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Sugars with nitrogen in the ring isolated from the leaves of *Morus bombycis*

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ABSTRACT

It is known that 1-deoxynojirimycin (**1**) is contained in the leaves and roots of *Morus* sp. The modified purification procedures of **1** from leaves of *Morus bombycis* led to the isolation of many polyhydroxylated alkaloids. These include **1**, *N*-methyl-1-deoxynojirimycin (**2**), 2-*O*-α-D-galactopyranosyl-1-deoxynojirimycin (**3**), fagomine (**4**), 1,4-dideoxy-1,4-imino-D-arabinitol (**5**), 1,4-dideoxy-1,4-imino-(2-*O*-β-D-glucopyranosyl)-D-arabinitol (**6**), and 1α,2β,3α,4β-tetrahydroxy-*nor*-tropane (**7**), designated nortropanoline. The isolation of **2** is the first report of its natural occurrence. Compounds **3** and **6** are the first naturally occurring glycosides of **1** and **5**, respectively. Natural alkaloidal glycosidase inhibitors are classified into five structural types: namely polyhydroxylated piperidines, pyrrolidines, pyrrolines, indolizidines, and pyrrolizidines. Nortropanoline is a novel tropane alkaloid and a new type of polyhydroxylated alkaloid.

INTRODUCTION

Glycosidases are involved in several important biological processes, such as digestion, the biosynthesis of glycoproteins and the lysosomal catabolism of glycoconjugates. Since it has been determined that certain alkaloidal inhibitors against glycosidases exhibit antiviral, insecticidal, anticancer, and antidiabetic effects, as well as immune modulatory properties, they have attracted a lot of attention. Such alkaloidal inhibitors have been isolated from a number of plants and microorganisms. These substances have been reviewed by Elbein[1], Legler[2], and Fellows et al.[3,4]. Natural alkaloidal glycosidase inhibitors isolated to date have been classified into five structural types: polyhydroxylated piperidines, pyrrolidines, pyrrolines, indolizidines, and pyrrolizidines. Various specific inhibitors against glycosidases, those especially involved in glycoprotein processing, have recently become available and have become useful tools in many areas of biochemical research. However, the discovery of new inhibitors has been decreasing rapidly. It is known that mixtures of different structural types of alkaloids can occur in the same

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species, e.g., DMDP [(2*R*,5*R*)-dihydroxymethyl-(3*R*,4*R*)-dihydroxypyrrolidine] with homonojirimycin and deoxymannojirimycin in *Omphalea diandra*[5], castanospermine with 6-epicastanospermine and the alexine series in *Castanospermum australe*[6–10]. This suggests that the improvement of purification procedures may lead to the discovery of new inhibitors other than known alkaloidal glycosidase inhibitors.

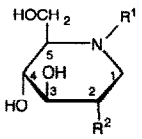
1-Deoxynojirimycin (**1**) is known to be contained in the leaves and roots of *Morus* sp.[11,12]. We then reexamined polyhydroxylated alkaloids in the leaves of *Morus bombycis* and found many polyhydroxylated alkaloids other than 1-deoxynojirimycin (**1**). This paper describes the isolation of polyhydroxylated alkaloids from the leaves of *M. bombycis* and their structural elucidation.

RESULTS AND DISCUSSION

Isolation and purification of alkaloids.—The alkaloid fraction was obtained by chromatography of the hot water extract of *Morus bombycis* leaves on an Amberlite IR-120 (H^+ form) ion-exchange column. The alkaloid fraction was further divided into four fractions of A, B, C, and D in order of elution from an Amberlite CG-50 (NH_4^+ form) column. Following ion-exchange chromatography on Dowex 50W-X8 (pyridine form), Dowex 1-X2, or CM Sephadex C-25 (NH_4^+ form), *N*-methyl-1-deoxynojirimycin (**2**) and 2-*O*-α-D-galactopyranosyl-1-deoxynojirimycin (**3**) were obtained from fraction A, notropanoline (**7**) and 1-deoxynojirimycin (**1**) from fractions B and C, respectively, and fagomine (**4**), 1,4-dideoxy-1,4-imino-D-arabinitol (**5**) and 1,4-dideoxy-1,4-imino-(2-*O*-β-D-glucopyranosyl)-D-arabinitol (**6**) from fraction D.

1-Deoxynojirimycin (**1**).—Comparison of 1-deoxynojirimycin (**1**) obtained from fraction C with an authentic sample by optical rotation, MS and NMR spectral analysis showed that they were identical in all respects. We obtained **1** in a yield

Structure A



1 R^1 = H R^2 = OH
2 R^1 = CH_3 R^2 = OH
3 R^1 = H R^2 = O-α-D-galactopyranosyl
4 R^1 = H R^2 = H

of 0.11% from the leaves of *M. bombycis*, whereas Yagi et al.[11] have reported the isolation of **1** in a yield of 0.14% from the roots of *Morus* sp. Evans et al.[12] have also reported compound **1** in a yield of 0.105% from the leaves of *M. nigra*.

