Nitrogen-Containing Furanose and Pyranose Analogues from *Hyacinthus orientalis*

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Aqueous methanol extracts from the bulbs of *Hyacinthus orientalis* were subjected to various ion-exchange column chromatographic steps to give 2(*R*),5(*R*)-bis(hydroxymethyl)-3(*R*),4(*R*)-dihydroxypyrrolidine (DMDP) (**1**), 2,5-dideoxy-2,5-imino-DL-*glycero*-D-*manno*-heptitol (homoDMDP) (**2**), 2,5-imino-2,5,6-trideoxy-D-*manno*-heptitol (6-deoxy-homoDMDP) (**3**), 2,5-imino-2,5,6-trideoxy-D-*gulo*-heptitol (**4**), 1-deoxynojirimycin (**5**), 1-deoxymannojirimycin (**6**), α-homonojirimycin (**7**), β-homonojirimycin (**8**), α-homomannojirimycin (**9**), β-homomannojirimycin (**10**), and 7-*O*-β-D-glucopyranosyl-α-homonojirimycin (MDL 25,637) (**11**). The structures of the new natural products **3** and **4** were determined by spectroscopic analysis, including extensive 1D and 2D NMR studies. Compound **2** was found to be a potent inhibitor of bacterial β-glucosidase, mammalian β-galactosidases, and mammalian trehalases, while **3** was a potent inhibitor of rice α-glucosidase and rat intestinal maltase. Compound **4** was observed to be a good inhibitor of α-L-fucosidase.

Glycosidase-inhibiting pyrrolidine alkaloids have recently been found in the leaves of bluebells, *Hyacinthoides nonscripta* (Hyacinthaceae).[1] These alkaloids were 2(*R*),5(*R*)-bis(hydroxymethyl)-3(*R*),4(*R*)-dihydroxypyrrolidine (DMDP, **1**), 1,4-dideoxy-1,4-imino-D-arabinitol (DAB), 2,5-dideoxy-2,5-imino-DL-*glycero*-D-*manno*-heptitol (homoDMDP, **2**), and homoDMDP-7-*O*-apioside.[1] DMDP (**1**) was seen to be the major alkaloid present at all growth stages of this plant and found at high concentrations in the leaves, seed pods, and bulbs.[1] This alkaloid has been reported from many disparate species of plants[2] and has more recently been isolated from the cultured broth of *Streptomyces* species.[3] The new alkaloid homoDMDP (**2**), whose stereochemistry at C-6 was not able to be determined from NMR data, was found to be the second most abundant alkaloid and also occurs as an apioside.[1] Compound **2** is a more potent inhibitor ($K_i = 1.5$ μM) of almond β-glucosidase than **1** ($K_i = 10$ μM), but it is a weak inhibitor ($K_i = 54$ μM) of yeast α-glucosidase compared to **1** ($K_i = 7$ μM).[1] We have reported that DAB is a good inhibitor with a broad inhibitory spectrum toward mammalian glycosidases, such as endoplasmic reticulum (ER) α-glucosidase II, Golgi α-mannosidases I and II, intestinal isomaltase, and trehalase.[4] Although rare, there are reports of livestock that have been poisoned by grazing on bluebells. Many polyhydroxylated alkaloids other than the pyrrolidine alkaloids described above have been detected in the leaves by GC−MS. Inhibition of glycosidase activities by these polyhydroxylated alkaloids could be responsible for the toxicity of this species to mammals.

In a search for polyhydroxylated alkaloids in other species of the Hyacinthaceae by GC−MS, we found many such alkaloids in the bulbs of *Hyacinthus orientalis* L., including α-homonojirimycin (**7**) as a major component. In this paper, we describe the isolation and characterization of 11 polyhydroxylated alkaloids (**1**−**11**) and their glycosidase inhibitory activity. The structures of the new natural products **3** and **4** were established on the analysis of their spectral data.

