New Polyhydroxylated Pyrrolidine, Piperidine, and Pyrrolizidine Alkaloids from *Scilla sibirica*

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Chromatographic separation of an extract of the bulbs of *Scilla sibirica* resulted in the isolation of five pyrrolidines, two pyrrolidine glycosides, six piperidines, one piperidine glycoside, and eight pyrrolizidines. 2,5-Dideoxy-2,5-imino-*glycero*-D-*manno*-heptitol (homoDMDP, **1**) is a common alkaloid in all plants of the Hyacinthaceae examined to date and was also found in *S. sibirica*. The structures of the new alkaloids were elucidated by spectroscopic methods as 7-deoxy-homoDMDP (**4**), 2,5-dideoxy-2,5-imino-*glycero*-D-*galacto*-heptitol (**5**), the 4-*O*-β-D-mannoside (**6**) and the 4-*O*-β-D-mannobioside (**7**) of 6-deoxy-homoDMDP (**2**), 7-deoxyhomonojirimycin (**12**), 7-deoxyhomomannojirimycin (**13**), and polyhydroxypyrrolizidines, hyacinthacines A_4 (**15**), A_5 (**16**), A_6 (**17**), A_7 (**18**), B_4 (**20**), B_5 (**21**), and B_6 (**22**). HomoDMDP (**1**) is a potent inhibitor of β-glucosidase and β-galactosidase, while 6-deoxy-homoDMDP (**2**) showed significantly less inhibition. However, 7-deoxygenation of **1**, leading to **4**, showed no effect on the inhibitory activity toward both enzymes. Although **2** is not an inhibitor of α-L-fucosidase, the monomannoside of **2** shows inhibitory activity toward α-L-fucosidase. Elongation of the β-mannopyranosyl chain of **6** to give **7** enhanced the inhibitory activity.

Glycosidase inhibitors are receiving considerable attention as potential therapeutic agents, e.g., as inhibitors of tumor metastasis and viral infections or as anti-diabetic agents.[1−3] Furthermore, novel oral treatment of lysosomal storage diseases with specific glycosidase and glycosyltransferase inhibitors is attracting great interest.[4−6] The discovery of diverse glycosidase and glycosyltransferase inhibitors, therefore, is an urgent task. Recently we found that plants in the Hyacinthaceae are a rich source of glycosidase inhibitors of structural diversity, such as polyhydroxylated pyrrolidines, piperidines, pyrrolizidines, and their glycosides.[7−10] 2,5-Dideoxy-2,5-imino-*glycero*-D-*manno*-heptitol (homoDMDP) (**1**), isolated from the Hyacinthaceae plants (*Hyacinthoides nonscripta*,[7,8] *Hyacinthus orientalis*,[9] *Scilla campanulata*,[8] and *Muscari armeniacum*[10]) examined to date, has been found to be a potent inhibitor of bacterial β-glucosidase (IC_{50} = 3.8 μM) and mammalian β-galactosidase (IC_{50} = 4.4 μM).[8] *H. orientalis* coproduces polyhydroxylated piperidine alkaloids such as α-homonojirimycin, its isomers, and its glycosides, while the genera other than *Hyacinthus* coproduce polyhydroxylated pyrrolizidine alkaloids, designated as hyacinthacines, in addition to polyhydroxylated pyrrolidine alkaloids.

Many species in the genera *Muscari* and *Scilla* are very common as garden plants, and the bulbs are commercially available. The GC−MS analysis of the extract of commercially available bulbs of *Scilla sibirica* showed the existence of many kinds of polyhydroxylated alkaloids, including homoDMDP (data not shown). In this paper, we describe the isolation and structural determination of 22 sugar-mimic alkaloids (**1**−**22**) and their glycosidase inhibitory activities. Furthermore, we attempted to determine which functional groups are essential for inhibitory activity and specificity.

Results and Discussion

The bulbs (9 kg) of *S. sibirica* were extracted with 60% aqueous EtOH. The chromatographic separation of the extract using various ion-exchange resins afforded 22 alkaloids (**1**−**22**). The ^{1}H NMR and ^{13}C NMR spectra of alkaloids **1**, **2**, and **3** were in accord with those of homoDMDP, 6-deoxy-homoDMDP, and 2,5-imino-2,5,6-trideoxy-D-*gulo*-heptitol, respectively, which have been isolated previously from *H. orientalis*.[9] Alkaloids **8**, **9**, **10**, **11**, and **14** were determined to be 1-deoxynojirimycin (DNJ), 4-*O*-α-D-glucopyranosyl-DNJ, 1-deoxymannojirimycin (DMJ), 1-deoxyaltronojirimycin (*altro*-DNJ), and β-homofuconojirimycin (β-HFJ), respectively, from ^{1}H NMR and ^{13}C NMR spectral data. We have isolated DNJ and 4-*O*-α-D-glucopyranosyl-DNJ from *Morus alba*[11] and DMJ, *altro*-DNJ, and β-HFJ from *Angylocalyx pynaertii*.[12,13] The optical rotation and ^{1}H NMR and ^{13}C NMR spectral data of alkaloid **19** were in accord with those of polyhydroxylated pyrrolizidine, hyacinthacine B_3 isolated from *M. armeniacum*.[10] The structural determination of the new alkaloids **4**−**7**, **12**, **13**, **15**−**18**, and **20**−**22** is described below.

The structure of homoDMDP (**1**) has been given as 2,5-dideoxy-2,5-imino-*glycero*-D-*manno*-heptitol or its enantiomer from its NMR spectral data.[7] However, the relative configuration at C-6 has not yet been determined since it cannot be determined from the NMR data. In 1999, in the course of the synthesis of a series of five-membered iminosugars, Takebayashi et al. reported the enantiospecific synthesis of (1′*S*,2*R*,3*R*,4*R*,5*R*)-3,4-dihydroxy-2-(1,2-hydroxyethyl)-5-hydroxymethyl)pyrrolidine.[14] The ^{1}H NMR

