Iminosugar-Producing Thai Medicinal Plants

Naoki Asano,*,† Taku Yamauchi,† Koichi Kagamifuchi,† Naoko Shimizu,† Sachiko Takahashi,† Hitoshi Takatsuka,† Kyoko Ikeda,† Haruhisa Kizu,† Wongsatit Chuakul,‡ Aikkarach Kettawan,§ and Tadashi Okamoto#

Faculty of Pharmaceutical Sciences, Hokuriku University, Ho-3 Kanagawa-machi, Kanazawa 920-1181, Japan, Department of Pharmaceutical Botany, Faculty of Pharmacy, Mahidol University, Thailand, The Food Chemistry Division, Institute of Nutrition, Mahidol University, Thailand, and Faculty of Pharmaceutical Sciences, Kobe Gakuin University, Kobe 651-2180, Japan

Received May 9, 2005

α-1-*C*-Hydroxymethylfagomine (**7**), 3-*O*-*β*-D-glucopyranosyl-DMDP (**12**), and 2,5-dideoxy-2,5-imino-D-glucitol (**13**) were isolated from the Thai traditional crude drug "Non tai yak" (*Stemona tuberosa*), which also contains a high concentration level of α-homonojirimycin (0.1% dry weight). "Thopthaep" (*Connarus ferrugineus*) and "Cha em thai" (*Albizia myriophylla*) contained 1-deoxymannojirimycin (DMJ) (**10**) at levels of 0.083% (dry weight) and 0.17% (dry weight), respectively. 2-*O*-α-D-Galactopyranosyl-DMJ (**20**), 3-*O*-*β*-D-glucopyranosyl-DMJ (**21**), 1,4-dideoxymannojirimycin (**17**), 1,4-dideoxyallonojirimycin (**18**), and 1,4-dideoxyaltronojirimycin (**19**) from *C. ferrugineus* and 2-*O*-*β*-D-glucopyranosyl-DMJ (**22**) and 4-*O*-*β*-D-glucopyranosyl-DMJ (**23**) from *A. myriophylla* were isolated as new compounds.

Glycosidase inhibitors are currently of great interest as potential therapeutic agents.[1−4] For example, the α-glucosidase inhibitors acarbose (Glucobay), voglibose (Basen), and miglitol (Glyset) have been introduced onto the market for the treatment of type 2 diabetes. Neuraminidase inhibitors such as zanamivir (Relenza) and oseltamivir (Tamiflu) are used for the treatment of influenza viral infections, and lysosomal glycosidase inhibitors have potential as therapeutic agents for lysosomal storage disorders.[5] 1-Deoxygalactonojirimycin (DGJ) (AT1001), a potent lysosomal α-galactosidase inhibitor, is currently in Phase I clinical trials for the treatment of Fabry disease.[6] Iminosugars such as 1-deoxynojirimycin (DNJ) and DGJ are a very important class of glycosidase inhibitors and bind specifically to the active sites of glycosidases by mimicking monosaccharides. Much effort has been devoted in recent years to develop methodologies for the asymmetric syntheses of iminosugars owing to their promising therapeutic potential, and the continuing search for lead compounds of this type from natural sources is still of high relevance.

In the course of a search for α-glucosidase inhibitors, it was found that iminosugars are present in some Thai traditional crude drugs.[7] For example, it was reported that α-homonojirimycin (α-HNJ) occurs in "Non tai yak", at a level of 0.1%, and 1-deoxymannojirimycin (DMJ) and 2*R*,5*R*-dihydroxymethyl-3*R*,4*R*-dihydroxypyrrolidine (DMDP) occur in "Thopthaep" at 0.05% and 0.04%, respectively. The "Non tai yak" sample is known to be *Stemona tuberosa* Lour. (Stemonaceae), which has been used in mainland China and Japan for various medicinal purposes. In particular, an extract from the fresh tuberous roots of *S. tuberosa* is used to treat respiratory disorders, including pulmonary tuberculosis and bronchitis, and also recommended as an insecticide.[8−10] The biological origin of "Thopthaep" is described as *Connarus ferruginens* (Combretaceae).[7] However, it was identified as *Connarus ferrugineus* Jack (Connaraceae) in the present work. "Thopthaep" is used traditionally to treat scabies, as an ointment, and to treat stomachache and constipation as an oral drug. We found that the DMJ content in "Cha em thai" (*Albizia myriophylla* Benth.) was very high (over 0.1% w/w). The roots and wood of *A. myriophylla* (Leguminosae) are used traditionally to relieve thirst and sore throats and to substitute for licorice owing to a sweet taste.[10] Several triterpene saponins (albiziasaponins A−E) have been characterized recently as sweet-tasting substances.[11] In the present report, we describe the isolation and structural determination of a number of iminosugars from the three Thai medicinal plants described above.

Results and Discussion

The roots (1 kg) of *S. tuberosa* were extracted with 50% aqueous MeOH, and the extract was subjected to a variety of ion-exchange resin chromatographic steps to afford alkaloids **1** (1010 mg), **2** (2 mg), **3** (29 mg), **4** (30 mg), **5** (165 mg), **6** (18 mg), **7** (2.4 mg), **8** (210 mg), **9** (14 mg), **10** (112 mg), **11** (344 mg), **12** (12 mg), and **13** (29 mg).