Results and Discussion

A 50% MeOH extract of the bulbs of *H. orientalis*, after preliminary purification by ion-exchange chromatography on Amberlite IR-120B [H^+ form] and Dowex 1 × 2 [OH^- form] resins, was silylated with Sigma Sil-A and analyzed by GC−MS. Compound **7**, a major component, was identified as α-homonojirimycin, which gave a pentabis(trimethylsilyl) (TMSi) derivative and a characteristic fragment ion at *m/z* 538 $[M - CH_3]^+$ and a base peak at *m/z* 450 $[M - CH_2OTMSi]^+$. A 50% MeOH extract of the bulbs (7.6 kg) was chromatographed with various ion-exchange resins to give compounds **1** (24 mg), **2** (325 mg), **3** (27 mg), **4** (15 mg), **5** (160 mg), **6** (31 mg), **7** (2.4 g), **8** (4 mg), **9** (29 mg), **10** (5 mg), and **11** (19 mg) (Chart 1).

The optical rotation, FABMS, and NMR spectra of compounds **1** and **7**−**11** were in accord with those of DMDP, α-homonojirimycin, β-homonojirimycin, α-homomannojirimycin, β-homomannojirimycin, and 7-*O*-β-D-glucopyranosyl-α-homonojirimycin (MDL 25,637) isolated from *Aglaonema treubii* (Araceae), respectively.[5] It was determined by the ^1H and ^{13}C NMR spectral data that compounds **5** and **6** were 1-deoxynojirimycin and 1-deoxymannojirimycin, respectively. The optical rotation and ^1H NMR and ^{13}C NMR (Table 1) spectral data of compound **2** were consistent with those reported for

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Chart 1

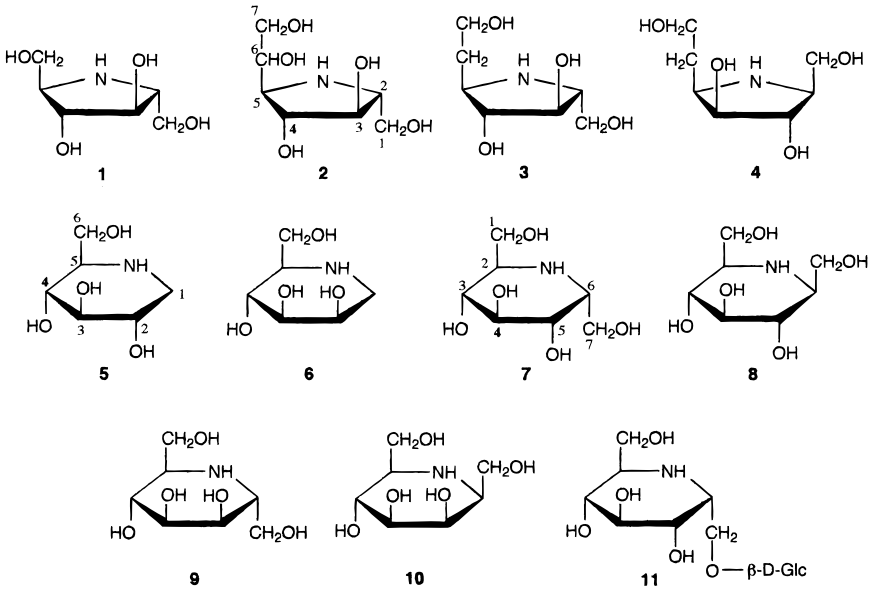

Table 1. ^{13}C NMR Data of Compounds **2**−**4** (in D_2O, 100 MHz)[a]

carbon	2	3	4
1	64.5	64.9	64.5
2	64.7	64.4	68.9
3	80.8	80.5	82.0
4	80.6	84.3	80.7
5	64.3	60.3	61.0
6	75.7	38.3	32.9
7	66.2	62.0	62.2

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

2,5-dideoxy-2,5-imino-DL-*glycero*-D-*manno*-heptitol (homoDMDP) isolated from *H. nonscripta*.[1]