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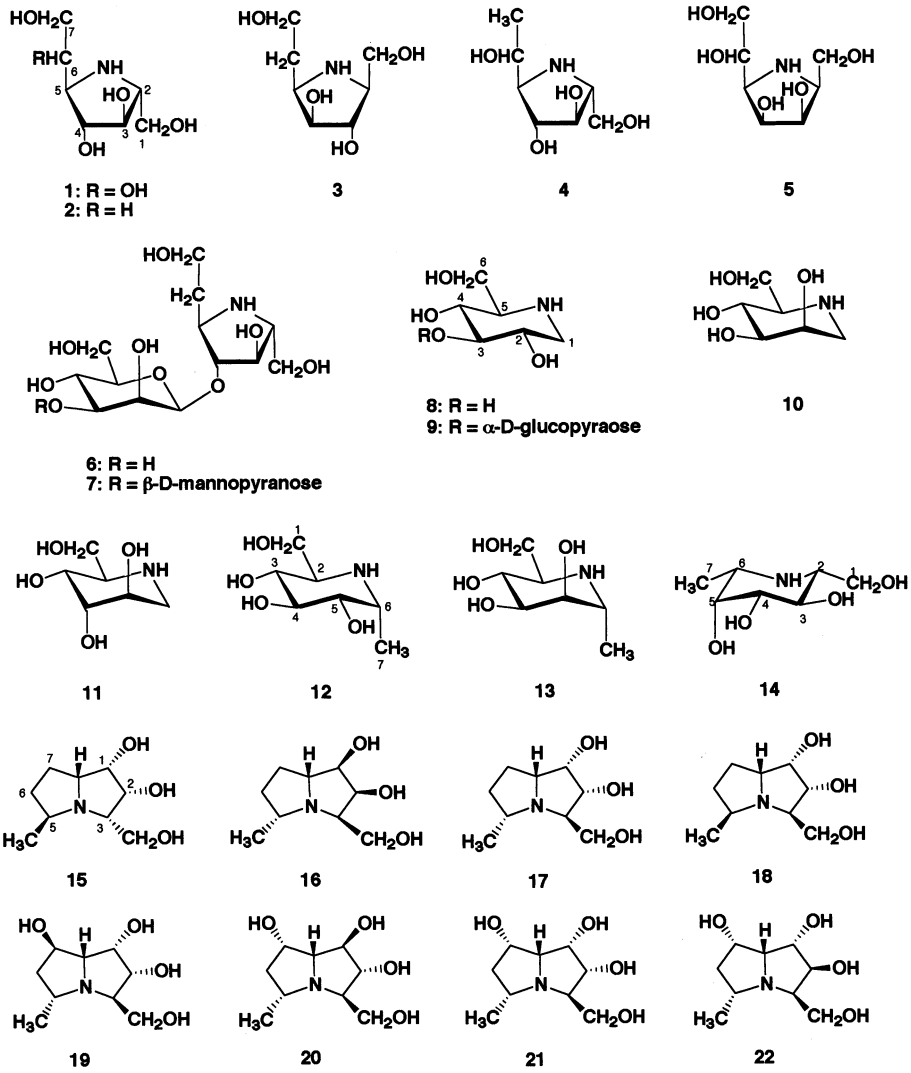
Chart 1



and ^{13}C NMR spectral data of homoDMDP reported in refs 7 and 9 were superimposable with those of the synthetic compound in ref 14, and the optical rotation ($[\alpha]_D$ +28.8° (*c* 3.75, H_2O)) of homoDMDP was also similar to that ($[\alpha]_D$ +25.6° (*c* 0.3, H_2O)) of the synthetic compound. Hence, we determined that these compounds are identical and the structure of homoDMDP is 2,5-dideoxy-2,5-imino-D-*glycero*-D-*manno*-heptitol.

The structure of 6-deoxy-homoDMDP (**2**) isolated from *H. orientalis* has been determined to be 2,5-dideoxy-2,5-imino-D-*manno*-heptitol or its enantiomer.[9] Recently, Fleet et al. have synthesized both enantiomers of 6-deoxy-homoDMDP (the detailed synthesis and their biological activities will be reported elsewhere). From comparison of the optical rotation values (natural product, $[\alpha]_D$ +59.2° (*c* 1.77, H_2O); D-enantiomer, $[\alpha]_D$ +62.2° (*c* 0.19, H_2O); L-enantiomer, $[\alpha]_D$ −89.1° (*c* 0.55, H_2O)), the natural product was determined to be the D-enantiomer.

Alkaloid **4** was determined to have the molecular formula $C_7H_{15}NO_4$ by HRFABMS. The ^{13}C NMR spectral data revealed the presence of a single methyl (δ 21.7), a single methylene (δ 64.6), and five methine (δ 64.7, 68.1, 71.6, 80.9, 81.0) carbon atoms. The methylene group at δ 64.6 (C-1) was attributed to the hydroxymethyl carbon. Decoupling experiments and COSY spectra elucidated a $CH_2OH-CH-CH-CH-CH-CH-CH_3$ moiety, showing a linear sequence from the C-1 hydroxymethyl group to the C-7 methyl. The relatively high-field chemical shifts of the methine carbon at δ 64.7 (C-2) and 68.1 (C-5) indicated that they must be bonded to the heterocyclic ring. The remaining methine carbons at δ 71.6, 80.9, and 81.0 were assigned to C-6, C-3, and C-4 bearing OH groups, respectively. The chemical shifts and $^3J_{H,H}$ couplings of H-2 (δ 3.04, J = 4.4, 6.1, 7.6 Hz), H-3 (δ 3.86, J = 6.8, 7.6 Hz), and H-4 (δ 4.03, J = 6.8, 7.1 Hz) on the five-membered ring were consistent with those (δ 3.05, J = 4.4, 6.1, 7.6 Hz, H-2; δ 3.86, J = 6.8, 7.6 Hz, H-3; δ 4.08, J = 6.8, 7.3 Hz, H-4) of homoDMDP. The relative configurations at the stereogenic centers in **4** were also corroborated by the definite NOE effects between H-2 and H-4 and between H-3 and H-5 (Figure 1a). From these NMR data, the $J_{5,6}$ values (5.8 Hz for **4** and 5.6 Hz for homoDMDP), and the specific rotation value ($[\alpha]_D$ +22.0° (*c* 0.39, H_2O) for **4**; $[\alpha]_D$ +28.0° (*c* 3.75, H_2O) for homoDMDP), the structure of **4** was determined to be 7-deoxy-homoDMDP (2,5-imino-2,5,7-trideoxy-D-*glycero*-D-*manno*-heptitol).

Alkaloid **5** was determined to have the molecular formula $C_7H_{15}NO_5$ by HRFABMS. The ^1H and ^{13}C NMR spectra of **5** were similar to those of homoDMDP (**1**), suggesting that **5** is an isomer of homoDMDP. Irradiation of H-2 enhanced the NOE intensity of H-3 and H-5, and irradiation of H-5 enhanced that of H-2 and H-4 (Figure 1b). These NOE interactions suggest that H-2, H-3, H-4, and H-5 are all in α orientations. The relative configuration at C-6 cannot be

