Glycosidase-Inhibiting Pyrrolidines and Pyrrolizidines with a Long Side Chain in *Scilla peruviana*

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2,5-Dideoxy-2,5-imino-D-*glycero*-D-*manno*-heptitol (homoDMDP) is widely distributed in Hyacinthaceae plants and can also be regarded as the α-1-*C*-(1,2-dihydroxyethyl) derivative of 1,4-dideoxy-1,4-imino-D-arabinitol (D-AB1). In a search for glycosidase inhibitors in this family of plants, we isolated three new D-AB1 derivatives bearing the 2-hydroxypropyl (**1**), 1,2-dihydroxypropyl (**2**), and 1,5,7,12,13-pentahydroxytridecyl (**3**) side chains at the C-1α position, respectively, from the bulbs of *Scilla peruviana*. Alkaloid **3** was a powerful inhibitor of bacterial *β*-glucosidase (IC_{50} = 80 nM) and bovine liver *β*-galactosidase (IC_{50} = 90 nM). This plant coproduced four new pyrrolizidine alkaloids, α-5-*C*-(3-hydroxybutyl)-7-*epi*-australine (**4**), α-5-*C*-(3-hydroxybutyl)hyacinthacine A_1 (**5**), α-5-*C*-(1,3-dihydroxybutyl)hyacinthacine A_1 (**6**), and α-5-*C*-(1,3,4-trihydroxybutyl)hyacinthacine A_1 (**7**). Alkaloids **4** and **6** were potent inhibitors of yeast α-glucosidase, with IC_{50} values of 6.6 and 6.3 *μ*M, respectively, and alkaloid **6** was also a potent inhibitor of bacterial *β*-glucosidase with an IC_{50} value of 5.1 *μ*M.

The alteration of glycosidase activity by inhibitors in vivo is of great interest because of the involvement of glycosidases in a wide range of anabolic and catabolic process, such as digestion, lysosomal catabolism of glycoconjugates, and biosynthesis, the endoplasmic reticulum (ER) quality control, and ER-associated degradation of glycoproteins. Thus, glycosidase inhibitors could have beneficial effects as therapeutic agents such as antidiabetics, antiobesities, antivirals, and therapeutic agents for some genetic disorders.[1−3] Recently, we found that plants in the Hyacinthaceae are a rich source of glycosidase inhibitors of structural diversity, such as polyhydroxylated pyrrolidines, piperidines, and pyrrolizidines.[4−8] Polyhydroxylated pyrrolidine alkaloids, (2*R*,5*R*)-bis(dihydroxymethyl)-(3*R*,4*R*)-dihydroxypyrrolidine (DMDP) and 1,4-dideoxy-1,4-imino-D-arabinitol (D-AB1), would appear to be fairly widespread secondary metabolites, as they have been reported from plant species of quite unrelated families.[1,2] On the other hand, the distribution of homoDMDP appears to be restricted to the Hyacinthaceae family, because it is found in all plants of Hyacinthaceae examined to date and has never been found in other families.[2,8] The structure of homoDMDP can be regarded as α-1-*C*-(1,2-dihydroxyethyl)-D-AB1, and its absolute configuration has been determined to be (1′*S*,2*R*,3*R*,4*R*,5*R*)-3,4-dihydroxy-2-(1,2-hydroxyethyl)-5-(hydroxymethyl)pyrrolidine by its enantiospecific synthesis.[8,9] D-AB1 derivatives bearing a longer side chain than that of homoDMDP have been isolated to date: α-1-*C*-(3,6-dihydroxyheptyl)-D-AB1 from bluebells (*Hyacinthoides non-scripta*)[5] and α-1-*C*-(1-hydroxypentyl)-D-AB1 from *Adenophora triphylla* var. *japonica* (Campanulaceae).[10]