The ^{13}C NMR spectral analysis of compound **3** revealed the presence of three methylene and four methine carbon atoms. This result and HRFABMS established that the molecular formula was $C_7H_{15}O_4N$. The ^1H NMR spectral data, combined with extensive decoupling experiments and 2D $^1H-^{13}C$ COSY spectral data, defined the complete connectivity of carbon and hydrogen atoms. Decoupling experiments suggested that the methylene carbon at δ 38.3 was located at the C-6 position. From these NMR results, the methylene triplets at δ 62.0 (C-7) and 64.9 (C-1) were attributed to the hydroxymethyl carbons, with the methine doublets at δ 80.5 and 84.3 assigned to C-3 and C-4 bearing the OH groups, respectively. The relatively high-field signals at C-2 (δ 64.4) and C-5 (δ 60.3) indicated that they must be bonded to the nitrogen of the heterocyclic ring. The large coupling constants ($J_{2,3} = J_{3,4} = 7.3$ Hz, $J_{4,5} = 7.8$ Hz) of the pyrrolidine ring protons suggested the all-trans configuration of H-2, H-3, H-4, and H-5, consistent with those ($J_{2,3} = J_{3,4} = J_{4,5} = 7.3$ Hz) reported for the all-trans configuration of homoDMDP (**2**).[1] The relative configurations at the stereogenic centers in **3** were established by definite NOE effects between H-2 and H-4 and between H-3 and H-5 or H-1 (Figure 1). Thus, compound **3** was determined to be 2,5-imino-2,5,6-trideoxy-D-*manno*-heptitol (6-deoxy-homoDMDP) or its enantiomer.

Compound **4** was found to be an isomer of **3** from its HRFABMS and ^{13}C NMR spectral data (Table 1). Since

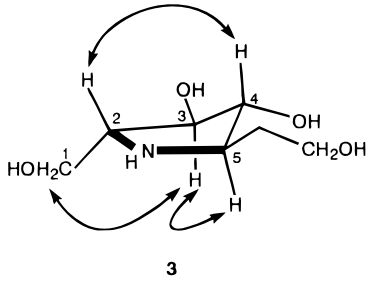

Figure 1. NOE interactions for 6-deoxy-homoDMDP (**3**).

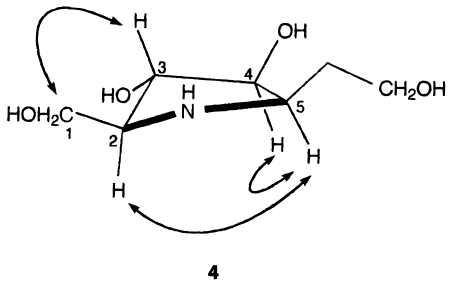

Figure 2. NOE interactions for 2,5-imino-2,5,6-trideoxy-D-*gulo*-heptitol (**4**).

the stereogenic centers of the pyrrolidine ring protons could not be determined from their coupling constants ($J_{2,3} = 4.4$ Hz, $J_{3,4} = 1.7$ Hz, $J_{4,5} = 4.1$ Hz), we performed extensive NOE experiments. Irradiation of H-5 of **4** enhanced the NOE intensity of H-2 and H-4, and definite NOE effects between H-3 and the C-1 (CH_2OH) protons were also observed (Figure 2). These results suggest that H-2, H-3, H-4, and H-5 are in the α, β, α, and α orientation, respectively. Thus, compound **4** was determined to be 2,5-imino-2,5,6-trideoxy-D-*gulo*-heptitol or its enantiomer.

The IC_{50} values of the natural nitrogen-containing furanoses against various glycosidases are shown in Table 2. Watson et al.[1] have reported that **2** is a more potent inhibitor ($K_i = 1.5$ μM) of almond β-glucosidase than **1** ($K_i = 10$ μM). The present work revealed that **2** was also a potent inhibitor of bacterial (*Caldocellum saccharolyticum*) β-glucosidase ($IC_{50} = 3.8$ μM) and mammalian trehalases ($IC_{50} = 5$ μM for porcine and 2

Table 2. Concentration of Nitrogen-Containing Furanoses Producing 50% Inhibition of Various Glycosidases

enzyme	IC_{50} (μM)			
	1	**2**	**3**	**4**
α-glucosidase				
rice	200	130	2.2	NI
rat intestinal maltase	290	400	2.5	NI
rat intestinal isomaltase	91	NI	NI	NI
rat intestinal sucrase	NI[a]	300	11	NI
rat liver lysosome	92	NI	7.2	NI
β-glucosidase				
Caldocellum saccharolyticum	11	3.8	380	NI
β-galactosidase				
bovine liver	2.5	4.4	60	NI
rat intestinal lactase	3.6	4.0	320	NI
α-L-fucosidase				
epididymis bovine	NI	NI	NI	50
α,α-trehalase				
porcine kidney	500	5.0	NI	NI
rat intestine	360	2.0	380	NI

[a] NI = less than 50% inhibition at 1000 μM.