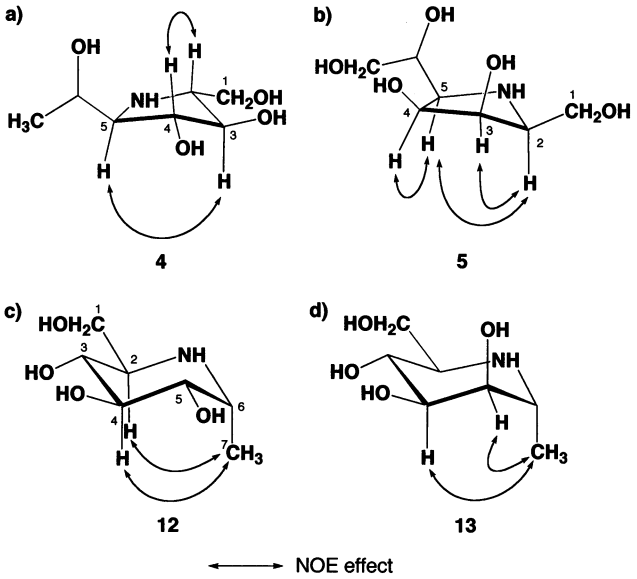


Figure 1. NOE interactions of 7-deoxy-homoDMDP (**4**) (a), 2,5-dideoxy-2,5-imino-DL-*glycero*-D-galactoheptitol (**5**) (b), α-7-deoxyhomonojirimycin (**12**) (c), and α-7-deoxyhomomannojirimycin (**13**) (d).

determined from the NMR data. Hence, alkaloid **5** was determined to be 2,5-dideoxy-2,5-imino-*glycero*-D-*galacto*-heptitol.

Alkaloid **6** was determined to have the molecular formula $C_{13}H_{25}NO_9$ by HRFABMS. The response to the naphthoresorcinol-sulfuric acid reagent and the characteristic carbon (C-1′, δ 103.0) signal in the ^{13}C NMR suggested that **6** was 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 water, the aglycone part was displaced from the resin with 0.5 M NH_4OH, concentrated to dryness, and confirmed as 6-deoxy-homoDMDP (**2**) from the ^{13}C NMR spectral data. The COSY and HMBC spectra of **6** elucidated that the carbon signals of δ 64.1, 70.0, 73.4, 75.7, 79.0, and 103.0 are derived from the sugar part, and these carbon chemical shifts were in good accord with those of β-D-mannopyranoside.[16] The chemical shift (δ 4.77) and characteristic $^3J_{H,H}$ coupling (0.8 Hz) of the anomeric proton also supported that the sugar part was β-D-mannose.[14] The HMBC spectrum showed a correlation peak between the anomeric proton and the aglycone C-4 carbon, defining the linkage site. Thus, the structure of **6** was determined to be 4-*O*-β-D-mannopyranosyl-6-deoxy-homoDMDP.

Alkaloid **7** was determined to have the molecular formula $C_{19}H_{35}NO_{14}$ by HRFABMS. The ^{13}C NMR spectra showed the presence of two anomeric carbon signals at δ 102.9 and 103.1, indicating the presence of two sugar units. The connectivity of the carbon and hydrogen atoms was defined from analysis of decoupling experiments and COSY and HMBC spectra. The ^{13}C NMR chemical shifts (δ 38.3, 59.8, 61.8, 64.4, 65.1, 80.0, 93.7) of the alkaloid part in **7** were quite identical to those of the 6-deoxy-homoDMDP moiety in **6**. The chemical shifts of the remaining 12 carbon signals were in good accord with those of the nonreducing end and central sugar units of β(1→4)-mannotriose.[15] Furthermore, an HMBC correlation between H-4 and C-1′ indicated a 1→4 linkage between the inner sugar and 6-deoxy-homoDMDP. Thus, alkaloid **7** was characterized as 4-*O*-[β-D-mannopyranosyl-(1→4)-β-D-mannopyranosyl]-6-deoxy-homoDMDP.

Alkaloid **12** was determined to have the molecular formula $C_7H_{15}NO_4$ by HRFABMS. The ^{13}C NMR spectral

Table 1. ^{13}C NMR Spectral Data of Polyhydroxylated Pyrrolizidines **15**−**22** at 125 MHz in D_2O[a]

C	15	16	17	18	19	20	21	22
1	72.5	77.3	72.4	73.6	72.2	76.9	74.8	79.86
2	75.7	80.7	75.4	77.1	77.4	81.4	76.9	79.89
3	66.6	62.7	64.3	71.3	63.8	64.8	65.5	63.4
5	62.6	60.0	63.2	68.5	57.1	57.7	58.5	57.0
6	37.6	36.6	35.7	37.0	44.4	42.4	43.9	44.2
7	27.4	24.8	25.2	24.8	71.5	72.8	75.0	75.2
7a	71.3	71.0	72.2	70.7	77.0	73.1	70.1	70.7
8	58.9	62.2	60.6	62.6	65.0	65.2	64.7	62.9
9	21.2	17.5	16.7	20.0	17.5	18.8	17.9	17.8

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

data revealed the presence of a single methyl (δ 13.9), a single methylene (δ 63.9), and five methine (δ 53.6, 56.6, 74.7, 75.3, 76.3) carbon atoms. The methylene group was attributed to the hydroxymethyl carbon (C-1). Decoupling experiments and COSY spectra elucidated a $CH_2OH-CH-CH-CH-CH-CH-CH_3$ moiety, showing a linear sequence from the C-1 hydroxymethyl group to the C-7 methyl. The relatively high-field chemical shifts of the methine carbon at δ 56.6 (C-2) and 53.6 (C-6) indicated that they must be bonded to the heterocyclic ring. The remaining methine carbons at δ 74.7, 75.3, and 76.3 were assigned to C-3, C-5, and C-4 bearing OH groups, respectively. In the ^{1}H NMR spectrum, the large $^3J_{H,H}$ values ($J_{2,3}$ = 9.8, $J_{3,4}$ = $J_{4,5}$ = 9.3 Hz) seen in the signals of H-3 and H-4 indicated an all *trans*-axial orientation of H-2, H-3, H-4, and H-5. An NOE effect between the C-7 methyl proton and H-2 or H-4 indicated an α orientation of the C-7 methyl group (Figure 1c). Thus, alkaloid **12** was determined to be α-7-deoxyhomonojirimycin. 7-Deoxy-homoDMDP (**4**) can be regarded as a ring-contracted form of α-7-deoxyhomonojirimycin.

Alkaloid **13** was determined to have the molecular formula $C_7H_{15}NO_4$ by HRFABMS. The ^{1}H and ^{13}C NMR spectra of **13** were similar to those of α-7-deoxyhomonojirimycin (**12**), suggesting that **13** was an isomer of **12**. The coupling patterns of H-3 (dd, $J_{2,3}$ = $J_{3,4}$ = 9.5 Hz) and H-4 (dd, $J_{3,4}$ = 9.5, $J_{4,5}$ = 3.0 Hz) indicated axial orientations of H-2, H-3, and H-4, and an equatorial orientation of H-5. An NOE effect between the C-7 methyl proton and H-4 indicated an α orientation of the C-7 methyl group (Figure 1d). Thus, alkaloid **13** was determined to be α-7-deoxyhomomannojirimycin.