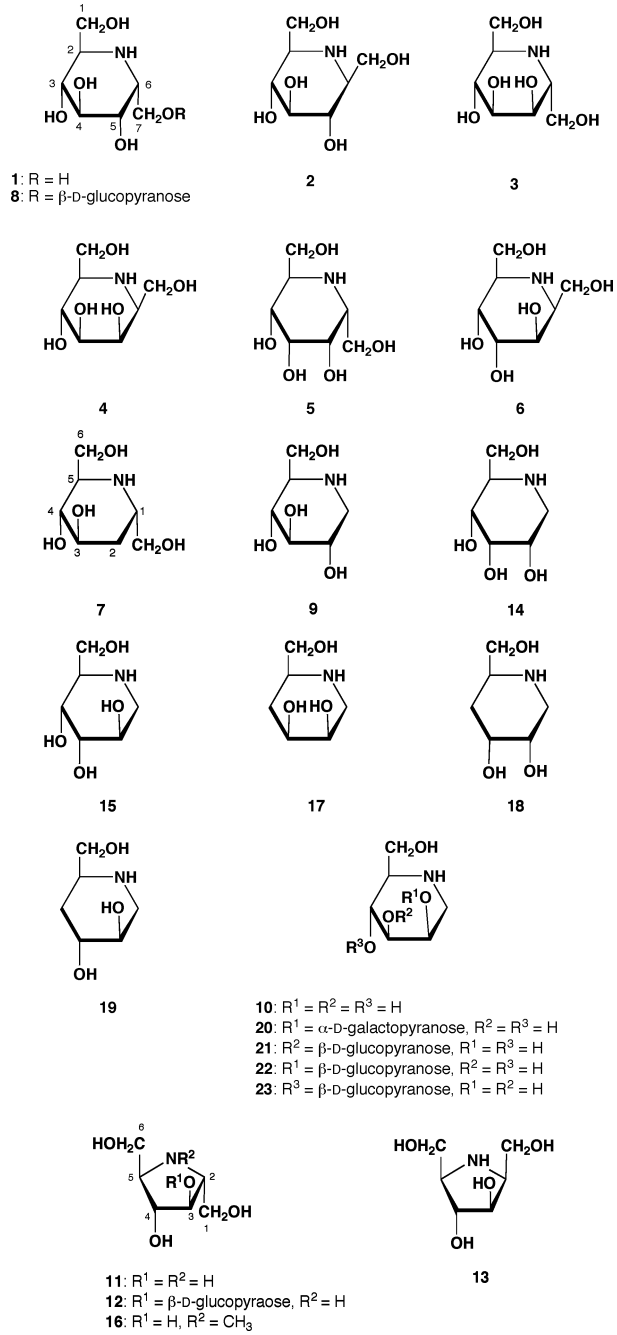
Alkaloids **1**−**6** and **8**−**11** were determined to be α-HNJ, *β*-HNJ, α-homomannojirimycin, *β*-homomannojirimycin, α-homoallonojirimycin, *β*-homoaltronojirimycin, 7-*O*-*β*-D-glucopyranosyl-α-HNJ, DNJ, DMJ, and DMDP, respectively, from their ^{1}H NMR and ^{13}C NMR spectroscopic data. We have isolated α-HNJ, its epimers, and its glycosides from *Aglaonema treubii* (Araceae) and *Hyacinthus orintalis* (Hyacinthaceae),[12,13] and DNJ, DMJ, and DMDP from *Adenophora triphylla* var. *japonica* (Campanulaceae) and *H. orientalis*.[13,14]

Alkaloid **7** was determined to have the molecular formula $C_7H_{15}NO_4$ by HRFABMS. The ^{1}H NMR and ^{13}C NMR spectroscopic data were superimposable with those of α-1-*C*-hydroxymethylfagomine (2,3,6-trideoxy-2,6-imino-D-*manno*-heptitol), which has been synthesized very recently.[15] The optical rotation {$[\alpha]_D$ +28.9° (*c* 0.20, H_2O)} of the natural compound was identical to that of the synthetic derivative {$[\alpha]_D$ +29.1° (*c* 0.40, H_2O)}. Hence, we determined that these compounds are identical, and the structure of **7** is α-1-*C*-hydroxymethylfagomine.

Alkaloid **12** was determined to have the molecular formula $C_{12}H_{23}NO_9$ by HRFABMS. The positive response

* To whom correspondence should be addressed. Fax: +81 76 229 2781. E-mail: n-asano@hokuriku-u.ac.jp.
† Hokuriku University.
‡ Mahidol University (Faculty of Pharmacy).
§ Mahidol University (Institute of Nutrition).
# Kobe Gakuin University.

10.1021/np050157a CCC: $30.25
Published on Web 08/09/2005



to the naphthoresorcinol−sulfuric acid reagent and a characteristic carbon signal (C-1′, δ_C 105.1) in the ^{13}C NMR spectrum suggested that **12** is a glycoside of an alkaloid. A small amount of this glycoside was subjected to acid hydrolysis (100 °C, 8 h) using Dowex 50W-X2 (H^+ form) resin. After washing the resin with H_2O, the aglycon part was displaced from the resin with 0.5 M NH_4OH, concentrated to dryness, and confirmed as DMDP from the ^{13}C NMR spectroscopic data. The sugar part of **12** in the washing was confirmed as D-glucose by the D-glucose oxidase−peroxidase method using the Glucose CII-test (Wako). From the value of the chemical shift (δ_H 4.61) and characteristic $^3J_{H,H}$ coupling (8.1 Hz) of the anomeric proton, the type of glucosidic linkage was determined to be β. The NMR spectra of DMDP are very simple (three peaks in the ^{13}C NMR spectrum, four spin systems in the ^1H NMR spectrum) due to its symmetrical structure. The ^{13}C NMR chemical shifts of C-3 and C-4 in DMDP were δ_C 80.7, while those in the glucoside were δ_C 80.1 and 90.3. Hence, the structure of **12** was determined as 3-*O*-β-D-glucopyranosyl-DMDP.

Alkaloid **13** was established as having the molecular formula $C_6H_{13}NO_4$ by HRFABMS. The ^1H NMR and ^{13}C NMR spectroscopic data were in accord with those of 2,5-dideoxy-2,5-imino-D-glucitol, which has been previously prepared by chemoenzymatic approaches.[16−20] The optical rotation {$[\alpha]_D$ +26.1° (*c* 0.84, H_2O)} was in accord with that of the synthetic compound {$[\alpha]_D$ +26.1° (*c* 1.2, H_2O)}.[18] Hence, alkaloid **13** was determined to be 2,5-dideoxy-2,5-imino-D-glucitol (DIDG).