μM for rat). Potencies of **1** and **2** toward mammalian β-galactosidases were very similar. Interestingly, the deoxygenation at C-6 of **2** relative to **3** significantly supressed its inhibitory activity toward β-glucosidase, β-galactosidase, and trehalase, whereas its potency toward rice and rat α-glucosidases was greatly enhanced. Compounds **1**−**3** exhibited no significant inhibitory activity toward α-fucosidase, whereas **4** was a very specific inhibitor of α-L-fucosidase (IC_{50} = 50 μM), lacking other glycosidase inhibitory activities.

Experimental Section

General Experimental Procedures. Alkaloids were chromatographed on HPTLC silica gel-60F_{254} (E. Merck) using the solvent system *n*-PrOH−AcOH−H_2O (4:1:1), and the chlorine−*o*-tolidine spray reagent[6,7] was used for detection. 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 internal reference. Mass spectra were measured on a JEOL JMS-SX 102A spectrometer. The GC−MS spectra were obtained on a QMASS 910 (Perkin-Elmer) mass spectrometer.

Plant Material. The bulbs of *H. orientalis* L. (variety name, "City of Haarlem") (Hyacinthaceae) were purchased in December 1995 from a flower shop. The supplier was Van Bloem Gardens (Zabo Plant B. V., 't Zand, Holland). A voucher specimen (no. NA970501) is deposited at the WPBS Herbarium of Institute of Grassland and Environmental Research.

GC−MS Analysis. Samples were dried and silylated at 20 °C for 60 min using 100 μL of Sigma Sil-A (Sigma Chemical Co.) per milligram of material. The column was a 25-m × 0.25 mm BPX5 (film thickness, 0.25 μm) capillary column (SGE), and the 25-min temperature program ran from 180 to 300 °C with an initial rate of increase of 10 °C/min and then held at 300 °C.

Extraction and Isolation. The bulbs (7.6 kg fresh wt) of *H. orientalis* were homogenized in 50% aqueous MeOH. The filtrate was applied to a column of Amberlite IR-120B [H^+ form, 1 L] prepared in 50% aqueous MeOH. A 0.5 M NH_4OH eluate was concentrated to give a brown oil (11.8 g), which was chromatographed over an Amberlite CG-50 column [3.8 × 90 cm, NH_4^+ form] with H_2O as eluant (fraction size 15 mL). The H_2O eluate was divided into four pools: A (fractions 14−31, 3.53 g), B (fractions 32−38, 358 mg), C (fractions 39−52, 522 mg), and D (fractions 53−62, 77 mg). The 0.5 M NH_4OH eluate from the same column was designated pool E. Each pool was further chromatographed on a Dowex 1 × 2 column [1.9 × 92 cm, OH^- form] to give **7** (2.4 g) and **8** (4 mg) from pool A, **5** (160 mg), **9** (29 mg), and **10** (5 mg) from pool B, **2** (325 mg) and **4** (15 mg) from pool C, **3** (27 mg) and **6** (31 mg) from pool D, and **1** (24 mg) and **11** (19 mg) from pool E.

2(*R*),5(*R*)-Bis(hydroxymethyl)-3(*R*),4(*R*)-dihydroxypyrrolidine (DMDP) (1): $[\alpha]_D$ +56.98 (*c* 0.6, H_2O); ^{13}C NMR (100 MHz, D_2O) δ 64.4 (C-2,5), 64.9 (C-1,6), 80.7 (C-3,4); FABMS *m/z* 164 $[M + H]^+$.