N-*Methyl-1-deoxynojirimycin* (**2**).—The ^{13}C NMR spectral analysis of *N*-methyl-1-deoxynojirimycin (**2**) revealed the presence of four methines, two methylenes, and an *N*-methyl carbon. The large *J* values ($J_{2,3} = J_{3,4} = J_{4,5} = 9.5$ Hz) seen in the H-2, H-3, and H-4 signals indicate all trans-axial orientations for H-2, H-3, H-4, and H-5, and hence the glucose configuration in the $^4C_1$ conformation. Further-

more, the ^{1}H NMR spectrum and optical rotation of **2** were completely in accord with those of the *N*-methylated derivative of 1-deoxynojirimycin (**1**) prepared by heating **1** with formaldehyde solution in the presence of formic acid[13]. *N*-Methyl-1-deoxynojirimycin (**2**) has been synthesized from **1** as an antidiabetic agent[4]. The isolation of **2** from the leaves of *M. bombycis* is a very interesting event and the first report of its natural occurrence. Compound **1** has been known to inhibit α-glucosidases I and II of the glycoprotein processing pathway; however, **1** inhibits α-glucosidase II more strongly. In contrast, **2** is a better inhibitor of α-glucosidase I[15–17], and **2**, to a lesser extent than **1**, has been reported to reduce the infectivity of human immunodeficiency virus (HIV) at concentration noncytopathic to T-lymphocytes[18,19].

2-O-α-D-Galactopyranosyl-1-deoxynojirimycin (**3**).—The glycoside **3** from fraction A was first detected on TLC by the naphthoresorcin–sulfuric acid reagent reaction that is characteristic of sugars and glycosides. Compound **3** was heated with Dowex 50W-X2 (H^+ form) resin in water, and the alkaloid moiety was adsorbed on the resin and eluted with 0.5 N aqueous ammonia. The chromatography of the eluate on Dowex 1-X2 (OH^- form) with water as eluant gave a free NH, free OH alkaloid, which was identified as 1-deoxynojirimycin (**1**) by direct comparison of its optical rotation, TLC analysis, and ^{13}C NMR spectrum with those of an authentic sample. The sugar moiety that was not adsorbed on the resin was analyzed by TLC. Its R_f value and color on TLC were the same as those of D-galactose. From the results described above and the large value of $[\alpha]_D$ of +118.8°, it was presumed that this glycoside **3** is the α-D-galactoside of **1**.

The details of the structure of **3** were determined on the basis of ^{1}H and ^{13}C NMR data, including two-dimensional ^{1}H–^{1}H and ^{1}H–^{13}C COSY spectra.

From the chemical shift and the coupling constant of the anomeric proton (H-1′, δ 5.09, $J_{1',2'}$ 3.7 Hz), the type of glycosidic linkage was determined to be α. The trans-axial orientations of the protons at positions 2, 3, 4, 5, 2′, and 3′ are all clearly indicated by the large vicinal *J* values. The H-4 proton occurred as a broad doublet having a small coupling constant (1.0 Hz) to H-5, which was revealed by a homo-spin decoupling experiment and was indicative of the galactopyranoside. The glycoside formation for **1** produced a 4.6-ppm downfield shift for C-2, and 3.2-ppm and 2.0-ppm upfield shifts for C-1 and C-3, respectively, in the ^{13}C NMR spectrum. By NMR studies mentioned above and the NOE enhancements observed between H-1′ and H-2 or H-1*eq*, the position of a glycosidic linkage was determined at C-2. Therefore the structure of **3** was shown to be 2-*O*-α-D-galactopyranosyl-1-deoxynojirimycin.

The glucosides of **1** have been prepared by microbial[20] and enzymic[21,22] transglucosylation reactions; however, no naturally occurring glycoside of **1** has been previously reported.

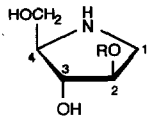
*Fagomine [1,2,5-trideoxy-1,5-imino-*D-arabino-*hexitol]* (**4**).—Fagomine (**4**) was isolated as a crystalline solid from fraction D adsorbed on Amberlite CG-50 (NH_4^+ form). The optical rotation, ^{1}H NMR and MS data of **4** were consistent with those

of a preparation isolated from buckwheat seeds (*Fagopyrum esculentum*)[23]. The ^{13}C NMR spectrum of **4** was measured, and assignments were confirmed by an $^{1}H-^{13}C$ COSY experiment.

Compound **4** has been found to occur with castanospermine in seed of *Castanospermum australe* (Leguminosae)[24], and a 4-*O*-β-D-glucoside of **4**, together with traces of its aglycon, has been shown to be contained in seeds of *Xanthocercis zambesiaca* (Leguminosae)[25]. Compound **4** has been shown to have some activity against mammalian gut α-glucosidases, but no other significant glycosidase inhibition has been reported[24–26].

*1,4-Dideoxy-1,4-imino-*D*-arabinitol* (**5**).—1,4-Dideoxy-1,4-imino-D-arabinitol (**5**) was isolated as a colorless oil from fraction D, as well as fagomine (**4**). The ^{1}H and ^{13}C NMR spectra of our preparation were completely in accord with those

Structure B



5 R = H
6 R = β-D-glucopyranosyl

reported for the alkaloid from fruits of *Angylocalyx boutiqueanus*[27]. The hydrochoride of a sample isolated from *Morus* had $[\alpha]_D$ +34.9° (*c* 1, H_2O), which was close to $[\alpha]_D$ +37.9° (*c* 0.53, H_2O) reported for a synthetic sample[28]. Therefore, our preparation was determined to be the D enantiomer. It has been reported that **5** is a potent inhibitor against yeast α-glucosidase[29] and that the unnatural synthetic enantiomer of **5** is a more effective inhibitor of mammalian gut α-glucosidases[26].