The leaves and twigs (3 kg) of *C. ferrugineus* were extracted with 50% aqueous MeOH, and the extract was subjected to a variety of ion-exchange resin chromatographic steps to give alkaloids **9** (DNJ) (5.3 mg), **10** (DMJ) (2.5 g), **11** (DMDP) (1.06 g), **13** (DIDG) (23 mg), **14** (9.1 mg), **15** (70 mg), **16** (7.0 mg), **17** (2.5 mg), **18** (11.2 mg), **19** (30 mg), **20** (50 mg), and **21** (28 mg).

The ^1H NMR and ^{13}C NMR spectroscopic data of **14** were in accord with those of 1-deoxyallonojirimycin, which has recently been synthesized.[21] From the ^1H NMR and ^{13}C NMR spectroscopic data of **15**, it was determined to be 1-deoxyaltronojirimycin, which has been isolated from *Angylocalyx pynaertii* (Leguminosae) and *Scilla sibirica* (Hyacinthaceae).[22,23] Recently, D- and L-enantiomers of DNJ and its six epimers other than 1-deoxytalonojirimycin have been synthesized enantiospecifically.[21,24,25] The absolute configurations of natural DNJ, DMJ, 1-deoxyallonojirimycin, and 1-deoxyaltronojirimycin were determined to be D from the value and sign of the optical rotation.[25] The ^1H NMR and ^{13}C NMR spectroscopic data of **16** were superimposable with those of *N*-methyl-DMDP prepared from DMDP.[26]

Alkaloids **17**−**19** were determined to have the molecular formula $C_6H_{13}NO_3$ by HRFABMS. The $^1H-^1H$ and $^1H-^{13}C$ COSY NMR spectroscopic data defined a unit, $CH_2-CH-CH-CH_2-CH-CH_2$, showing a linear sequence from the C-1 to the C-6 methylene groups. The coupling patterns (a doublet of doublets) of H-1 and H-1′ and the relatively high-field chemical shift of the methine carbon at δ_C 53−58 (C-5) indicated that they must be bonded to the heterocyclic ring. These results suggested that alkaloids **17**−**19** are 4-deoxy derivatives of DNJ or its epimers. The coupling patterns of H-3 (ddd, $J_{2,3}$ = 2.8, $J_{3,4ax}$ = 11.9, $J_{3,4eq}$ = 5.0 Hz) and H-4ax (dt, $J_{3,4ax} = J_{4ax,5}$ = 11.9, $J_{4ax,4eq}$ = 12.4 Hz) in **17** indicate the axial orientations of H-3 and H-5 and the equatorial orientation of H-2. Therefore, the structure of **17** was deduced as 1,4-dideoxymannojirimycin. The coupling patterns of H-2 (ddd, $J_{1ax,2}$ = 11.0, $J_{1eq,2}$ = 5.0, $J_{2,3}$ = 3.2 Hz) and H-4ax (ddd, $J_{3,4ax}$ = 2.8, $J_{4ax,5}$ = 12.5, $J_{4ax,4eq}$ = 13.7 Hz) in **18** indicated the axial orientations of H-2 and H-5 and the equatorial orientation of H-3. Accordingly, the structure of **18** was characterized as 1,4-dideoxyallonojirimycin. In the last 1,4-dideoxyiminosugar, **19**, the coupling patterns of H-1ax (dd, $J_{1ax,2}$ = 2.7, $J_{1ax,1eq}$ = 13.8 Hz) and H-1eq (dd, $J_{1eq,2}$ = 4.6, $J_{1ax,1eq}$ = 13.8 Hz) and a large coupling (9.5 Hz) between H-4ax and H-5 indicated the equatorial orientations of H-2 and H-3 and the axial orientation of H-5. Therefore, the structure of **19** was determined as 1,4-dideoxyaltronojirimycin.

Alkaloids **20** and **21** were shown to have the molecular formula $C_{12}H_{23}NO_9$ by HRFABMS. The connectivity of the carbon and hydrogen atoms was defined from COSY and HMBC spectroscopic data. Again, a positive response to the naphthoresorcinol−sulfuric acid reagent suggested that **20** and **21** are glycosides of alkaloids. Their acid hydrolysis using Dowex 50W-X2 (H^+ form) demonstrated that their

aglycons are both DMJ. In the ^{1}H NMR spectrum of **20**, the coupling patterns of H-2′ (dd, $J_{1',2'} = 3.7$, $J_{2',3'} = 10.5$ Hz), H-3′ (dd, $J_{2',3'} = 10.5$, $J_{3',4'} = 3.7$ Hz), and H-4′ (dd, $J_{3',4'} = 3.7$, $J_{4',5'} = 1.0$ Hz) suggested that this glycoside is α-D-galactopyranosyl-DMJ. The fact that this glycoside was hydrolyzed by coffee bean α-D-galactosidase also supported that **20** is an α-D-galactoside. The HMBC spectrum showed a correlation peak between the anomeric proton and the aglycon C-2 carbon, defining the linkage site. Thus, the structure of **20** was determined to be 2-*O*-α-D-galactopyranosyl-DMJ. From the acid hydrolysis of **21**, the sugar part in the washing of the resin was confirmed as D-glucose by the D-glucose oxidase−peroxidase method. As a result of the value of the chemical shift (δ_H 4.61) and characteristic $^3J_{H,H}$ coupling (7.8 Hz) of the anomeric proton, the type of glucosidic linkage was determined to be β and the HMBC measurement elucidated that the glucosyl group is located at C-3. Therefore, the structure of **21** was determined to be 3-*O*-β-D-glucopyranosyl-DMJ.