A variety of D-AB1 and 1,4-dideoxy-1,4-imino-D-lyxitol (D-Lyx) derivatives with a very long side chain at the C-1α position have been isolated from *Broussonetia kajinoki* (Moraceae) and were designated as broussonetines and broussonetinines.[11−19] These compounds have C_{13} side chains at the C-1α position of D-AB1 or D-Lyx. For instance, broussonetine S is the α-1-*C*-(1,10,13-trihydroxytridecyl) derivative of D-AB1. This plant also coproduces a pyrrolizidine alkaloid with the C_{10} side chain at the C-5α position, which was designated as broussonetine N.[16] Broussonetine N can also be regarded as the α-5-*C*-(1,19-dihydroxy-6-oxodecyl) derivative of hyacinthacine A_2 isolated from *Muscari armeniacum*.[7] In this paper, we describe the isolation and structural determination of three new D-AB1 derivatives bearing 2-hydroxypropyl, 1,2-dihydroxypropyl, and 1,5,7,-12,13-pentahydroxytridecyl side chains, respectively, and four new hyacinthacines A_1 and 7-*epi*-australine derivatives with the hydroxylated butyl side chain at the C-5α position from *Scilla peruviana* (Hyacinthaceae), which belongs to a family that is quite unrelated to *B. kajinoki* (Moraceae). Furthermore, we report their inhibitory activities toward glycosidases.

Results and Discussion

The bulbs (5 kg) of *S. peruviana* were extracted with 50% aqueous EtOH, and the extract was subjected to a variety of ion-exchange resin chromatographic steps to give alkaloids **1** (70 mg), **2** (86 mg), **3** (160 mg), **4** (70 mg), **5** (142 mg), **6** (832 mg), and **7** (72 mg).

Alkaloid **1** was determined to have the molecular formula $C_8H_{17}NO_4$ by HRFABMS. The ^{13}C NMR spectroscopic data showed the presence of a single methyl (*δ* 24.7), two methylene (*δ* 44.4, 64.6), and five methine (*δ* 61.0, 64.6, 68.8, 80.3, 84.3) carbon atoms. The relatively high-field chemical shifts of the methine carbons at *δ* 61.0 (C-1) and 64.6 (C-4) indicated that they must be bonded to the heterocyclic ring. The methylene group at *δ* 64.6 was attributed to the hydroxymethyl carbon (C-5). The methine carbons at *δ* 80.3 and 84.3 were assigned to C-3 and C-2, respectively, from decoupling experiments and COSY spectra, and the HMBC spectra elucidated the presence of a 2-hydroxypropyl side chain (*δ* 24.7, 44.4, 68.8) at C-1. The $^3J_{H,H}$ couplings ($J_{1,2}$ = 7.8, $J_{2,3}$ = 6.9, $J_{3,4}$ = 7.3, $J_{4,5a}$ = 4.6, $J_{4,5b}$ = 6.4 Hz) on the five-membered ring were almost consistent with those ($J_{1,2}$ = 7.3, $J_{2,3}$ = 6.8, $J_{3,4}$ = 7.6, $J_{4,5a}$ = 4.4, $J_{4,5b}$ = 6.1 Hz) of homoDMDP.[4,8] The strong NOE correlations between H-1 and H-3 and between H-2 and H-4 indicate that H-1 and H-3 are on the same side of the ring and H-2 and H-4 are on the opposite side. Hence, the structure of **1** was determined to be α-1-*C*-(2-hydroxypropyl)-D-AB1.