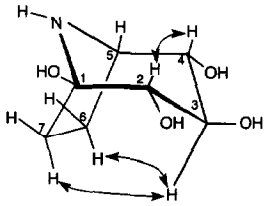
*1,4-Dideoxy-1,4-imino-(2-*O*-β-*D*-glucopyranosyl)-*D*-arabinitol* (**6**).—The glucoside **6** from fraction D responding to naphthoresorcinol–sulfuric acid reagent was hydrolyzed with Dowex 50W-X2 (H^+ form) as well as **3**. The alkaloid fraction that eluted from the resin was chromatographed on a column of Dowex 1-X2 (OH^- form) and was shown to be 1,4-dideoxy-1,4-imino-D-arabinitol (**5**) by comparison of its optical rotation and ^{1}H and ^{13}C NMR spectra with **5** described above. The sugar moiety was presumed to be D-glucose as determined by TLC analysis, and its amount was determined by the D-glucose oxidase–peroxidase method using the Glucose B-test (Wako). Acid hydrolysis of **6** with Dowex 50W-X2 (H^+ form) showed that **6** consisted of equimolar amounts of D-glucose and **5**. From these results and the small value of $[\alpha]_D$ of −29.8°, glucoside **6** was presumed to be the β-D-glucoside of **5**.

From the value of the chemical shift and the coupling constant of the anomeric proton (H-1′, δ 4.51, $J_{1',2'}$ 8.0 Hz), the type of glucosidic linkage was determined to be β. The glucoside formation for **5** produced a 8.1-ppm downfield shift for C-2, and 1.7-ppm and 1.4-ppm upfield shifts for C-1 and C-3, respectively, in the ^{13}C

NMR spectrum. Furthermore, NOE enhancements of the H-2, H-3′, H-5′, and H-1a signals were observed upon irradiation of H-1′. Therefore, the structure of **6** was shown to be 1,4-dideoxy-1,4-imino-(2-*O*-β-D-glucopyranosyl)-D-arabinitol. No glycosides of polyhydroxylated pyrrolidine alkaloids have been previously reported.

*Nortropanoline [1α,2β,3α,4β-tetrahydroxy-*nor*-tropane]* (**7**).—The ^{13}C NMR spectral analysis of nortropanoline (**7**) isolated from fraction B revealed the presence of four methines, two methylenes, and a quaternary carbon. The ^{1}H NMR spectrum, together with information from extensive homo-spin decoupling experiments. ^{13}C NMR, and two-dimensinal ^{1}H–^{13}C COSY spectral data, defines the complete connectivity of the carbon and hydrogen atoms. Acetylation of **7** with acetic anhydride in pyridine afforded an *O,N*-tetraacetyl derivative (acetyl resonances at δ 2.03, 2.08, 2.09, and 2.24 in $CDCl_3$). The broad singlet at δ 6.30 disappeared on deuterium oxide exchange and was assigned to the tertiary alcoholic OH that remained unacetylated. From the NMR studies described above, and EIMS (*m/z* 343, $[M]^+$) of the acetyl derivative, **7** must be tetrahydroxy-*nor*-tropane with a bridgehead OH group. The large *J* values ($J_{2,3} = J_{3,4} = 8.4$ Hz) seen in the H-2, H-3, and H-4 signals indicate all trans–axial orientations 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 stereoconfiguration of **7** was corroborated by definite NOE effects between H-3 and H-6endo or H-7endo and between H-2 and H-4. In addition, the presence of W-shape long-range couplings ($J_{2,7exo}$ 1.8 Hz, $J_{4.6exo}$ 1.5 Hz) between H-2 and H-7exo and between H-4 and H-6exo provided further configurational support. Therefore the relative structure of nortropanoline **7** was shown to be 1α,2β,3α,4β-tetrahydroxy-*nor*-tropane.

Structure C



7

⟷ NOE Effects

The tropane alkaloids are a well-recognized group of structurally related natural products and occur in the seven plant families: Solanaceae, Convolvulaceae, Erythroxylaceae, Proteaceae, Rhizophoraceae, Euphorbiaceae, and Cruciferae[30]. Nortropanoline (**7**) is the first tropane alkaloid isolated from Moraceae, and it is a very novel tetrahydroxylated tropane alkaloid.

In 1988 Tepfer et al.[31] reported the presence of secondary metabolites of plants, designated calystegins. These substances are abundant in the underground organs

and root exudates of *Calystegium sepium, Convolvulus arvensis* (Convolvulaceae), and *Atropa belladonna* (Solanaceae). They suggest that calystegins are novel, low-molecular-weight, nitrogen-containing, bicyclic compounds. In 1990 Goldmann et al.[32] and Ducrot et al.[33] reported that three of the six calystegins shown to be present in *C. sepium* have been structurally characterized as polyhydroxy-*nor*-tropane alkaloids by the ^1H and ^{13}C NMR spectral data and mass spectral data. Calystegin A_3 was identified as the trihydroxy-*nor*-tropane, whereas calystegins B_1 and B_2 were established as the tetrahydroxy-*nor*-tropanes. The relative structure of nortropanoline was the same as that of calystegin B_2. Duclos et al.[34] reported the synthesis of enantiomerically pure (+)- and (−)-calystegins B_2 and stated that the specific rotation values in H_2O of (+)- and (−)-calystegins B_2 are +12° (*c* 0.25) and −14° (*c* 0.275), respectively, whereas that of the natural sample is +2.9°. The specific rotation values in H_2O for the free base and the hydrochloride of nortropanoline were +27.2° (*c* 0.5) and +22.3° (*c* 0.53), respectively. These values lead to the conclusion that the absolute configuration of nortropanoline is the same as that of (+)-calystegin B_2.