Alkaloid **15** was determined to have the molecular formula $C_9H_{17}NO_3$ by HRFABMS. The ^{13}C NMR spectral data revealed the presence of a single methyl, three methylene, and five methine carbon atoms, as shown in Table 1. These spectral data suggested that **15** is an isomer of polyhydroxylated pyrrolizidine alkaloid, hyacinthacine A_3, which has been found in the bulbs of *M. armeniacum*.[10] The complete connectivity of the carbon and hydrogen atoms was defined from analysis of decoupling experiments, COSY, and HMBC spectral data. The NOE interactions, obtained from 1D difference NOE experiments, are shown in Figure 2a. The definite NOE between H-1 and H-3 and between H-1 and H-2 indicate that H-1, H-2, and H-3 are on the same side of the ring. Irradiation of H-5 enhanced the NOE signal intensity of H-7α and the C-8 (CH_2OH) proton. These NOE interactions indicate that the hydroxymethyl group and H-5 are on the opposite side. The relative configurations at the stereogenic centers in **15** were also corroborated by the NOE effects between the methyl group and H-6β and between H-6β and H-7a (Figure 2a). Thus, alkaloid **15** was determined to be (1*S*,2*R*,3*S*,5*S*,7a*R*)-1,2-dihydroxy-3-hydroxymethyl-5-methylpyrrolizidine or its enantiomer and designated hyacinthacine A_4.

Table 2. ^{1}H NMR Spectral Data of Hyacinthacines A_4 (**15**), A_5 (**16**), A_6 (**17**), and A_7 (**18**) at 500 MHz in D_2O[a]

position	15	16	17	18
1	4.30 dd (7.3, 4.4)[b]	4.01 dd (4.5, 3.0)	4.23 dd (4.5, 4.0)	4.13 t (4.0)
2	4.35 t (4.4)	4.32 dd (4.5, 3.0)	4.22 dd (9.5, 4.0)	4.19 dd (9.5, 4.0)
3	3.62 ddd (6.5, 5.0, 4.4)	3.39 ddd (8.0, 5.0, 4.5)	3.48 ddd (9.5, 4.0, 3.5)	3.22 ddd (9.5, 4.9, 3.9)
5	3.83 m	3.35 m	3.77 m	3.35 m
6α	2.26 m	2.00 m	1.91 m	2.21 m
6*β*	1.63 m	1.67 m	2.10 m	1.69 m
7α	2.13 m	1.89 m	2.18 m	2.17 m
7*β*	1.91 m	1.72 m	1.91 m	1.98 m
7a	4.05 dt (9.5, 7.3)	3.90 m	4.14 ddd (8.5, 5.5, 4.4)	3.98 ddd (8.6, 7.0, 4.0)
8	4.03 dd (12.5, 6.5)	3.71 dd (11.0, 5.0)	3.84 dd (12.7, 4.0)	3.82 dd (12.5, 4.9)
8′	4.06 dd (12.5. 5.0)	3.81 dd (11.0, 8.0)	3.92 dd (12.7, 3.5)	3.92 dd (12.5, 3.9)
9	1.32 d (6.3)	1.21 d (7.0)	1.35 d (7.0)	1.31 d (6.5)

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

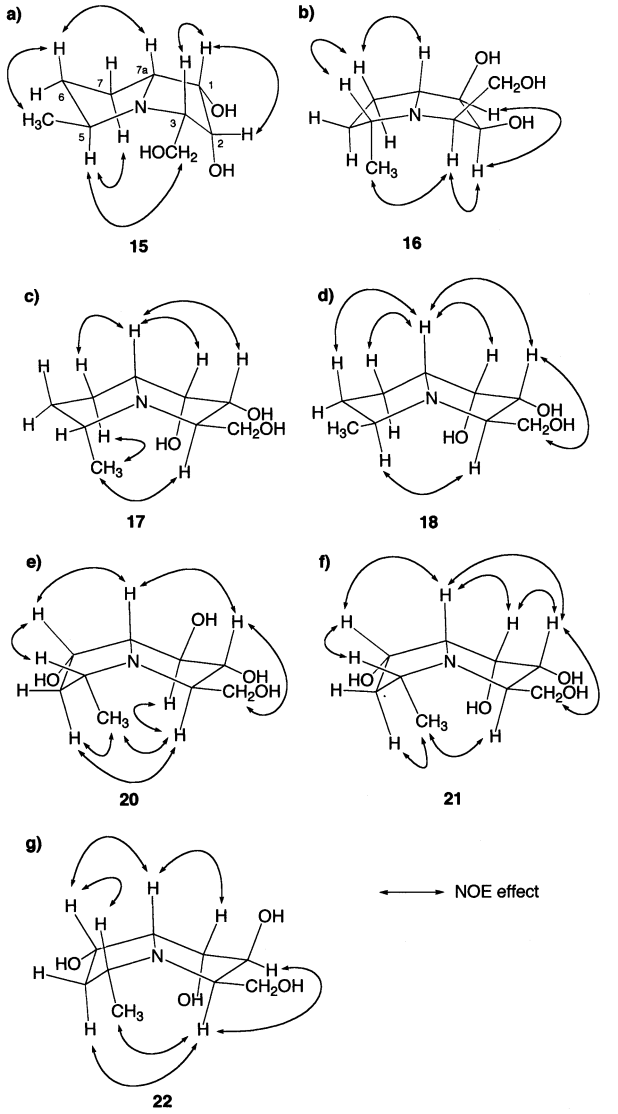


Figure 2. NOE interactions of of hyacinthacines A_4 (**15**) (a), A_5 (**16**) (b), A_6 (**17**) (c), A_7 (**18**) (d), B_4 (**20**) (e), B_5 (**21**) (f), and B_6 (**22**) (g).

Alkaloids **16**−**18** consist of the same molecular formula, $C_9H_{17}NO_3$, as **15**, as determined by HRFABMS. Their ^{1}H and ^{13}C NMR spectral features are closely similar to those of **15**. The signals in the ^{1}H and ^{13}C NMR spectra were assigned from analysis of decoupling experiments, COSY, and HMBC spectral data. The relative configurations at the stereogenic centers in **16**−**18** were corroborated by the NOE effects (Figure 2b−d) and $^3J_{H,H}$ coupling constants. In alkaloid **16**, the NOE effects between H-2 and H-1 or H-3, and between H-3 and the C-9 (CH_3) proton, were observed. The NOE interactions of **17** were observed between H-7a and H-1 or H-2, and between H-3 and the C-9 (CH_3) proton. A large coupling constant (9.5 Hz) observed between H-2 and H-3 indicates that H-2 and H-3 are in a pseudo trans-*axial* position. The difference of the observed NOEs among **17** and **18** is the presence of the NOE between H-3 and the C-9 (CH_3) proton in **17** and between H-3 and the H-5 proton in **18**. A large coupling constant (9.5 Hz) between H-2 and H-3 was observed in **18** as well as **17**. Thus, alkaloids **16**−**18** were determined to be (1*R*,2*S*,3*R*,5*R*,7a*R*)-, (1*S*,2*R*,3*R*,5*R*,7a*R*)-, and (1*S*,2*R*,-3*R*,5*S*,7a*R*)-1,2-dihydroxy-3-hydroxymethyl-5-methylpyrrolizidines or their enantiomers and designated hyacinthacines A_5, A_6, and A_7, respectively.