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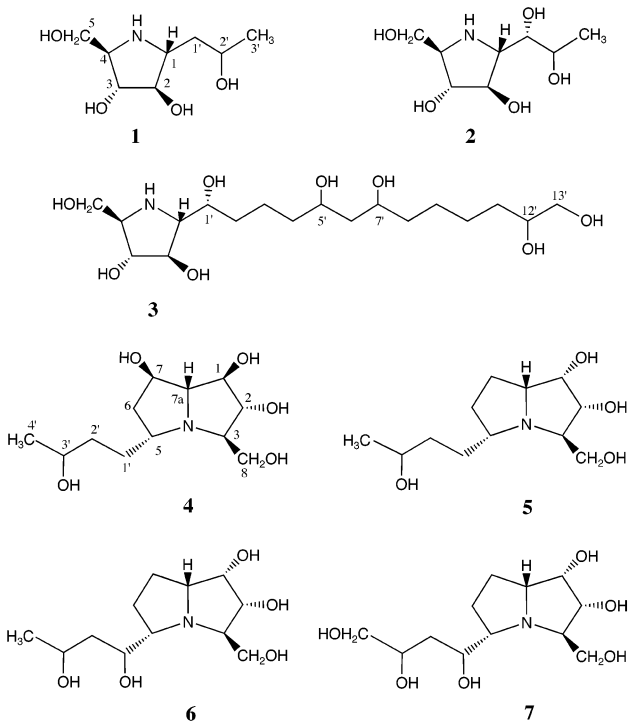

Alkaloid **2** was determined to have the molecular formula $C_8H_{17}NO_5$ by HRFABMS. The ^{13}C NMR spectroscopic data showed the presence of a single methyl (δ 20.3), a single methylene (δ 64.4), and six methine (δ 64.5, 64.9, 71.5, 81.0, 81.0) carbon atoms. Analysis of decoupling experiments and COSY and HMBC spectra elucidated that alkaloid **2** was the D-AB1 or its isomer derivative bearing a 1,2-dihydroxypropane side chain at the C-1 position. The $^3J_{H,H}$ couplings ($J_{1,2} = 7.3$, $J_{2,3} = 7.3$, $J_{3,4} = 6.4$, $J_{4,5a} = 4.6$, $J_{4,5b} = 6.0$ Hz) on the five-membered ring were similar to those of homoDMDP. The configurations at the stereogenic centers on the five-membered ring were also corroborated by the strong NOE correlations between H-1 and H-3 and between H-2 and H-4. Hence, the structure of **2** was determined to be α-1-*C*-(1,2-dihydroxypropyl)-D-AB1. Furthermore, the $J_{1,1'}$ values (6.0 Hz for **2** and 5.6 Hz for homoDMDP) imply the *S* configuration of C-1′.

Alkaloid **3** was determined to have the molecular formula $C_{18}H_{37}NO_8$ by HRFABMS. The ^{13}C NMR spectroscopic data showed the presence of 10 methylene (δ 23.8, 27.3, 27.5, 35.0, 35.8, 38.8, 38.9, 45.7, 64.5, 68.3) and eight methine (δ 64.6, 66.9, 72.6, 72.7, 74.6, 75.2, 80.6, 80.9) carbon atoms. The decoupling experiments and COSY spectral data implied the presence of the same five-membered ring as alkaloids **1** and **2**. The chemical shifts (δ 66.9 (C-1), 80.6 (C-2), 80.9 (C-3), 64.6 (C-4), and 64.5 (C-5)) of the ^{13}C NMR spectrum and $^3J_{H,H}$ couplings ($J_{1,2} = 7.3$, $J_{2,3} = 6.8$, $J_{3,4} = 7.7$, $J_{4,5a} = 4.4$, $J_{4,5b} = 6.3$ Hz) on the five-membered ring were quite similar to those of homoDMDP. These NMR data indicate that alkaloid **3** is the D-AB1 derivative bearing a pentahydroxytridecyl side chain at the C-1α position. From the ^{13}C NMR chemical shifts and HMBC correlations (Figure 1), the binding sites of the OH groups in the side chain were determined to be C-1, C-5, C-7, C-12, and C-13. Hence, the structure of **3** was determined to be α-1-*C*-(1,5,7,12,13-pentahydroxytridecyl)-D-AB1. In addition, the $J_{1,1'}$ values (5.2 Hz for **3** and 5.6 Hz for homoDMDP) imply the *R* configuration of C-1′. Recently, broussonetine S was isolated from the branches of *Broussonetia kazinoki*, and its absolute stereostructure was determined to be (2*R*,3*R*,4*R*,5*R*)-2-hydroxymethyl-3,4-dihydroxy-5-[(1*R*,10*S*)-1,10,13-trihydroxytridecyl]pyrrolidine.[18] This plant coproduces a series of pyrrolidine alkaloids bearing a variety of the C_{13} side chains, designated broussonetines and broussonetinines.[11−19] Interestingly, broussonetine S can be regarded as α-1-*C*-(1,10,13-trihydroxytridecyl)-D-AB1. Although *B. kazinoki* and *S. peruviana* belong to quite unrelated families, Moraceae and Hyacinthaceae, respectively, broussonetine S and alkaloid **3** could be synthesized from a common precursor in the biosynthetic pathway.