Glycosidase inhibitory activities in addition to pharmacological actions can be expected for nortropanoline and calystegins because they can be viewed as a nitrogen-in-the-ring pyranose with an ethano bridge across the 1,5-positions. Molyneux et al.[35] in fact have just reported that calystegins B were potent inhibitors of almond β-glucosidase ($K_i = 3 \times 10^{-6}$ M) and *Aspergillus niger* α-galactosidase ($K_i = 7 \times 10^{-6}$ M). Nortropanoline also had the K_i values of 1.2×10^{-6} M for almond β-glucosidase and 2.3×10^{-6} M for *A. niger* α-galactosidase. The biological activities of (+)- and (−)-calystegins will determine the absolute structure of nortropanoline and calystegin B_2.

The biological activities of sugars with nitrogen in the ring isolated from *Morus* will be reported in the near future.

EXPERIMENTAL

General methods.—1-Deoxynojirimycin was purchased from Sigma Chemical Co. The alkaloids were monitored by HPTLC Silica Gel-60F_{254} (E. Merck) using solvent system I (4:1:1 PrOH–AcOH–H_2O) or II (5:3:2 BuOH–Pyridine–0.1 M HCl) with detection by spraying with the chlorine–*o*-tolidine reagent for alkaloids and the naphthoresorcinol–sulfuric acid reagent for sugars. 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 as indicated in D_2O and $CDCl_3$ using sodium 3-(trimethylsilyl)propionate and Me_4Si, respectively, as internal standards.

Isolation and purification of alkaloids.—Finely ground leaves of *Morus bombycis* (1 kg) were extracted with hot H_2O (6 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, 250 mL)

prepared in 50% MeOH. The brown oil (12 g) obtained by concentration of an 0.5 N NH_4OH eluate was applied to an Amberlite CG-50 column (3.8 × 90 cm, NH_4^+ form) and eluted with H_2O. The fraction size was 20 mL. The H_2O eluate was separated into three fractions, A (fractions 52–62, 0.95 g), B (fractions 63–74, 0.12 g), and C (fractions 75–116, 1.3 g). The 0.5 N NH_4OH eluate from the same column was designated fraction D (0.6 g).

Fraction A was chromatographed on a Dowex 1-X2 column (1.5 × 95 cm, OH^- form) and eluted with H_2O. Fractions 18–25 (fraction size, 10 mL) were concentrated and further chromatographed on a Dowex 50W-X8 column (1.4 × 46 cm, pyridine form) with 0.1 M pyridinium acetate buffer (pH 6.0) as an eluant to give 2-*O*-α-D-galactopyranosyl-1-deoxynojirimycin (600 mg, **3**). The M pyridine eluate from this column was concentrated and further chromatographed on a short column of Dowex 1-X2 (OH^- form) (H_2O elution) to give *N*-methyl-1-deoxynojirimycin (18 mg, **2**). Fractions B and C were chromatographed on a Dowex 1-X2 column (1.5 × 95 cm, OH^- form) (H_2O elution) to give nortropanoline (40 mg, **7**) and 1-deoxynojirimycin (1.1 g, **1**), respectively. Fraction D was chromatographed on a Dowex 1-X2 column (1.5 × 95 cm, OH^- form) (H_2O elution) and separated into three fractions, D-1 (fractions 14–18), D-2 (fractions 23–30), and D-3 (fractions 33–43). The fraction size was 10 mL. 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 to give fagomine (200 mg, **4**). Fractions D-2 and D-3 were concentrated to give 1,4-dideoxy-1,4-imino-D-arabinitol (42 mg, **5**) and 1,4-dideoxy-1,4-imino-(2-*O*-β-D-glucopyranosyl)-D-arabinitol (76 mg, **6**), respectively.

1-Deoxynojirimycin (**1**).—Compound **1** was isolated as a crystalline solid with R_f 0.37 (system I); $[\alpha]_D$ +42.1° (*c* 1, H_2O) (lit.[11] $[\alpha]_D$ +45.1°); EIMS *m/z* 163 (1%) $[M]^+$, 132 (100%) $[M - CH_2OH]^+$; ^{13}C NMR (D_2O) δ 51.5 (C-1), 63.3 (C-5), 64.2 (C-6), 73.6 (C-2), 74.4 (C-4), 81.3 (C-3).

N-*Methyl-1-deoxynojirimycin* (**2**).—Compound **2** was isolated as a crystalline solid with R_f 0.26 (System I); $[\alpha]_D$ +12.6° (*c* 1, H_2O); EIMS *m/z* 146 (100%) $[M - CH_2OH]^+$; FABMS *m/z* 178 $[M + H]^+$; ^1H NMR (D_2O) δ 1.99 (dt, 1 H, $J_{4,5}$ 9.5, $J_{5,6a}$ 2.6 Hz, H-5), 2.23 (dd, 1 H, $J_{1ax,2}$ 10.8, $J_{1ax,1eq}$ 11.6 Hz, H-1*ax*), 2.35 (s, 3 H, NCH_3), 2.97 (dd, 1 H, $J_{1eq,2}$ 4.9, $J_{1ax,1eq}$ 11.6 Hz, H-1*eq*), 3.29 (t, 1 H, $J_{2,3} = J_{3,4} = 9.5$ Hz, H-3), 3.40 (t, 1 H, $J_{3,4} = J_{4,5} = 9.5$ Hz, H-4), 3.57 (ddd, 1 H, $J_{1ax,2}$ 10.8, $J_{1eq,2}$ 4.9, $J_{2,3}$ 9.5 Hz, H-2), 3.83 (dd, 1 H, $J_{5,6a}$ 2.6, $J_{6a,6b}$ 12.8 Hz, H-6a), 3.91 (dd, 1 H, $J_{5,6b}$ 2.6, $J_{6a,6b}$ 12.8 Hz, H-6b); ^{13}C NMR (D_2O) δ 43.8 (NCH_3), 60.3 (C-6), 62.3 (C-1), 70.9 (C-5), 71.5 (C-2), 72.6 (C-4), 81.1 (C-3).