The HRFABMS of alkaloids **20**−**22** showed the same molecular formula, $C_9H_{17}NO_4$. Their ^{1}H and ^{13}C NMR spectral features were similar to those of hyacinthacine B_3 (**19**). From analysis of decoupling experiments, COSY, and HMBC spectra, alkaloids **20**−**22** were found to be isomers of hyacinthacine B_3. The relative configurations at the stereogenic centers in **20**−**22** were determined from the NOE effects (Figure 2e−g) and $^3J_{H,H}$ coupling constants. In the ^{1}H NMR spectrum of **20**, the large coupling constants ($J_{1,2} = 7.6$ Hz, $J_{2,3} = 8.1$ Hz) seen in H-2 indicate that H-1, H-2, and H-3 are all in pseudo trans-*axial* positions. Irradiation of H-7 in **20** enhanced the NOE signal intensity of H-5 and H-7a, and the NOE between H-7a and H-2 was also observed. These results in **20** indicate that H-2, H-5, H-7, and H-7a are on the same side of the ring and H-1 and H-3 on the opposite side. The difference of the observed NOEs between **20** and **21** is the presence of the NOE between H-1 and H-20 and the absence of such a NOE in **21**. The NOE between H-7a and H-2, which was observed in **20** and **21**, was not observed in **22**. The NOEs between H-3 and the C-9 methyl proton, between H-5 and H-7, and between H-7 and H-7a were observed in all of **20**, **21**, and **22**. Consequently, the structures of alkaloids **20**, **21**, and **22** were determined to be (1*R*,2*R*,3*R*,5*R*,7*S*,7a*R*)-, (1*S*,-2*R*,3*R*,5*R*,7*S*,7a*R*)-, and (1*S*,2*S*,3*R*,5*R*,7*S*,7a*R*)-3-hydroxymethyl-5-methyl-1,2,7-trihydroxypyrrolizidines or their enantiomers and designated hyacinthacines B_4, B_5, and B_6, respectively.

The IC_{50} values of polyhydroxylated pyrrolidines, their glycosides, and piperidines toward various glycosidases are shown in Table 4. We previously reported that 6-deoxy-homoDMDP (**2**) showed potent inhibitory activities toward rice, rat intestinal, and rat liver α-glucosidases.[8] However, we concluded that the previous inhibitory activities of **2** toward α-glucosidases are due to contaminant(s) since pure 6-deoxy-homoDMDP obtained in the present work showed no significant inhibition toward α-glucosidases. HomoDMDP (**1**) is a potent inhibitor of bacterial (*Caldocellum*

sacchrolyticum) β-glucosidase and mammalian β-galactosidase.[8] The present work revealed that deoxygenation at C-6 of **1** to give **2** markedly lowers its inhibition toward both enzymes, whereas deoxygenation at C-7 to give **4** has no effect on β-glycosidases tested. These results indicate that the C-6 OH group in **1** has a very important role in the inhibition of **1** toward β-glycosidases. Although 6-deoxy-homoDMDP (**2**) is not an inhibitor of α-L-fucosidase, the β-monomannoside (**6**) of **2** is inhibitory toward this enzyme; furthermore, the elongation of the β-mannopyranosyl chain of **2** to give **7** enhanced the inhibitory activity.

We have previously reported that α-homonojirimycin (α-HNJ) is a potent and very specific inhibitor of rice and mammalian α-glucosidases.[17] Although deoxygenation at C-7 in α-HNJ to give **12** caused a significant decrease of inhibition toward α-glucosidases, **12** was a moderate inhibitor of α-L-rhamnosidase (Table 2). This result indicates that the OH group of the hydroxymethyl at the pseudoanomeric position plays an important role in the potent inhibition and specificity.

With respect to the glycosidase inhibitory activity of polyhydroxylated pyrrolizidine alkaloids, we have reported that hyacinthacine B_3 (**19**) is a moderate inhibitor of rat intestinal lactase (IC_{50} = 18 μM) and amyloglucosidase (IC_{50} = 51 μM).[10] Hyacinthacines A_5 (**16**) (IC_{50} = 110 μM), B_4 (**20**) (IC_{50} = 89 μM), and B_5 (**21**) (IC_{50} = 110 μM) were moderate inhibitors of amyloglucosidase, and hyacinthacine B_4 was also an inhibitor of bovine epididymis α-L-fucosidase (IC_{50} = 23 μM). Other hyacinthacines obtained in the present work showed no significant inhibitory activity toward any of the enzymes tested.

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 a chlorine-*o*-tolidine reagent was used for detection. Optical rotations were measured with a Jasco DIP-370 digital polarimeter. ^1H NMR (500 MHz) and ^{13}C NMR (125 MHz) spectra were recorded on a JEOL ECP-500 spectrometer. Chemical shifts are expressed in ppm downfield from sodium 3-(trimethylsilyl)-propionate (TSP) in D_2O as an internal standard. The assignment of proton and carbon signals in NMR was determined from extensive homonuclear decoupling experiments, DEPT, $^1H-^{13}C$ COSY, and HMBC spectral data. FABMS were measured using glycerol as a matrix on a JEOL JMS-SX 102A spectrometer.

Plant Materials. The bulbs of *S. sibirica* were purchased in November 1999 from a flower shop. The supplier was Floribon International (Voorhout, Holland). The voucher specimen (no. NA000601) is deposited in the Herbarium of the Medicinal Plants Garden, Hokuriku University.

Extraction and Isolation. The bulbs (9 kg) of *S. sibirica* were homogenized in 60% aqueous EtOH. The filtrate was applied to a column of Amberlite IR-120B (1000 mL, H^+ form). The 0.5 M NH_4OH eluate was concentrated to give a brown syrup (64.6 g), which was applied to Dowex 1-X2 (OH^- form, 500 mL) to remove amino acids and pigments and eluted with 50% aqueous MeOH (2.5 L). The eluate was concentrated to give a yellowish syrup (27 g). This syrup was chromatographed over an Amberlite CG-50 column (3.8 × 95 cm, NH_4^+ form) with H_2O as eluant (fraction size 15 mL). Fractions were divided into four pools: I (fractions 13−33, 15 g), II (fractions 34−40, 960 mg), III (fractions 41−69, 1.6 g), and IV (fractions 70−164, 460 mg). The 0.5 M NH_4OH eluate from the same column was divided into two pools: V (fractions 27−31, 1.2 g) and VI (fractions 32−55, 3.0 g). 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 M NH_4OH as eluant to give alkaloids **6** (13 mg), **7** (16 mg), **8** (790 mg), **9** (3 mg), and **12** (290 mg) from pool I, **1** (845 mg), **4** (41 mg), and **5** (4 mg) from pool II, **2** (110 mg), **10** (330 mg), **13** (4 mg), and **14** (174 mg) from pool III, **3** (18 mg), **11** (10 mg), and **20** (51 mg) from pool IV, **18** (5 mg), **19** (23 mg), **21** (3 mg), and **22** (11 mg) from pool V, and **15** (91 mg), **16** (12 mg), and **17** (6 mg) from pool VI.