Alkaloid **4** was determined to have the molecular formula $C_{12}H_{23}NO_5$ by HRFABMS. The ^{13}C NMR spectroscopic data showed the presence of a single methyl (δ 24.7), four methylene (δ 28.6, 39.0, 40.0, 65.5), and seven methine (δ 64.4, 65.5, 70.5, 76.7, 80.0, 80.6, 80.8) carbon atoms. The connectivity of the carbon and hydrogen atoms was defined from COSY and HMBC spectroscopic data. The relatively high-field chemical shifts of the methines at δ 65.5 (C-3) and 64.4 (C-5) suggest that they are bonded to the nitrogen of the heterocyclic ring. The methylene signal at δ 65.5 (C-8) was attributed to the hydroxymethyl carbon, which was bonded to C-3. The COSY and HMBC spectroscopic data showed the presence of the 3-hydroxybutyl group (δ 24.7, 28.6, 39.0, 70.5) at C-5. The methine signals at δ 76.7, 80.6, and 80.8 were assigned to C-7, C-2, and C-1 bearing the OH groups, respectively, due to the appearance in the low-field region of H-7 (δ 4.33), H-2 (δ 3.97), and H-1 (δ 3.82). The last methine signal at δ 80.0, with the doublet of doublets at δ 3.22 ($J_{7,7a} = 1.8$, $J_{7a,1} = 6.0$ Hz) in the ^1H NMR spectrum, was identified as the bridgehead C-7a. These data suggest that alkaloid **4** has a pyrrolizidine ring system. The relative configurations at the stereogenic centers in **4** were determined from the NOE correlations (Figure S1, Supporting Information) because they cannot be determined from $^3J_{H,H}$ coupling constants ($J_{7a,1} = 6.0$, $J_{1,2} = 6.4$, $J_{2,3} = 6.4$ Hz). Irradiation of H-7a enhanced the NOE signal intensity of H-2 and H-5, and irradiation of H-1 enhanced the NOE signals of H-7 and H-3. These results indicate that H-7a, H-2, and H-5 are on the same side of the ring, and H-1, H-3, and H-7 are on the opposite side. Hence, alkaloid **4** was determined to be α-5-*C*-(3-hydroxybutyl)-7-*epi*-australine. 7-*epi*-Australine has been

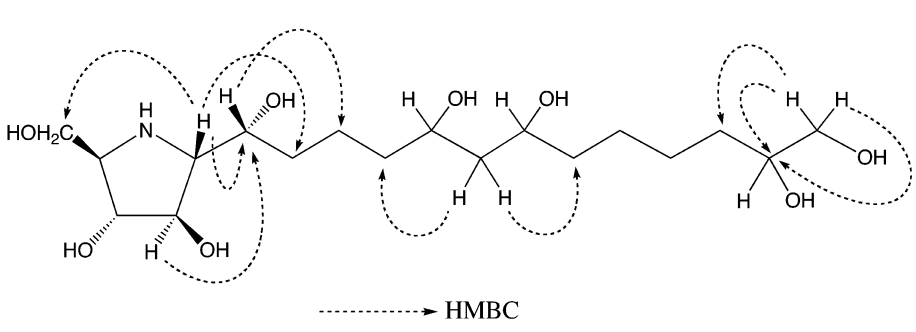

Figure 1. Selected HMBC correlations of alkaloid **3**.