N-*Methylation of 1-deoxynojirimycin* (**1**).—A solution of **1** (520 mg), 37% HCHO (1 mL), and 80% HCOOH (2 mL) was stirred at 80°C and then evaporated. The residue was chromatographed on a Dowex 1-X2 column (1.5 × 95 cm, OH^- form) and eluted with H_2O to give **2** (540 g, 96%) as a crystalline solid. The product was identical by the ^1H and ^{13}C NMR spectra with an authentic sample of **2**.

2-O-α-D-*Galactopyranosyl-1-deoxynojirimycin* (**3**).—Compound **3** was isolated as a colorless powder with R_f 0.26 (system I); $[\alpha]_D$ +118.8° (*c* 0.1 H_2O); FABMS

m/z 326 $[M + H]^+$; ^1H NMR (D_2O) δ 2.46 (dd, 1 H, $J_{1ax,2}$ 10.6, $J_{1ax,1eq}$ 12.5 Hz, H-1*ax*), 2.57 (ddd, 1 H, $J_{4,5}$ 9.5, $J_{5,6a}$ 6.2, $J_{5,6b}$ 2.9 Hz, H-5), 3.30 (t, 1 H, $J_{3,4} = J_{4,5} = 9.5$ Hz, H-4), 3.32 (dd, 1 H, $J_{1eq,2}$ 4.8, $J_{1ax,1eq}$ 12.5 Hz, H-1*eq*), 3.47 (t, 1 H, $J_{2,3} = J_{3,4} = 9.5$ Hz, H-3), 3.58 (ddd, 1 H, $J_{1eq,2}$ 4.8, $J_{1ax,2}$ 10.6, $J_{2,3}$ 9.5 Hz, H-2), 3.65 (dd, 1 H, $J_{5,6a}$ 6.2, $J_{6a,6b}$ 11.7 Hz, H-6a), 3.75 (d, 2 H, J 6.2 Hz, H-6′a, H-6′b), 3.83 (dd, 1 H, $J_{1',2'}$ 3.7, $J_{2',3'}$ 10.2 Hz, H-2′), 3.84 (dd, 1 H, $J_{5,6b}$ 2.9, $J_{6a,6b}$ 11.7 Hz, H-6b), 3.91 (dd, 1 H, $J_{2',3'}$ 10.2, $J_{3',4'}$ 3.3 Hz, H-3′), 4.01 (br d, 1 H, $J_{3',4'}$ 3.3, $J_{4',5'}$ 1.0 Hz [revealed by decoupling experiments], H-4′), 4.19 (br t, 1 H, $J_{4',5'}$ 1.0, $J_{5',6'a} = J_{5',6'b} = 6.2$ Hz, H-5′), 5.09 (d, 1 H, $J_{1',2'}$ 3.7 Hz, H-1′); ^{13}C NMR (D_2O) δ 48.3 (C-1), 63.3 (C-5), 63.8 (C-6′), 64.1 (C-6), 70.9 (C-2′), 72.0 (C-4′), 72.1 (C-3′), 73.6 (C-5′), 74.3 (C-4), 78.2 (C-2), 79.3 (C-3), 98.6 (C-1′).

Acid hydrolysis of 2-O-α-D-galactopyranosyl-1-deoxynojirimycin (**3**).—Compound **3** (24 mg) was heated at 100°C with Dowex 50W-X2 (1 g, H^+ form) in H_2O for 8 h. The resin was filtered off and packed into a short column. The alkaloid moiety was eluted with 0.5 N NH_4OH and concentrated. The residue was chromatographed on a short column of Dowex 1-X2 (OH^- form) with H_2O to yield 11.5 mg (96%) of crystalline free base. Comparison of this alkaloid with authentic 1-deoxynojirimycin by optical rotation, TLC, and NMR analysis showed that they were identical. The sugar moiety in the filtrate was analyzed by TLC with solvent system II and naphthoresorcinol–sulfuric acid reagent for detection. Its R_f (0.54) and color (blue) were the same as those of D-galactose (D-glucose, 0.60, violet; D-mannose, 0.65, blue grey; D-fructose, 0.62, purple).