2,5-Dideoxy-2,5-imino-DL-*glycero*-D-*manno*-heptitol (homoDMDP) (2): $[\alpha]_D$ +33.38 (*c* 0.95, H_2O); ^{13}C NMR, see Table 1; HRFABMS *m/z* 194.1032 $[M + H]^+$ ($C_7H_{16}O_5N$ requires 1194.1028).

2,5-Imino-2,5,6-trideoxy-D-*manno*-heptitol (6-deoxyhomoDMDP) (3): $[\alpha]_D$ +98.48 (*c* 1.13, H_2O); ^{1}H NMR (400 MHz, D_2O) δ 1.71 (1H, m, H-6a), 1.93 (1H, m, H-6b), 3.01 (1H, ddd, *J* = 4.9, 7.8, 8.8 Hz, H-5), 3.05 (1H, ddd, *J* = 4.4, 6.1, 7.3 Hz, H-2), 3.65 (1H, dd, *J* = 6.1, 11.7 Hz, H-1a), 3.64−3.77 (2H, m, H-7a, H-7b), 3.71 (1H, dd, *J* = 4.4, 11.7 Hz, H-1b), 3.74 (1H, dd, *J* = 7.3, 7.8 Hz, H-4), 3.84 (1H, t, *J* = 7.3 Hz, H-3); ^{13}C NMR, see Table 1; HRFABMS *m/z* 178.1076 $[M + H]^+$ ($C_7H_{16}O_4N$ requires 178.1079).

2,5-Imino-2,5,6-trideoxy-D-*gulo*-heptitol (4): $[\alpha]_D$ +41.38 (*c* 0.56, H_2O); ^{1}H NMR (400 MHz, D_2O) δ 1.79 (1H, m, H-6a), 1.90 (1H, m, H-6b), 3.07 (1H, ddd, *J* = 4.4, 5.1, 6.4 Hz, H-2), 3.34 (1H, ddd, *J* = 4.1, 6.6, 7.8 Hz, H-5), 3.71 (1H, dd, *J* = 6.4, 11.7 Hz, H-1a), 3.72 (2H, H-7a, H-7b), 3.78 (1H, dd, *J* = 5.1, 11.7 Hz, H-1b), 3.92 (1H, dd, *J* = 1.7, 4.4 Hz, H-3), 4.03 (1H, dd, *J* = 1.7, 4.1 Hz, H-4); ^{13}C NMR, see Table 1; HRFABMS *m/z* 178.1081 $[M + H]^+$ ($C_7H_{16}O_4N$ requires 178.1079).

1-Deoxynojirimycin (5): $[\alpha]_D$ +42.18 (*c* 1, H_2O); ^{13}C NMR (100 MHz, D_2O) δ 51.5 (C-1), 63.3 (C-5), 64.2 (C-6), 73.7 (C-2), 74.3 (C-4), 81.2 (C-3); FABMS *m/z* 164 $[M + H]^+$.

1-Deoxymannojirimycin (6): $[\alpha]_D$ −41.48 (*c* 0.74, H_2O); ^{13}C NMR (100 MHz, D_2O) δ 51.5 (C-1), 63.4 (C-5), 63.7 (C-6), 71.3 (C-4), 72.1 (C-2), 77.5 (C-3); FABMS *m/z* 164 $[M + H]^+$.

α-Homonojirimycin (7): $[\alpha]_D$ +77.28 (*c* 0.57, H_2O); ^{13}C NMR (100 MHz, D_2O) δ 56.9 (C-2), 59.1 (C-7), 59.7 (C-6), 64.8 (C-1), 74.4 (C-5), 74.9 (C-3), 77.1 (C-4); FABMS *m/z* 194 $[M + H]^+$.

***β*-Homonojirimycin (8):** $[\alpha]_D$ −1.78 (*c* 0.35, H_2O); ^{13}C NMR (100 MHz, D_2O) δ 62.6 (C-2, C-6), 64.3 (C-1, C-7), 74.3 (C-3, C-5), 81.0 (C-4); FABMS *m/z* 194 $[M + H]^+$.

α-Homomannojirimycin (9): $[\alpha]_D$ +4.38 (*c* 0.55, H_2O); ^{13}C NMR (100 MHz, D_2O) δ 58.6 (C-2), 61.4 (C-6), 62.2 (C-7), 63.9 (C-1), 71.4 (C-3), 71.6 (C-5), 74.7 (C-4); FABMS *m/z* 194 $[M + H]^+$.