2,5-Dideoxy-2,5-imino-D-*glycero*-D-*manno*-heptitol (homoDMDP) (1): colorless syrup; $[\alpha]_D$ +28.8° (*c* 3.75, H_2O); ^1H NMR, see ref 9; ^{13}C NMR (500 MHz, D_2O) δ 64.3 (C-5), 64.5 (C-1), 64.7 (C-2), 66.2 (C-7), 75.7 (C-6), 80.6 (C-4), 80.8 (C-3); FABMS *m/z* 194 $[M + H]^+$.

2,5-Imino-2,5,6-trideoxy-D-*manno*-heptitol (6-deoxy-homoDMDP) (2): colorless solid; $[\alpha]_D$ +59.2° (*c* 1.77, H_2O); ^1H NMR, see ref 9; ^{13}C NMR (500 MHz, D_2O) δ 38.3 (C-6), 60.3 (C-5), 62.0 (C-7), 64.4 (C-2), 64.9 (C-1), 80.5 (C-3), 84.3 (C-4); FABMS *m/z* 178 $[M + H]^+$.

2,5-Imino-2,5,7-trideoxy-D-*glycero*-D-*manno*-heptitol (7-deoxy-homoDMDP) (4): colorless solid; $[\alpha]_D$ +22.0° (*c* 0.39, H_2O); ^1H NMR (500 MHz, D_2O) δ 1.24 (3H, d, *J* = 6.3 Hz, CH_3), 2.91 (1H, dd, *J* = 5.8, 7.1 Hz, H-5), 3.04 (1H, ddd, *J* = 4.4, 6.1, 7.6 Hz, H-2), 3.64 (1H, dd, *J* = 6.1, 11.7 Hz, H-1a), 3.72 (1H, dd, *J* = 4.4, 11.7 Hz, H-1b), 3.86 (1H, dd, *J* = 6.8, 7.6 Hz, H-3), 3.89 (1H. dq, *J* = 5.8, 6.3 Hz, H-6), 4.03 (1H, dd, *J* = 6.8, 7.1 Hz, H-4); ^{13}C NMR (125 MHz, D_2O) δ 21.7 (C-7), 64.6 (C-1), 64.7 (C-2), 68.1 (C-5), 71.6 (C-6), 80.9 (C-3), 81.0 (C-4); HRFABMS *m/z* 178.1075 $[M + H]^+$ ($C_7H_{16}NO_4$ requires 178.1079).

2,5-Dideoxy-2,5-imino-*glycero*-D-*galacto*-heptitol (5): colorless syrup; $[\alpha]_D$ +33.6° (*c* 0.19, H_2O); ^1H NMR (500 MHz, D_2O) δ 3.13 (1H, ddd, *J* = 4.6, 5.0, 5.9 Hz, H-2), 3.29 (1H, dd, *J* = 5.0, 6.9 Hz, H-5), 3.62 (1H, dd, *J* = 6.9, 11.9 Hz, H-7a), 3.75 (1H, dd, *J* = 5.9, 11.4 Hz, H-1a), 3.75 (1H, dd, *J* = 3.7, 11.9 Hz, H-7b), 3.79 (1H, dd, *J* = 5.0, 11.4 Hz, H-1b), 3.94 (1H. dd, *J* = 2.3, 4.6 Hz, H-3), 3.96 (1H, ddd, *J* = 3.7, 6.9, 6.9 Hz, H-6), 4.11 (1H, dd, *J* = 2.3, 5.0 Hz, H-4); ^{13}C NMR (125 MHz, D_2O) δ 64.4 (C-5), 64.5 (C-1), 66.3 (C-7), 68.1 (C-2), 72.9 (C-6), 79.9 (C-4), 81.8 (C-3); HRFABMS *m/z* 194.1029 $[M + H]^+$ ($C_7H_{16}NO_5$ requires 194.1028).

4-*O*-β-D-Mannopyranosyl-6-deoxy-homoDMDP (6): colorless powder; $[\alpha]_D$ +2.0° (*c* 0.32, H_2O); ^1H NMR (500 MHz, D_2O) δ 1.80 (1H, m, H-6a), 1.92 (1H, m, H-6b), 3.12 (1H, ddd, *J* = 4.7, 5.6, 6.3 Hz, H-2), 3.24 (1H, m, H-5), 3.44 (1H, ddd, *J* = 2.4, 7.8, 9.6 Hz, H-5′), 3.55 (1H, dd, *J* = 9.6, 9.6 Hz, H-4′), 3.66 (1H, dd, *J* = 3.2, 9.6 Hz, H-3′), 3.68 (1H, dd, *J* = 6.3, 11.8 Hz, H-1a), 3.66−3.72 (2H, H-7a, H-7b), 3.69 (1H, dd, *J* = 7.8, 11.9 Hz, H-6′a), 3.74 (1H, dd, *J* = 4.7, 11.8 Hz, H-1b), 3.89 (1H, dd, *J* = 5.6, 7.1 Hz, H-4), 3.98 (1H, dd, *J* = 2.4, 11.9 Hz, H-6′b), 4.04 (1H, dd, *J* = 5.6, 6.9 Hz, H-3), 4.05 (1H, dd, *J* = 0.8, 3.2 Hz, H-2′), 4.77 (1H, d, *J* = 0.8 Hz, H-1′); ^{13}C NMR (125 MHz, D_2O) δ 38.3 (C-6), 59.7 (C-5), 61.8 (C-7), 64.1 (C-6′), 64.4 (C-1), 65.0 (C-2), 70.0 (C-4′), 73.4 (C-2′), 75.7 (C-3′), 79.0 (C-5′), 80.0 (C-3), 93.9 (C-4), 103.0 (C-1′); HRFABMS *m/z* 340.1604 $[M + H]^+$ ($C_{13}H_{26}NO_9$ requires 340.1608).