*Fagomine [1,2,5-trideoxy-1,5-imino-*D-arabino-*hexitol]* (**4**).—Compound **4** was isolated as a crystalline solid with R_f 0.36 (system I); $[\alpha]_D$ +19.5° (*c* 1, H_2O) (lit.[23] $[\alpha]_D$ +23°, lit.[24,25] $[\alpha]_D$ +24.7°); EIMS *m/z* 147 (2%) $[M]^+$, 116 (100%) $[M - CH_2OH]^+$; ^1H NMR (D_2O) δ 1.45 (ddt, 1 H, $J_{1ax,2ax} = J_{2ax,2eq} = 13.0$, $J_{1eq,2ax}$ 4.5, $J_{2ax,3}$ 11.6 Hz, H-2*ax*), 1.99 (dddd, 1 H, $J_{1eq,2eq}$ 2.2, $J_{1ax,2eq}$ 2.6, $J_{2ax,2eq}$ 13.0, $J_{2eq,3}$ 5.1 Hz, H-2*eq*), 2.52 (ddd, 1 H, $J_{4,5}$ 9.5, $J_{5,6a}$ 6.6, $J_{5,6b}$ 3.0 Hz, H-5), 2.60 (dt, 1 H, $J_{1ax,1eq} = J_{1ax,2ax}$ 13.0, $J_{1ax,2eq}$ 2.6 Hz, H-1*ax*), 3.00 (ddd, 1 H, $J_{1ax,1eq}$ 13.0, $J_{1eq,2ax}$ 4.5, $J_{1eq,2eq}$ 2.2 Hz, H-1*eq*), 3.17 (dd, 1 H, $J_{3,4}$ 9.2, $J_{4,5}$ 9.5 Hz, H-4), 3.54 (ddd, 1 H, $J_{2ax,3}$ 11.6, $J_{2eq,3}$ 5.1, $J_{3,4}$ 9.2 Hz, H-3), 3.63 (dd, 1 H, $J_{5,6a}$ 6.6, $J_{6a,6b}$ 11.7 Hz, H-6a), 3.86 (dd, 1 H, $J_{5,6b}$ 3.0, $J_{6a,6b}$ 11.7 Hz, H-6b); ^{13}C NMR (D_2O) δ 35.6 (C-2), 45.4 (C-1), 63.7 (C-5), 64.5 (C-6), 76.1 (C-3,4).

*1,4-Dideoxy-1,4-imino-*D-*arabinitol* (**5**).—Compound **5** was isolated as a colorless oil with R_f 0.45 (system I); $[\alpha]_D$ +6.3° (*c* 1, H_2O) (lit.[28] $[\alpha]_D$ +7.8°); FABMS *m/z* 134 $[M + H]^+$; ^1H NMR (D_2O) δ 2.86 (dd, 1 H, $J_{1a,1b}$ 12.1, $J_{1a,2}$ 4.0 Hz, H-1a), 3.01 (ddd, 1 H, $J_{3,4}$ 5.5, $J_{4,5a}$ 6.3, $J_{4,5b}$ 4.8 Hz, H-4), 3.14 (dd, 1 H, $J_{1a,1b}$ 12.1, $J_{1b,2}$ 5.8 Hz, H-1b), 3.67 (dd, 1 H, $J_{4,5a}$ 6.3, $J_{5a,5b}$ 11.7 Hz, H-5a), 3.75 (dd, 1 H, $J_{4,5b}$ 4.8, $J_{5a,5b}$ 11.7 Hz, H-5b), 3.85 (dd, 1 H, $J_{2,3}$ 3.7, $J_{3,4}$ 5.5 Hz, H-3), 4.16 (ddd, 1 H, $J_{1a,2}$ 4.0, $J_{1b,2}$ 5.8, $J_{2,3}$ 3.7 Hz, H-2); ^{13}C NMR (D_2O) δ 53.4 (C-1), 64.8 (C-5), 68.0 (C-4), 80.2 (C-2), 81.8 (C-3).

*1,4-Dideoxy-1,4-imino-*D-*arabinitol hydrochloride.*—The free base **5** was dissolved in H_2O (3 mL) and acidified to pH 4 with dil aq HCl. The solution was then

freeze-dried to give 1,4-dideoxy-1,4-imino-D-arabinitol hydrochloride as a solid. $[\alpha]_D$ +34.9° (*c* 1, H_2O) (lit.[28] $[\alpha]_D$ +37.9°); EIMS *m/z* 133 (2%) $[M]^+$, 102 (100%) $[M - CH_2OH]^+$; ^{13}C NMR (D_2O) δ 52.8 (C-1), 61.8 (C-5), 69.4 (C-4), 77.1 (C-2), 78.5 (C-3).

1,4-Dideoxy-1,4-imino-(2-O-β-D-glucopyranosyl)-D-arabinitol (**6**).—Compound **6** was isolated as a colorless powder with R_f 0.36 (system I); $[\alpha]_D$ −29.8° (*c* 0.73, H_2O); FABMS *m/z* 296 $[M + H]^+$; ^1H NMR (D_2O) δ 3.05 (2 H, H-1a and H-4), 3.21 (dd, 1 H, $J_{1a,1b}$ 12.5, $J_{1b,2}$ 5.8 Hz, H-1b), 3.29 (dd, 1 H, $J_{1',2'}$ 8.0, $J_{2',3'}$ 9.2 Hz, H-2'), 3.38 (dd, 1 H, $J_{3',4'}$ 9.2, $J_{4',5'}$ 9.5 Hz, H-4'), 3.48 (ddd, 1 H, $J_{4',5'}$ 9.5, $J_{5',6'a}$ 6.2, $J_{5',6'b}$ 2.2 Hz, H-5'), 3.50 (t, 1 H, $J_{2',3'} = J_{3',4'} = 9.2$ Hz, H-3'), 3.68 (dd, 1 H, $J_{4,5a}$ 6.6, $J_{5a,5b}$ 11.7 Hz, H-5a), 3.71 (dd, 1 H, $J_{5',6'a}$ 6.2, $J_{6'a,6'b}$ 12.5 Hz, H-6'a), 3.77 (dd, 1 H, $J_{4,5b}$ 4.8 Hz, $J_{5a,5b}$ 11.7 Hz, H-5b), 3.94 (dd, 1 H, $J_{5',6'b}$ 2.2, $J_{6'a,6'b}$ 12.5 Hz, H-6'b), 4.06 (dd, 1 H, $J_{2,3}$ 3.3, $J_{3,4}$ 5.9 Hz, H-3), 4.30 (ddd, 1 H, $J_{1a,2}$ 4.0, $J_{1b,2}$ 5.8, $J_{2,3}$ 3.3 Hz, H-2), 4.51 (d, 1 H, $J_{1',2'}$ 8.0 Hz, H-1'); ^{13}C NMR (D_2O) δ 51.7 (C-1), 63.7 (C-6'), 64.2 (C-5), 68.1 (C-4), 72.6 (C-4'), 75.9 (C-2'), 78.4 (C-3'), 78.8 (C-5'), 80.4 (C-3), 88.3 (C-2), 104.4 (C-1').