The wood (1 kg) of *A. myriophylla* was extracted with 50% aqueous MeOH, and the extract was subjected to a variety of ion-exchange resin chromatographic steps to give alkaloids **10** (DMJ) (1.68 g), **11** (DMDP) (175 mg), **12** (3-*O*-β-D-glucopyranosyl-DMDP) (7.5 mg), **13** (DIDG) (30 mg), **22** (15 mg), and **23** (16 mg).

Alkaloids **22** and **23** were both determined to have the molecular formula $C_{12}H_{23}NO_9$ by HRFABMS and to be the D-glucosides of DMJ from their positive response to the naphthoresorcinol−sulfuric acid reagent, by acid hydrolysis using the Dowex 50W-X2 (H^+ form) resin, and from the analysis of the washing using the D-glucose oxidase−peroxidase method. The chemical shifts (δ_H 4.49 in **22** and δ_H 4.57 in **23**) and characteristic $^3J_{H,H}$ couplings (7.8 Hz in **22** and **23**) of the anomeric protons indicated that their glucosidic linkages are β. The linkage sites of the glucosyl groups in **22** and **23** were determined to be C-2 and C-4, respectively, by HMBC measurements. Accordingly, the structures of **22** and **23** were determined to be 2-*O*-β-D-glucopyranosyl-DMJ and 4-*O*-β-D-glucopyranosyl-DMJ, respectively.

DMJ is a competitive inhibitor of mammalian α-L-fucosidases with a K_i value in the μM range.[22,27] α-L-Rhamnopyranosyl-DMJ, which is the first naturally occurring glycoside of DMJ, is a more potent inhibitor of α-L-fucosidases than DMJ, with a K_i value of 0.06 μM, and enzymatically prepared 6-*O*-α-D-glucopyranosyl-DMJ and 6-*O*-α-isomaltosyl-DMJ are also inhibitors of the enzyme, with K_i values of 19 and 6.5 μM, respectively.[22] However, enzymatically prepared 2-*O*-α-D-glucopyranosyl-DMJ was not an inhibitor of the enzyme.[22] In the present work, we isolated four glycosides of DMJ, 2-*O*-α-D-galactopyranosyl-DMJ (**20**), 2-*O*-β-D-glucopyranosyl-DMJ (**22**), 3-*O*-β-D-glucopyranosyl-DMJ (**21**), and 4-*O*-β-D-glucopyranosyl-DMJ (**23**), but none of them showed inhibition toward α-L-fucosidase, and the deoxygenation at C-4 of DMJ to afford **17** nullified its inhibitory activity toward α-L-fucosidase. From studies on α-L-fucosidase inhibitors, it is concluded that the minimum structural feature for the inhibition is the identical configuration of three hydroxyl groups on the piperidine ring corresponding to C-2, C-3, and C-4 of L-fucose.[22,27,28] The present results reinforce the suggestion that the hydroxyl groups at C-2, C-3, and C-4 in DMJ must be intact for inhibition.[22] Liu et al.[17] have reported that DIDG (**13**) is a potent inhibitor of α- and β-glucosidases, with K_i values in the μM range. In the present work, DIDG was found to be a very weak inhibitor of α- and β-glucosidases, with IC_{50} values ranging from 300 to 1000 μM (320 μM toward almond β-glucosidase; 1000 μM toward rat intestinal sucrase and isomaltase). Furthermore, Liu et al. have reported that DIDG is also an inhibitor of coffee bean α-galactosidase with a K_i value of 50 μM, but in our present study, DIDG showed no significant inhibition toward the same enzyme, even at 1000 μM. DMDP is a potent inhibitor of bovine liver β-galactosidase and almond β-glucosidase, with IC_{50} values of 3.3 and 7.8 μM,[29,30] but the introduction of the β-glucosyl residue to C-3 (or C-4) of DMDP, to give **12**, markedly lowered its inhibitory activity toward β-galactosidase (IC_{50} = 270 μM) or abolished its inhibition of almond β-glucosidase.

Experimental Section

General Experimental Procedures. 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 chlorine−*o*-tolidine reagent or iodine vapor was used for detection. Optical rotations were measured with a JASCO DIP-370 digital polarimeter (Tokyo, Japan). ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) spectra were recorded on a JEOL ECP-500 spectrometer (Tokyo, Japan). Chemical shifts are expressed in ppm downfield from sodium 3-(trimethylsilyl)-propionate (TSP) in D_2O as an internal standard. FABMS were measured using glycerol as a matrix on a JEOL JMS-700 spectrometer.

Plant Material. Thai medicinal plants "Non tai yak", "Thopthaep", and "Cha em thai" were purchased from the Thai crude drug shop, Chao-Krom-Per, Bangkok, Thailand, in March 2001, and identified as *S. tuberosa* Lour., *C. ferrugineus* Jack., and *A. myriophylla* Benth., respectively, by one of the authors. (W.C.). The voucher specimens of *S. tuberosa* Lour. and *A. myriophylla* Benth. are retained at the Department of Pharmaceutical Botany, Faculty of Pharmacy, Mahidol University, Thailand, and that of *C. ferrugineus* Jack. is deposited at Kaeng Krachan National Park, Petchburi Province, Thailand.

Extraction and Isolation. The roots (1 kg) of *S. tuberosa* were extracted twice with 50% aqueous MeOH (5 L). The extract was applied to a column of Amberlite IR-120B (300 mL, H^+ form). The 0.5 M NH_4OH eluate was concentrated to give a brown syrup (23 g), which was further chromatographed over a Dowex 1-X2 (200 mL, OH^- form) column with H_2O (2 L) as eluant to give a colorless syrup (9.8 g). The syrup was applied to a 90 cm × 2.8 cm Amberlite CG-50 column (NH_4^+ form) with H_2O as eluant (fraction size 18 mL). The H_2O eluate was divided into five pools: I (fractions 14−26, 2.3 g); II (fractions 27−36, 650 mg), III (fractions 45−54, 150 mg), IV (fractions 62−100, 0.32 g), and V (fractions 123−134, 400 mg). Each pool was further chromatographed with Dowex 1-X2 (OH^- form) with H_2O as eluant and/or CM-Sephadex C-25 (NH_4^+ form) with 0.01−0.10 M NH_4OH as eluent to give alkaloids **1** (1010 mg), **8** (210 mg), and **12** (12 mg) from pool I, **3** (29 mg), **4** (30 mg), **5** (165 mg), and **9** (14 mg) from pool II, **2** (2 mg), **6** (18 mg), and **7** (2.4 mg) from pool III, **10** (112 mg) from pool IV, and **11** (344 mg) and **13** (29 mg) from pool V.