***β*-Homomannojirimycin (10):** $[\alpha]_D$ +12.08 (*c* 0.27, H_2O); ^{13}C NMR (100 MHz, D_2O) δ 60.8 (C-6), 63.0 (C-2), 63.8 (C-1), 64.2 (C-7), 71.3 (C-3), 71.8 (C-5), 77.8 (C-4); FABMS *m/z* 194 $[M + H]^+$.

7-*O*-*β*-D-Glucopyranosyl-α-homonojirimycin (MDL 25,637) (11): $[\alpha]_D$ +24.68 (*c* 0.70, H_2O);^{13}C NMR (100 MHz, D_2O) δ 57.2 (C-2), 58.2 (C-6), 63.6 (C-6′), 64.6 (C-1), 68.4 (C-7), 72.5 (C-4′), 74.1 (C-5), 74.7 (C-3), 76.0 (C-2′), 77.2 (C-4), 78.5 (C-3′), 78.8 (C-5′), 105.8 (C-1′); FABMS *m*/*z* 356 $[M + H]^+$.

Glycosidase Inhibitory Activities. Rice α-glucosidase (EC 3.2.1.20), *Caldocellum saccharolyticum* *β*-glucosidase (EC 3.2.1.21), bovine liver *β*-galactosidase (EC 3.2.1.23), α,α-trehalase (EC 3.2.1.28), α-L-fucosidase (EC 3.2.1.51), *p*-nitrophenyl glycosides, and disaccharides were purchased from Sigma Chemical Co. Brush border membranes, prepared from the intestines of male Wister rats by the method of Kessler et al.,[8] were used as the source of rat digestive glycosidases. The partially purified lysosomal fraction prepared by the procedures of Tsuji et al.[9] was used as a source of lysosomal α-glucosidase.

The activities of rice α-glucosidase and rat digestive glycosidases were determined using the appropriate disaccharide as substrate at the optimum pH of each enzyme. The released D-glucose was determined colorimetrically using the commercially available Glucose B-test Wako (Wako Pure Chemical Industries). Other glycosidase activities were determined using the appropriate *p*-nitrophenyl glycoside as substrate at the optimum pH of each enzyme. The reaction was stopped by adding 400 mM Na_2CO_3. The released *p*-nitrophenol was measured spectrometrically at 400 nm.

References and Notes

(1) Watson, A. A.; Nash, R. J.; Wormald, M. R.; Harvey, D. J.; Dealler, S.; Lees, E.; Asano. N.; Kizu, H.; Kato, A.; Griffiths, R. C.; Cairns, A. J.; Fleet, G. W. J. *Phytochemistry* **1997**, *46*, 255−259.
(2) Nash, R. J.; Watson, A. A.; Asano, N. In *Alkaloids: Chemical and Biological Perspectives*; Pelletier, S. W., Ed.; Elsevier: Oxford, 1996; Vol. 11, Chapter 5, pp 344−376.
(3) Watanabe, S.; Kato, H.; Nagayama, K.; Abe, H. *Biosci. Biotech. Biochem.* **1995**, *59*, 936−937.
(4) Asano, N.; Oseki, K.; Kizu, H.; Matsui, K. *J. Med. Chem.* **1994**, *37*, 3701−3706.
(5) Asano, N.; Nishida, M.; Kizu, H.; Matsui, K.; Watson, A. A.; Nash, R. J. *J. Nat. Prod.* **1997**, *60*, 98−101.
(6) Barrollier, J. *Naturwissenschften* **1961**, *48*, 554.
(7) Pataki, G. *J. Chromatogr.* **1963**, *12*, 541.
(8) Kessler, M.; Acuto, O.; Strelli, C.; Murer, H.; Semenza, G. A. *Biochim. Biophys. Acta* **1978**, *506*, 136−154.
(9) Tsuji, H.; Hattori, N.; Yamamoto, T.; Kato, K. *J. Biochem.* **1977**, *82*, 619−636.

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