4-*O*-[β-D-Mannopyranosyl-(1→4)-β-D-mannopyranosyl]-6-deoxy-homoDMDP (7): colorless powder; $[\alpha]_D$ +10.4° (*c* 0.69, H_2O); ^1H NMR (500 MHz, D_2O) δ 1.80 (1H, m, H-6a), 1.92 (1H, m, H-6b), 3.14 (1H, ddd, *J* = 4.7, 6.1, 6.7 Hz, H-2), 3.26 (1H, m, H-5), 3.45 (1H, ddd, *J* = 2.3, 6.9, 9.7 Hz, H-5″), 3.57 (1H, m, H-5′), 3.58 (1H, dd, *J* = 9.7, 9.7 Hz, H-4″), 3.66 (1H, dd, *J* = 3.2, 9.7 Hz, H-3″), 3.67 (1H, dd, *J* = 6.3, 12.0 Hz, H-1a), 3.68−3.75 (2H, H-7a, H-7b), 3.73 (1H, dd, *J* = 7.8, 11.9 Hz, H-1b), 3.74 (1H, dd, *J* = 6.9, 11.9 Hz, H-6′a), 3.96 (1H, dd, *J* = 6.9, 11.9 Hz, H-6″a), 3.80 (1H, dd, *J* = 9.3, 9.3 Hz, H-4′), 3.82 (1H, dd, *J* = 2.8, 9.3 Hz, H-3′), 3.91 (1H, dd, *J* = 5.6, 6.9 Hz, H-4), 3.95 (1H, dd, *J* = 2.3, 11.9 Hz, H-6′b), 3.96 (1H, dd, *J* = 2.3, 11.9 Hz, H-6″b), 4.06 (1H, dd, *J* = 5.6, 6.7 Hz, H-3), 4.07 (1H, dd, *J* = 0.9, 3.2 Hz, H-2″), 4.12 (1H, dd, *J* = 0.9, 2.8 Hz, H-2′), 4.73 (1H, d, *J* = 0.9 Hz, H-1″), 4.8 (1H, H-1′, overlapped with the HDO signal); ^{13}C NMR (125 MHz, D_2O) δ 38.3 (C-6), 59.8 (C-5), 61.8 (C-7), 63.7 (C-6′), 63.9 (C-6″), 64.4 (C-1), 65.1 (C-2), 69.6 (C-4″), 72.8 (C-2′), 74.4 (C-3′), 75.6 (C-3″), 77.7 (C-5′), 79.3 (C-5″), 79.98 (C-3), 80.00 (C-4′), 93.7 (C-4), 102.9 (C-1′), 103.1 (C-1″); HRFABMS *m/z* 502.2143 $[M + H]^+$ ($C_{19}H_{36}NO_{14}$ requires 502.2136).

Table 3. ^{1}H NMR Spectral Data of Hyacinthacines B_3 (**19**), B_4 (**20**), B_5 (**21**), and B_6 (**22**) at 500 MHz in D_2O[a]

position	19	20	21	22
1	4.03 dd (4.6, 4.2)[b]	4.18 t (7.6)	4.37 dd (4.4, 4.2)	4.28 dd (4.6, 3.0)
2	3.91 dd (7.3, 4.2)	3.98 dd (8.1, 7.6)	4.08 dd (8.0, 4.2)	4.32 dd (5.0, 3.0)
3	3.08 ddd (7.3, 4.9, 4.4)	3.19 dt (8.1, 4.9)	3.48 ddd (5.1, 4.6, 4.2)	3.58 ddd (9.2, 5.0, 4.4)
5	3.50 m	3.34 m	3.44 m	3.26 m
6α	1.82 m	1.72 ddd (13.2, 7.8, 6.3)	1.86 ddd (12.5, 10.0, 8.0)	1.74 ddd (12.4, 9.9, 7.8)
6*β*	1.82 m	2.17 dt (13.2, 5.9)	2.22 ddd (12.5, 6.4, 6.0)	2.18 ddd (12.4, 6.7, 5.6)
7	4.52 ddd (6.8, 4.6, 3.9)	4.45 ddd (6.6, 6.3, 5.9)	4.55 ddd (8.0, 7.6, 6.4)	4.55 ddd (7.8, 7.3, 6.7)
7a	3.30 t (4.6)	3.39 dd (7.6, 6.6)	3.66 dd (7.6, 4.4)	3.64 dd (7.3, 4.6)
8	3.53 dd (11.0, 4.4)	3.70 d (4.9)	3.70 dd (12.0, 5.1)	3.68 dd (11.0, 4.4)
8′	3.57 dd (11.0, 4.9)	3.70 d (4.9)	3.73 dd (12.0, 4.6)	3.77 dd (11.0, 9.2)
9	1.17 d (6.8)	1.27 d (7.1)	1.32 d (7.0)	1.30 d (7.0)

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

Table 4. Concentration of Polyhydroxylated Pyrrolidines and Piperidines Giving 50% Inhibition of Various Glycosidases

	IC_{50} (*μ*M)								
enzyme	**1**	**2**	**3**	**4**	**6**	**7**	**8**	**11**	**12**
α-glucosidase									
rice	95	–	–	9.2	–	–	0.05	–	8.0
yeast	220	250	–	85	–	–	330	–	–
rat intestinal maltase	400	–	–	nd[b]	–	–	0.36	–	18
rat liver lysosome	–[a]	–	–	–	–	–	0.40	–	19
β-glucosidase									
Caldocellum saccharolyticum	3.2	350	–	6.7	nd	nd	100	–	–
β-mannosidase									
rat epididymis	53	320	–	35	160	180	–	–	–
β-galactosidase									
bovine liver	4.4	88	–	3.4	nd	nd	–	700	260
α-L-fucosidase									
bovine epididymis	–	–	840	–	78	13	–	–	–
α-L-rhamnosidase									
Penicillium decumbens	–	94	–	–	nd	nd	850	1.8	54

[a] – indicates no inhibition (less than 50% inhibition at 1000 *μ*M). [b] Not determined.

α-7-Deoxyhomonojirimycin (12): colorless powder; $[\alpha]_D$ +78.9° (*c* 0.46, H_2O); ^{1}H NMR (500 MHz, D_2O) *δ* 1.17 (3H, d, *J* = 7.1, CH_3), 2.88 (1H, ddd, *J* = 2.9, 5.6, 9.8 Hz, H-2), 3.27 (1H, dd, *J* = 9.3, 9.8 Hz, H-3), 3.37 (1H, dq, *J* = 5.5, 7.1 Hz, H-6), 3.59 (1H, dd, *J* = 9.3, 9.3 Hz, H-4), 3.62 (1H, dd, *J* = 5.5, 9.3 Hz, H-5), 3.69 (1H, dd, *J* = 5.6, 11.7 Hz, H-1a), 3.81 (1H, dd, *J* = 2.9, 11.7 Hz, H-1b); ^{13}C NMR (125 MHz, D_2O) *δ* 13.9 (C-7), 53.6 (C-6), 56.6 (C-2), 63.9 (C-1), 74.7 (C-3), 75.3 (C-5), 76.3 (C-4); HRFABMS *m*/*z* 178.1081 $[M + H]^+$ ($C_7H_{16}NO_4$ requires 178.1079).