Acid hydrolysis of 1,4-dideoxy-1,4-imino-(2-O-β-D-glucopyranosyl)-D-arabinitol (**6**).—Compound **6** (19 mg) was heated at 100°C with Dowex 50W-X2 (1 g, H^+ form) in H_2O for 8 h. The resin was filtered off and packed into a short column. The alkaloid moiety was eluted with 0.5 N NH_4OH and concentrated. The residue was chromatographed on a Dowex 1-X2 (0.8 ×27 cm, OH^- form) with H_2O to yield 6.9 mg (80.5%) of a colorless oily base. Comparison of the hydrochloride salt of this alkaloid with that of **5** by optical rotation, TLC, and NMR analysis showed that they were identical. The R_f value (0.60) and color (violet) on TLC of the sugar component in the filtrate were the same as those of D-glucose. After neutralizing the filtrate, the released D-glucose was determined to be 10.2 mg (88%) by the D-glucose oxidase–peroxidase method using commercially available Glucose B-test (Wako Pure Chemical Industries). Consequently, acidic hydrolysis of **6** with Dowex 50W-X2 (H^+ form) gave equimolar amounts of D-glucose and **5**.

*Nortropanoline [1α,2β,3α,4β-tetrahydroxy-*nor-*tropane]* (**7**).—Compound **7** was isolated as a colorless powder with R_f 0.44 (system I); $[\alpha]_D$ +27.2° (*c* 0.5, H_2O); FABMS *m/z* 176 $[M + H]^+$; ^1H NMR (D_2O) δ 1.54 (m, 1 H, H-7exo), 1.76 (m, 1 H, H-6endo), 1.95 (m, 1 H, H-6exo), 2.00 (m, 1 H, H-7endo), 3.32 (br dd, 1 H, $J_{4,5}$ 4.0, $J_{5,6endo}$ 1.5 [revealed by decoupling experiments], $J_{5,6exo}$ 7.0 Hz, H-5), 3.35 (t, 1 H, $J_{2,3} = J_{3,4} = 8.4$ Hz, H-3), 3.42 (dd, 1 H, $J_{2,7exo}$ 1.8, $J_{2,3}$ 8.4 Hz, H-2), 3.58 (ddd, 1 H, $J_{3,4}$ 8.4, $J_{4,5}$ 4.0, $J_{4,6exo}$ 1.5 Hz, H-4); ^{13}C NMR (D_2O) δ 24.5 (C-2), 31.5 (C-7), 58.6 (C-5), 77.6 (C-4), 77.7 (C-3), 80.4 (C-2), 93.2 (C-1).

Acetylation of nortropanoline (**7**).—A solution of **7** (4.5 mg) in pyridine (1 mL) and Ac_2O (0.2 mL) was stirred overnight at room temperature and then evaporated. The residue was chromatographed on a short column of silica gel with 2:1 hexane–acetone as a developing solvent to give a colorless powder (5.4 mg, 61%); $[\alpha]_D$ −18.8° (c 0.53, $CHCl_3$); EIMS *m/z* 343 (12%) $[M]^+$; ^1H NMR ($CDCl_3$) δ 1.86 (m, 1 H, H-7exo), 1.95 (m, 1 H, H-6endo), 2.01–2.07 (m, 1 H, H-6 exo), 2.03 (s,

3 H, Ac), 2.08 (s, 3 H, Ac), 2.09 (s, 3 H, Ac), 2.24 (s, 3 H, Ac), 2.41 (m, 1 H, H-7endo), 4.30 (br dd, 1 H, $J_{4,5}$ 4.0, $J_{5,6exo}$ 7.0 Hz, H-5), 4.73 (br dd, 1 H, $J_{3,4}$ 8.8, $J_{4,5}$ 4.0 Hz, H-4), 5.25 (t, 1 H, $J_{2,3} = J_{3,4} = 8.8$ Hz, H-3), 5.37 (dd, 1 H, $J_{2,3}$ 8.8, $J_{2,7exo}$ 1.5 Hz, H-2), 6.30 (br s, 1 H, OH).