The leaves and twigs (3 kg) of *C. ferrugineus* and wood (1 kg) of *A. myriophylla* were also extracted twice with 50% aqueous MeOH. Their 50% MeOH extracts were also chromatographed in a similar manner, and alkaloids **9** (5.3 mg), **10** (2.5 g), **11** (1.06 g), **13**, (23 mg), **14** (9.1 mg), **15** (70 mg), **16** (7 mg), **17** (2.5 mg), **18** (11.2 mg), **19** (30 mg), **20** (50 mg), and **21** (28 mg) were isolated from *C. ferrugineus*, and alkaloids **10** (1.68 g), **11** (175 mg), **12** (7.5 mg), **13** (30 mg), **22** (15 mg), and **23** (16 mg) from *A. myriophylla*.

α-1-*C*-Hydroxymethylfagomine (7): colorless syrup; $[\alpha]_D$ +28.9° (*c* 0.20, H_2O); ^{1}H NMR (D_2O, 500 MHz) δ 1.65 (1H, ddd, J = 6.0, 11.9, 13.3 Hz, H-2ax), 2.01 (1H, ddd, J = 1.8, 5.0, 13.3 Hz, H-2eq), 2.80 (1H, ddd, J = 2.8, 7.8, 9.2 Hz, H-5), 3.17 (1H, t, J = 9.2 Hz, H-4), 3.15−3.19 (1H, m, H-1), 3.59 (1H, dd, J = 7.8, 11.5 Hz, H-6a), 3.60 (1H, dd, J = 6.0, 11.5 Hz, H-7a), 3.68 (1H, ddd, J = 5.0, 9.2, 11.9 Hz, H-3), 3.77 (1H,

Table 1. ^{13}C NMR Spectroscopic Data of 1-Deoxymannojirimycin (**10**), 1-Deoxyallonojirimycin (**14**), 1-Deoxyaltronojirimycin (**15**), and Their 4-Deoxy Derivatives **17**−**19**, at 125 MHz in D_2O[a]

carbon	10	14	15	17	18	19
1	51.5	46.5	47.3	51.4	47.3	47.6
2	72.1	71.0	72.0	69.6	71.4	71.0
3	77.5	74.3	73.4	71.8	70.1	70.4
4	71.3	71.5	68.9	32.5	36.0	32.8
5	63.4	57.3	58.5	58.4	53.0	54.1
6	63.7	64.2	63.5	67.0	67.7	66.0

[a] Chemical shifts are expressed in ppm downfield from sodium 3-(trimethylsilyl)propionate (TSP).

dd, J = 9.2, 11.5 Hz, H-7b), 3.90 (1H, dd, J = 2.8, 11.5 Hz, H-6b); ^{13}C NMR (D_2O, 125 MHz) δ 34.9 (C-2), 54.9 (C-1), 57.7 (C-5), 63.2 (C-7), 65.0 (C-6), 72.4 (C-3), 76.4 (C-4); HRFABMS *m/z* 178.1077 $[M + H]^+$ (calcd for $C_7H_{16}NO_4$ 178.1079).

2,5-Dideoxy-2,5-imino-(3-*O*-β-D-glucopyranosyl)-D-mannitol (3-*O*-β-D-glucopyranosyl-DMDP) (12): colorless hygroscopic powder; $[\alpha]_D$ +7.2° (*c* 0.26, H_2O); ^{1}H NMR (D_2O, 500 MHz) δ 3.21 (1H, m, H-5), 3.34 (1H, m, H-2), 3.40 (1H, dd, J = 8.1, 9.3 Hz, H-2′), 3.47 (1H, dd, J = 9.3, 9.8 Hz, H-4′), 3.58 (1H, ddd, J = 2.6, 6.6, 9.8 Hz, H-5′), 3.59 (1H, t, J = 9.3 Hz, H-3′), 3.74 (1H, dd, J = 6.1, 11.7 Hz, H-6a), 3.78 (1H, dd, J = 6.6, 12.0 Hz, H-6′a), 3.75−3.80 (2H, H-1a, H-1b), 3.82 (1H, dd, J = 4.4, 11.7 Hz, H-6b), 4.03 (1H, dd, J = 2.6, 12.0 Hz, H-6′b), 4.10 (1H, t, J = 5.6 Hz, H-3), 4.13 (1H, t, J = 5.6 Hz, H-4), 4.61 (1H, d, J = 8.1 Hz, H-1′); ^{13}C NMR (D_2O, 125 MHz) δ 63.8 (C-6′), 64.0 (C-2), 64.6 (C-6), 64.7 (C-1), 65.0 (C-5), 72.8 (C-4′), 76.0 (C-2′), 78.5 (C-3′), 78.8 (C-5′), 80.1 (C-4), 90.3 (C-3), 105.1 (C-1′); HRFABMS *m/z* 326.1453 $[M + H]^+$ (calcd for $C_{12}H_{24}NO_9$ 326.1451).

