 12.4 Hz, H-6'b), and 4.73 (d, 1 H, $J_{1',2'}$ 8.1 Hz, H-1'); ^{13}C NMR (D_2O): δ 51.4 (C-1), 63.2 (C-5), 63.5 (C-6'), 64.1 (C-6), 72.4 (C-4'), 72.8 (C-2), 73.4 (C-4), 76.4 (C-2'), 78.4 (C-3'), 78.9 (C-5'), 90.6 (C-3), and 105.8 (C-1').

6-O-(β-D-Glucopyranosyl)-1-deoxynojirimycin (**18**).—The glycoside **18** was isolated as a colorless powder with R_f 0.30; FABMS *m/z* 326 $[M + H]^+$; ^1H NMR (D_2O): δ 2.46 (dd, 1 H, $J_{1ax,1eq}$ 12.4, $J_{1ax,2}$ 10.9 Hz, H-1*ax*), 2.69 (m, 1 H, H-5), 3.12 (dd, 1 H, $J_{1ax,1eq}$ 12.4, $J_{1eq,2}$ 5.1 Hz, H-1*eq*), 3.30 (dd, 1 H, $J_{1',2'}$ 8.0, $J_{2',3'}$ 9.2 Hz, H-2'), 3.32 (t, 1 H, $J_{3,4} = J_{4,5} = 9.0$ Hz, H-4), 3.33 (t, 1 H, $J_{2,3} = J_{3,4}$ 9.0 Hz, H-3), 3.39 (dd, 1 H, $J_{3',4'}$ 9.2, $J_{4',5'}$ 9.5 Hz, H-4'), 3.47 (ddd, 1 H, $J_{4',5'}$ 9.5, $J_{5',6'a}$ 5.9, $J_{5',6'b}$ 2.4 Hz, H-5'), 3.51 (t, 1 H, $J_{2',3'} = J_{3',4'} = 9.2$ Hz, H-3'), 3.72 (dd, 1 H, $J_{5',6'a}$ 5.9, $J_{6'a,6'b}$ 12.4 Hz, H-6'a), 3.76 (dd, 1 H, $J_{5,6a}$ 6.3, $J_{6a,6b}$ 10.7 Hz, H-6a), 3.95 (dd, 1 H, $J_{5',6'b}$ 2.4, $J_{6'a,6'b}$ 12.4 Hz, H-6'b), 4.14 (dd, 1 H, $J_{5,6b}$ 2.7, $J_{6a,6b}$ 10.7 Hz, H-6b), and 4.45 (d, 1 H, $J_{1',2'}$ 8.0 Hz, H-1'); ^{13}C NMR (D_2O): δ 51.4 (C-1), 62.0 (C-5), 63.6 (C-6'), 72.6 (C-4'), 72.9 (C-6), 73.7 (C-2), 74.1 (C-4), 76.0 (C-2'), 78.4 (C-3'), 78.8 (C-5'), 81.1 (C-3), and 105.8 (C-8).

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