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REFERENCES

1 A.D. Elbein, *Annu. Rev. Biochem.*, 56 (1987) 497–534.
2 G. Legler, *Adv. Carbohydr. Chem. Biochem.*, 48 (1990) 319–384.
3 L.E. Fellows, G.C. Kite, R.J. Nash, M.S.J. Simmonds, and A.M. Scofield, in J.E. Poulton, J.T. Romero, and E.E. Conn (Eds.) *Plant Nitrogen Metabolism*, Plenum, New York, 1989, pp. 395–427.
4 L.E. Fellows, G.C. Kite, R.J. Nash, M.S.J. Simmonds, and A.M. Scofield, in K. Mengel and D.J. Pibeam (Eds.) *Nitrogen Metabolism of Plants*, Clarendon Press, Oxford, 1992, pp. 271– 284.
5 G.C. Kite, L.E. Fellows, G.W.J. Fleet, P.S. Liu, A.M. Scofield, and N.G. Smith, *Tetrahedron Lett.*, 29 (1988) 6483–6486.
6 L.D. Hohenschulz, E.A. Bell, P.J. Jewess, D.P. Leworthy, R.J. Pryce, E. Arnold, and J. Clardy, *Phytochemistry*, 20 (1981) 811– 814.
7 R.J. Molyneux, J.N. Roitman, G. Dunnheim, T. Szumilo, and A.D. Elbein, *Arch. Biochem. Biophys.*, 251 (1986) 450–457.
8 J.E. Tropea, R.J. Molyneux, G.P. Kaushal, Y.T. Pan, M. Mitchell, and A.D. Elbein, *Biochemistry*, 28 (1989) 2027–2034.
9 R.J. Nash, L.E. Fellows, J.V. Dring, G.W.J. Fleet, A. Girdhar, N.G. Ramsden, J.M. Peach, M.P. Hegarty, and A.M. Scofield, *Phytochemistry*, 29 (1990) 111–114.
10 R.J. Molyneux, Y.T. Pan, J.E. Tropea, M. Benson, G.P. Kaushal, and A.D. Elbein, *Biochemistry*, 30 (1991) 9981–9987.
11 M. Yagi, T. Kouno, Y. Aoyagi, and H. Murai, *Nippon Nogei Kagaku Kaishi*, 50 (1976) 571–572.
12 S.V. Evans, L.E. Fellows, T.K.M. Shing, and G.W.J. Fleet, *Phytochemistry*, 24 (1985) 1953–1955.
13 H. Kato, E. Kosinaka, Y. Arata, and M. Hanaoka, *Chem. Pharm. Bull.*, 21 (1973) 2661–2666.
14 B. Junge, H.P. Krause, L. Mueller, and W. Plus, Ger. Offen. 2 738 717 (1979); *Chem. Abstr.*, 91 (1979) 39716s.
15 H. Hettkamp, G. Legler, and E. Bause, *Eur. J. Biochem.*, 142 (1984) 85–90.
16 J. Schweden, C. Borgmann, G. Legler, and E. Bause, *Arch. Biochem. Biophys.*, 248 (1986) 335–340.
17 R. Datema, S. Olofsson, and P.A. Romero, *Pharmacol. Ther.*, 33 (1987) 221–286.
18 G.W.J. Fleet, A. Karpas, R.A. Dwek, L.E. Fellows, A.S. Tyms, S. Petursson, S.K. Namgoong, N.G. Ramsden, P.W. Smith, J.C. Son, F. Wilson, D.R. Witty, G.S. Jacob, and T.W. Rademacher, *FEBS Lett.*, 237 (1988) 128–132.
19 A. Karpas, G.W.J. Fleet, R.A. Dwek, S. Petursson, S.K. Namgoong, N.G. Ramsden, G.S. Jacob, and T.W. Rademacher, *Proc. Natl. Acad. Sci. USA*, 85 (1988) 9229–9233.
20 M. Arai, M. Sumida, S. Nakatani, and S. Murao, *Agric. Biol. Chem.*, 47 (1983) 183–185.
21 Y. Ezure, *Agric. Biol. Chem.*, 49 (1985) 2159–2165.
22 M. Arai, M. Sumida, K. Fukuhara, N. Kainosho, and S. Murao, *Agric. Biol. Chem.*, 50 (1986) 639–644.
23 M. Koyama, and S. Sakamura, *Agric. Biol. Chem.*, 38 (1974) 1111– 1112.

24 R.J. Molyneux, M. Benson, R.Y. Wong, J.E. Tropea, and A.D. Elbein, *J. Nat. Prod.*, 51 (1988) 1198–1206.
25 S.V. Evans, A.R. Hayaman, L.E. Fellows, T.K.M. Shing, A.E. Derome, and G.W.J. Fleet, *Tetrahedron Lett.*, 26 (1985) 1465–1468.
26 A.M. Scofield, L.E. Fellows, R.J. Nash, and G.W.J. Fleet, *Life Sci.*, 39 (1986) 645–650.
27 R.J. Nash, E.A. Bell, and J.M. Williams, *Phytochemistry*, 24 (1985) 1620–1622.
28 G.W.J. Fleet and P.W. Smith, *Tetrahedron*, 42 (1986) 5685–5692.
29 G.W.J. Fleet, S.J. Nicolas, P.W. Smith, S.V. Evans, L.E. Fellows, and R.J. Nash, *Tetrahedron Lett.*, 26 (1985) 3127–3130.
30 M. Launasmaa, *Alkaloids*, 33 (1988) 1–81.
31 D. Tepfer, A. Goldmann, N. Pamboukdjian, M. Maille, A. Lepingle, D. Chevalier, J. Dénarié, and C. Rosenberg, *J. Bacteriol.*, 170 (1988) 1153–1161.
32 A. Goldmann, M.L. Milat, P.H. Ducrot, J.Y. Lallemand, M. Maille, A. Lepingle, I. Charpin, and D. Tepfer, *Phytochemistry*, 29 (1990) 2125–2127.
33 P.H. Ducrot and J.Y. Lallemand, *Tetrahedron Lett.*, 31 (1990) 3879–3882.
34 O. Duclos, M. Mondange, A. Duréault, and J.C. Depezay, *Tetrahedron Lett.*, 33 (1992) 8061–8064.
35 R.J. Molyneux, Y.T. Pan, A. Goldmann, D.A. Tepfer, and A.D. Elbein, *Arch. Biochem. Biophys.*, 304 (1993) 81–88.