2,5-Dideoxy-2,5-imino-D-glucitol (13): colorless solid; mp 127−130 °C (lit. 132−136 °C);[18] $[\alpha]_D$ +26.1° (*c* 0.84, H_2O); ^{1}H NMR (D_2O, 500 MHz) δ 3.08 (1H, ddd, J = 2.9, 4.4, 4.9 Hz, H-5), 3.38 (1H, ddd, J = 2.9, 4.9, 6.0 Hz, H-2), 3.69 (1H, dd, J = 4.4, 11.5 Hz, H-6a), 3.71 (1H, dd, J = 4.9, 11.3 Hz, H-1a), 3.77 (1H, dd, J = 4.9, 11.5 Hz, H-6b), 3.82 (1H, dd, J = 6.0, 11.3 Hz, H-1b), 3.90 (1H, dd, J = 2.9, 5.2 Hz, H-4), 4.14 (1H, dd, J = 2.9, 5.2 Hz, H-3); ^{13}C NMR (D_2O, 125 MHz) δ 62.8 (C-1), 63.7 (C-2), 64.9 (C-6), 67.7 (C-5), 80.0 (C-3), 81.8 (C-4); HRFABMS *m/z* 164.0923 $[M + H]^+$ (calcd for $C_6H_{14}NO_4$ 164.0923).

1,4-Dideoxymannojirimycin (17): colorless syrup; $[\alpha]_D$ −37.5° (*c* 0.14, H_2O); ^{1}H NMR (D_2O, 500 MHz) δ 1.52 (1H, dt, J = 11.9, 12.4 Hz, H-4ax), 1.75 (1H, br ddd, J = 2.8, 5.0, 12.4 Hz, H-4eq), 2.78 (1H, dd, J = 1.5, 14.2 Hz, H-1eq), 2.78 (1H, m, H-5), 3.11 (1H, dd, J = 2.8, 14.2 Hz, H-1ax), 3.57 (1H, dd, J = 5.6, 11.5 Hz, H-6a), 3.61 (1H, dd, J = 4.6, 11.5 Hz, H-6b), 3.84 (1H, ddd, J = 2.8, 5.0, 11.9 Hz, H-3), 3.90 (1H, br s, H-2); ^{13}C NMR, see Table 1; HRFABMS *m/z* 148.0975 $[M + H]^+$ (calcd for $C_6H_{14}NO_3$ 148.0974).

1,4-Dideoxyallonojirimycin (18): colorless solid; mp 166−170 °C; $[\alpha]_D$ +62.0° (*c* 0.92, H_2O); ^{1}H NMR (D_2O, 500 MHz) δ 1.43 (1H, ddd, J = 2.8, 12.4, 13.7 Hz, H-4ax), 1.83 (1H, ddd, J = 2.8, 3.7, 13.7 Hz, H-4eq), 2.78 (1H, t, J = 11.0 Hz, H-1ax), 2.88 (1H, br dd, J = 5.0, 11.0 Hz, H-1eq), 2.92 (1H, dddd, J = 2.8, 4.6, 6.9, 12.4 Hz, H-5), 3.43 (1H, dd, J = 6.9, 11.4 Hz, H-6a), 3.54 (1H, dd, J = 4.6, 11.4 Hz, H-6b), 3.69 (1H, ddd, J = 3.2, 5.0, 11.0 Hz, H-2), 4.11 (1H, m, H-3); ^{13}C NMR, see Table 1; HRFABMS *m/z* 148.0973 $[M + H]^+$ (calcd for $C_6H_{14}NO_3$ 148.0974).

1,4-Dideoxyaltronojirimycin (19): colorless hygroscopic solid; $[\alpha]_D$ −9.0° (*c* 1.16, H_2O); ^{1}H NMR (D_2O, 500 MHz) δ 1.63 (1H, br ddd, J = 3.7, 5.6, 14.2 Hz, H-4eq), 1.83 (1H, ddd, J = 3.7, 9.5, 14.2 Hz, H-4ax), 2.83 (1H, br dd, J = 4.6, 13.8 Hz, H-1eq), 3.04 (1H, dd, J = 2.7, 13.8 Hz, H-1ax), 3.04 (1H, m, H-5), 3.17 (2H, H-6a, H-6b), 3.62 (1H, br ddd, J = 2.7, 4.6, 5.1 Hz, H-2), 3.89 (1H, m, H-3); ^{13}C NMR, see Table 1; HRFABMS *m/z* 148.0973 $[M + H]^+$ (calcd for $C_6H_{14}NO_3$ 148.0974).

2-*O*-α-D-Galactopyranosyl-1-deoxymannojirimycin (20): colorless hygroscopic powder; $[\alpha]_D$ +51.1° (*c* 0.96, H_2O); ^{1}H NMR, see Table 2; ^{13}C NMR, see Table 3; HRFABMS *m/z* 326.1450 $[M + H]^+$ (calcd for $C_{12}H_{24}NO_9$ 326.1451).

2-*O*-β-D-Glucopyranosyl-1-deoxymannojirimycin (21): colorless hygroscopic powder; $[\alpha]_D$ −27.9° (*c* 0.35, H_2O); ^{1}H NMR, see Table 2; ^{13}C NMR, see Table 3; HRFABMS *m/z* 326.1449 $[M + H]^+$ (calcd for $C_{12}H_{24}NO_9$ 326.1451).

3-*O*-β-D-Glucopyranosyl-1-deoxymannojirimycin (22): colorless hygroscopic powder; $[\alpha]_D$ −28.3° (*c* 0.86, H_2O); ^{1}H NMR, see Table 2; ^{13}C NMR, see Table 3; HRFABMS *m/z* 326.1448 $[M + H]^+$ (calcd for $C_{12}H_{24}NO_9$ 326.1451).

4-*O*-β-D-Glucopyranosyl-1-deoxymannojirimycin (23): colorless hygroscopic powder; $[\alpha]_D$ −26.3° (*c* 0.41, H_2O); ^{1}H NMR, see Table 2; ^{13}C NMR, see Table 3; HRFABMS *m/z* 326.1450 $[M + H]^+$ (calcd for $C_{12}H_{24}NO_9$ 326.1451).