α-7-Deoxyhomomannojirimycin (13): colorless syrup; $[\alpha]_D$ −11.1° (*c* 0.44, H_2O); ^{1}H NMR (500 MHz, D_2O) *δ* 1.19 (3H, d, *J* = 7.3, CH_3), 2.76 (1H, ddd, *J* = 2.9, 4.4, 9.5 Hz, H-2), 3.21 (1H, dq, *J* = 3.0, 7.3 Hz, H-6), 3.67 (1H, dd, *J* = 9.5, 9.5 Hz, H-3), 3.73 (1H, dd, *J* = 3.0, 11.7 Hz, H-1a), 3.79 (1H, dd, *J* = 3.0, 9.5 Hz, H-4), 3.85 (1H, dd, *J* = 4.4, 11.7 Hz, H-1b), 3.86 (1H, dd, *J* = 3.0, 3.0 Hz, H-5); ^{13}C NMR (125 MHz, D_2O) *δ* 17.1 (C-7), 55.2 (C-6), 57.4 (C-2), 63.4 (C-1), 71.4 (C-3), 74.0 (C-4), 75.8 (C-5); HRFABMS *m*/*z* 178.1075 $[M + H]^+$ ($C_7H_{16}NO_4$ requires 178.1079).

Hyacinthacine A_4 ((1*S,2*R**,3*S**,5*S**,7a**R*)-1,2-dihydroxy-3-hydroxymethyl-5-methylpyrrolizidine) (15):** colorless syrup; $[\alpha]_D$ −49.5° (*c* 0.48, H_2O); ^{1}H NMR, see Table 2; ^{13}C NMR, see Table 1; HRFABMS *m*/*z* 188.1286 $[M + H]^+$ ($C_9H_{18}NO_3$ requires 188.1287).

Hyacinthacine A_5 ((1*R,2*S**,3*R**,5*R**,7a**R*)-1,2-dihydroxy-3-hydroxymethyl-5-methylpyrrolizidine) (16):** colorless powder; $[\alpha]_D$ −39.4° (*c* 0.30, H_2O); ^{1}H NMR, see Table 2; ^{13}C NMR, see Table 1; HRFABMS *m*/*z* 188.1286 $[M + H]^+$ ($C_9H_{18}NO_3$ requires 188.1287).

Hyacinthacine A_6 ((1*S,2*R**,3*R**,5*R**,7a**R*)-1,2-dihydroxy-3-hydroxymethyl-5-methylpyrrolizidine) (17):** colorless syrup; $[\alpha]_D$ +16.3° (*c* 0.22, H_2O); ^{1}H NMR, see Table 2; ^{13}C NMR, see Table 1; HRFABMS *m*/*z* 188.1287 $[M + H]^+$ ($C_9H_{18}NO_3$ requires 188.1287).

Hyacinthacine A_7 ((1*S,2*R**,3*R**,5*S**,7a**R*)-1,2-dihydroxy-3-hydroxymethyl-5-methylpyrrolizidine) (18):** colorless syrup; $[\alpha]_D$ −51.8° (*c* 0.45, H_2O); ^{1}H NMR, see Table 2; ^{13}C NMR, see Table 1; HRFABMS *m*/*z* 188.1285 $[M + H]^+$ ($C_9H_{18}NO_3$ requires 188.1287).

Hyacinthacine B_4 ((1*R,2*R**,3*R**,5*R**,7*S**,7a**R*)-3-hydroxymethyl-5-methyl-1,2,7-trihydroxypyrrolizidine) (20):** colorless powder; $[\alpha]_D$ −6.7° (*c* 1.19, H_2O); ^{1}H NMR, see Table 3; ^{13}C NMR, see Table 1; HRFABMS *m*/*z* 204.1236 $[M + H]^+$ ($C_9H_{18}NO_4$ requires 204.1236).

Hyacinthacine B_5 ((1*S,2*R**,3*R**,5*R**,7*S**,7a**R*)-3-hydroxymethyl-5-methyl-1,2,7-trihydroxypyrrolizidine) (21):** colorless syrup; $[\alpha]_D$ −25.4° (*c* 0.26, H_2O); ^{1}H NMR, see Table 3; ^{13}C NMR, see Table 1; HRFABMS *m*/*z* 204.1234 $[M + H]^+$ ($C_9H_{18}NO_4$ requires 204.1236).

Hyacinthacine B_6 ((1*S,2*S**,3*R**,5*R**,7*S**,7a**R*)-3-hydroxymethyl-5-methyl-1,2,7-trihydroxypyrrolizidine) (22):** colorless syrup; $[\alpha]_D$ −61.2° (*c* 0.97, H_2O); ^{1}H NMR, see Table 3; ^{13}C NMR, see Table 1; HRFABMS *m*/*z* 204.1239 $[M + H]^+$ ($C_9H_{18}NO_4$ requires 204.1236).

Glycosidase Inhibitory Activities. The enzymes α-glucosidases (from rice, assayed at pH 5.0; from yeast, assayed at pH 6.8), *β*-glucosidase (from *Caldocellum saccharolyticum*, assayed at pH 5.0), *β*-galactosidase (from bovine liver, assayed at pH 6.8), α-L-fucosidase (from bovine epididymis, assayed at pH 5.5), α-L-rhamnosidase (from *Penicillium decumbens*, assayed at pH 4.5), *p*-nitrophenyl glycosides, and disaccharides were purchased from Sigma Chemical Co. The rat epididymal fluid was purified from epididymis according to the method of Skudlarek et al.[18] and assayed at pH 5.2 for *β*-mannosidase using *p*-nitrophenyl-*β*-D-mannoside. Brush border membranes prepared from rat small intestine according to the method of Kessler et al.[19] were assayed at pH 5.8 for rat intestinal maltase using maltose. The partially purified lysosomal fraction prepared by the procedures of Tsuji et al.[20] was assayed at pH 4.0 for lysosomal α-glucosidase using *p*-nitrophenyl-α-D-glucoside. The activity of rice α-glucosidase and rat intestinal maltase was determined using maltose as substrate at the optimum pH of each enzyme. Substrates, suitably diluted enzyme solutions, and inhibitors were incubated together for 30 min at 37 °C, but the incubation time for rice α-glucosidase

was 10 min. The released D-glucose was determined colorimetrically using the Glucose B-test Wako (Wako Pure Chemical Ind.). Other glycosidase activities were determined using an appropriate *p*-nitrophenyl glycoside as a substrate at the optimum pH of each enzyme. The reaction was stopped by adding 400 mM Na_2CO_3. The released *p*-nitrophenol was measured spectrometrically at 400 nm.

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