Acid Hydrolysis of Glycosides. A small amount (3−5 mg) of each glycoside was heated at 100 °C with Dowex 50W-X2 (0.5 g, H^+ form) in water for 8 h. The resin was filtered off and packed into a short column. The alkaloid moiety was eluted with 0.5 M NH_4OH and concentrated. From its NMR spectroscopic data, the alkaloid moiety of **12** was identified to be DMDP, and those of **20**, **21**, **22**, and **23** were determined to be DMJ. The response of the neutralized filtrate to the D-glucose oxidase−peroxidase method using the Glucose CII-test (Wako) was studied, and D-glucose was detected from the filtrates of **12**, **21**, **22**, and **23**.

Enzymatic Hydrolysis of 2-*O*-α-D-Galactopyranosyl-1-deoxymannojirimycin (20) by Coffee Bean α-D-Galactosidase. A small amount (below 1 mg) of 2-*O*-α-D-galactopyranosyl-1-deoxymannojirimycin and coffee bean α-D-galactosidase (Sigma Chemical Co.) were incubated at 37 °C for 24 h in a phosphate buffer (pH 6.5). The reaction mixture was analyzed by HPTLC using the solvent system PrOH−AcOH−H_2O (4:1:1). The alkaloid and sugar in the hydrolysate were detected by the chlorine−*o*-tolidine and naphthoresorcinol−sulfuric acid reagents, respectively.

Glycosidase Inhibitory Activities. The enzymes β-glucosidase (from almond, pH 5.0), α-galactosidase (from coffee

Table 2. ^{1}H NMR Spectroscopic Data of 1-Deoxymannojirimycin Glycosides **20**−**23** at 500 MHz in D_2O[a]

position	20	21	22	23
1ax	2.75 dd (1.4, 14.7)[b]	2.76 dd (1.4, 14.7)	2.77 dd (1.4, 14.6)	2.80 dd (1.4, 14.7)
1eq	3.24 dd (2.7, 14.7)	3.31 dd (2.8, 14.7)	3.22 dd (2.8, 14.6)	3.04 dd (2.8, 14.7)
2	4.03 m	4.20 m	4.20 m	4.10 ddd (1.4, 2.8, 3.2)
3	3.63 dd (3.2, 9.6)	3.81 dd (3.2, 9.6)	3.64 dd (3.7, 9.2)	3.71 dd (3.2, 9.6)
4	3.70 t (9.6)	3.74 t (9.6)	3.60 t (9.2)	3.84 t (9.6)
5	2.49 ddd (3.2, 4.1, 9.6)	2.53 ddd (3.2, 5.5, 9.6)	2.60 ddd (3.2, 5.5, 9.2)	2.68 ddd (2.8, 4.1, 9.6)
6a	3.76 dd (3.2, 11.9)	3.73 dd (5.5, 12.4)	3.70−3.77	3.79 dd (6.0, 12.4)
6b	3.84 dd (4.1, 11.9)	3.91 dd (3.2, 12.4)	3.84 dd (3.2, 11.5)	3.98 dd (2.7, 12.4)
1′	5.18 d (3.7)	4.61 d (7.8)	4.49 d (7.8)	4.57 d (7.8)
2′	3.83 dd (3.7, 10.5)	3.38 dd (7.8, 9.6)	3.55 dd (7.8, 9.6)	3.37 dd (7.8, 9.6)
3′	3.95 dd (3.7, 10.5)	3.52 dd (8.7, 9.6)	3.51 dd (8.7, 9.6)	3.57 dd (9.2, 9.6)
4′	3.99 dd (1.0, 3.7)	3.43 dd (8.7, 9.6)	3.41 dd (8.7, 9.6)	3.47 dd (9.2, 9.6)
5′	4.05 m	3.46 m	3.44 m	3.58 ddd (3.2, 4.1, 9.6)
6′a	3.74 m	3.77−3.84	3.70−3.77	3.85 dd (3.2, 11.5)
6′b	3.74 m	3.77−3.84	3.92 dd (2.3, 12.4)	3.94 dd (4.1, 11.5)

[a] Chemical shifts are expressed in ppm downfield from sodium 3-(trimethylsilyl)propionate (TSP). [b] J in Hz.

Table 3. ^{13}C NMR Spectroscopic Data of 1-Deoxymannojirimycin Glycosides at 125 MHz in D_2O[a]

carbon	**20**	**21**	**22**	**23**
1	50.0	50.7	48.5	50.6
2	81.8	69.4	79.4	71.4
3	77.8	84.9	76.5	75.9
4	71.4	69.4	71.7	81.8
5	63.1	63.3	63.3	62.0
6	63.3	63.5	63.7	63.4
1′	103.3	103.0	103.4	105.5
2′	71.7	75.8	75.7	76.0
3′	72.1	78.4	78.4	78.3
4′	72.2	72.4	72.5	72.3
5′	74.7	78.8	78.7	78.8
6′	64.1	63.4	63.6	62.8

[a] Chemical shifts are expressed in ppm downfield from sodium 3-(trimethylsilyl)propionate (TSP).

bean, pH 6.5), β-galactosidase (from bovine liver, pH 6.8), and α-L-fucosidase (from bovine epididymis, pH 5.5), *p*-nitrophenyl glycosides, and disaccharides were purchased from Sigma Chemical Co. Brush border membranes prepared from rat small intestine according to the method of Kessler et al.[31] were assayed at pH 5.8 for rat intestinal sucrase and isomaltase using sucrose and isomaltose as substrate. The released D-glucose was determined colorimetrically using the Glucose CII-test Wako (Wako Pure Chemical Ind.). Other glycosidase activities were determined using an appropriate *p*-nitrophenyl glycoside as substrate. The reaction was stopped by adding 400 mM Na_2CO_3. The released *p*-nitrophenol was measured spectrometrically at 400 nm.

References and Notes

(1) Asano, N.; Nash, R. J.; Molyneux, R. J.; Fleet, G. W. J. *Tetrahedron: Asymmetry* **2000**, *11*, 1645−1680.
(2) Watson, A. A.; Fleet, G. W. J.; Asano, N.; Molyneux, R. J.; Nash, R. J. *Phytochemistry* **2001**, *56*, 265−295.
(3) Butters, T. D.; Dwek, R. A.; Platt, F. M. *Chem. Rev.* **2000**, *100*, 4683−4696.
(4) Asano, N. *Glycobiology* **2003**, *13*, 93R-104R.
(5) Fan, J.-Q. *Trends Pharmacol. Sci.* **2003**, *24*, 355−360.
(6) Fan, J.-Q.; Ishii, S.; Asano, N.; Suzuki, Y. *Nat. Med.* **1999**, *5*, 112−115.
(7) Kitaoka, M.; Ichikawa, K.; Sakurai, Y.; Matsushita, Y.; Iijima, Y.; Akiyama, T.; Boriboon, M. *Annu. Rep. Sankyo Res. Lab.* **1993**, *45*, 99−104, and references therein.
(8) Terada, M.; Sano, M.; Ishii, A. I.; Kino, H.; Fukushima, S.; Noro, T. *Nippon Yakurigaku Zasshi* **1982**, *79*, 93−103.
(9) Sakata, K.; Aoki, K.; Chang, C. F.; Sakurai, A.; Murakoshi, J. *Agric. Biol. Chem.* **1978**, *42*, 457−463.
(10) Saralamp, P., Chuakul, W., Temsiririrkkul, R., Clayton, T., Eds. *Medicinal Plants in Thailand*; Mahidol University: Bangkok, 1996; Vol. 1.
(11) Yoshikawa, M.; Morikawa, T.; Nakano, K.; Pongpiriyadacha, Y.; Murakami, T.; Matsuda, H. *J. Nat. Prod.* **2002**, *65*, 1638−1642.
(12) Asano, N.; Nishida, M.; Kizu, H.; Matsui, K. *J. Nat. Prod.* **1997**, *60*, 98−101.
(13) Asano, N.; Kato, A.; Miyauchi, M.; Kizu, H.; Kameda, Y.; Watson, A. A.; Nash, R. J.; Fleet, G. W. J. *J. Nat. Prod.* **1998**, *61*, 625−628.
(14) Asano, N.; Nishida, M.; Miyauchi, M.; Ikeda, K.; Yamamoto, M.; Kizu, H.; Kameda, Y.; Watson, A. A.; Nash, R. J.; Fleet, G. W. J. *Phytochemistry* **2000**, *53*, 379−382.
(15) Goujon, J.-Y.; Gueyrard, D.; Compain, P.; Martin, O. R.; Ikeda, K.; Asano, N. *Bioorg. Med. Chem.* **2005**, *13*, 2313−2324.
(16) Reiz, A.; Baxter, E. W. *Tetrahedron Lett.* **1990**, *31*, 6777−6780.
(17) Liu, K. K.-C.; Kajimoto, T.; Chen, L.; Zhong, Z.; Ichikawa, Y.; Wong, C.-H. *J. Org. Chem.* **1991**, *56*, 6280−6289.
(18) Legler, G.; Korth, A.; Berger, A.; Ekhart, C.; Gradnig, G.; Stütz, A. E. *Carbohydr. Res.* **1993**, *250*, 67−77.
(19) Baxter, E. W.; Reitz, A. B. *J. Org. Chem.* **1994**, *59*, 3175−3185.
(20) Takayama, S.; Martin, R.; Wu, J.; Laslo, K.; Siuzdak, G.; Wong, C.-H. *J. Am. Chem. Soc.* **1997**, *119*, 8146−8151.
(21) Takahata, H.; Banba, Y.; Sasatani, M.; Nemoto, H.; Kato, A.; Adachi, I. *Tetrahedron* **2004**, *60*, 8199−8205.
(22) Asano, N.; Yasuda, K.; Kizu, H.; Kato, A.; Fan, J.-Q.; Nash, R. J.; Fleet, G. W. J.; Molyneux, R. J. *Eur. J. Biochem.* **2001**, *268*, 35−41.
(23) Yamashita, T.; Yasuda, K.; Kizu, H.; Kameda, Y.; Watson, A. A.; Nash, R. J.; Fleet, G. W. J.; Asano, N. *J. Nat. Prod.* **2002**, *65*, 1875−1881.
(24) Takahata, H.; Banba, Y.; Ouchi, H.; Nemoto, H. *Org. Lett.* **2003**, *5*, 2527−2529.
(25) Kato, A.; Kato, N.; Kano, E.; Adachi, I.; Ikeda, K.; Yu, L.; Okamoto, T.; Banba, Y.; Ouchi, H.; Takahata, H.; Asano, N. *J. Med. Chem.* **2005**, *48*, 2036−2044.
(26) Asano, N.; Kizu, H.; Oseki, K.; Tomioka, E.; Matsui, K.; Okamoto, M.; Baba. M. *J. Med. Chem.* **1995**, *38*, 2349−2356.
(27) Winchester, B.; Barker, C.; Baines, S.; Jacob, G. S.; Namgoong, S. K.; Fleet, G. W. J. *Biochem. J.* **1990**, *265*, 277−282.
(28) Winchester, B.; Fleet, G. W. J. *Glycobiology* **1992**, *2*, 199−210.
(29) Asano, N.; Oseki, K.; Kizu, H.; Matsui, K. *J. Med. Chem.* **1994**, *37*, 3701−3706.
(30) Fleet, G. W. J.; Nicholas, S. J.; Smith, P. W.; Evans, S. V.; Fellows, L. E.; Nash, R. J. *Tetrahedron Lett.* **1985**, *26*, 3127−3130.
(31) Kessler, M.; Acuto, O.; Strelli, C.; Murer, H.; Semenza, G. A. *Biochim. Biophys. Acta* **1978**, *506*, 136−154.

NP050157A