Table 1. Concentration of Polyhydroxylated Pyrrolidines (**1**−**3**) and Pyrrolizidines (**4**−**7**) Giving 50% Inhibition of Various Glycosidases

	IC_{50} (μM)						
enzyme	**1**	**2**	**3**	**4**	**5**	**6**	**7**
α-glucosidase							
rice	210	650	265	−	−	300	390
yeast	100	630	220	6.6	−	3.6	650
rat intestinal maltase	−[a]	−	740	−	−	350	−
β-glucosidase							
Caldocellum saccharolyticum	700	20	0.08	200	136	5.1	11.4
almond	−	120	6.6	−	49	9.5	25.4
human placenta (β-glucocerebrosidase)	−	−	970	−	−	−	−
α-galactosidase							
coffee bean	−	−	−	−	−	−	−
β-galactosidase							
bovine liver	260	130	0.09	320	−	830	920
rat epididymis	−	−	−	−	−	−	−
α-mannosidase							
rat epididymis	−	−	−	−	−	680	−
β-mannosidase							
rat epididymis	610	150	9.3	−	−	−	−
α-L-fucosidase							
bovine epididymis	−	−	−	−	−	−	−
α-L-rhamnosidase							
Penicillium decumbens (naringinase)	−	−	−	−	−	180	−

[a] − indicates no inhibition (less than 50% inhibition at 1000 μM).

unambiguously synthesized but has not yet been found as a natural product.[20−22]

Alkaloid **5** was determined to have the molecular formula $C_{12}H_{23}NO_4$ by HRFABMS. The ^{13}C NMR spectrum was similar to that of **4**, except for an additional methylene signal and disappearance of an oxymethine signal. The molecular formula and ^{13}C NMR spectroscopic data suggest that alkaloid **5** is the deoxygenated derivative of **4**, or its isomer. The connectivity of the carbon and hydrogen atoms was defined from COSY and HMBC spectroscopic data. The $^1H-^1H$ COSY and HMBC spectra indicated the deoxygenation at C-7 and the presence of the 3-hydroxybutyl group at C-5. The coupling pattern ($J_{1,2} = 4.6$, $J_{2,3} = 8.5$ Hz) of H-2 in the ^1H NMR spectrum suggests that H-1 and H-2 are in a *cis* arrangement, and H-2 and H-3 are a *trans* diaxial pair. Irradiation of H-7a enhanced the NOE signal intensity of H-2 and H-5 (Figure S1, Supporting Information). These results indicate that H-7a, H-1, H-2, and H-5 are on the same side of the ring. Hence, alkaloid **5** was determined to be α-5-*C*-(3-hydroxybutyl)hyacinthacine A_1. Hyacinthacine A_1 has been isolated from the bulbs of *Muscari armeniacum* (Hyacinthaceae).[7]

Alkaloid **6** was determined to have the molecular formula $C_{12}H_{23}NO_5$ by HRFABMS. The ^{13}C NMR spectrum was similar to that of **5**, except for an additional oxymethine signal and disappearance of a methylene signal. The connectivity of the carbon and hydrogen atoms was defined from COSY and HMBC spectroscopic data. The $^1H-^1H$ COSY and HMBC spectra indicated the presence of the 1,3-dihydroxybutyl group at C-5 and suggested that alkaloid **6** is hyacinthacine A_1 or its isomer bearing the 1,3-dihydroxybutyl side chain at C-5. The coupling pattern ($J_{1,2} = 3.7$, $J_{2,3} = 9.1$ Hz) of H-2 in the ^1H NMR spectrum and the strong NOE correlations between H-7a and H-2 (Figure S1, Supporting Information) indicate that H-7a, H-1, and H-2 are on the same side of the ring. The NOE between H-3 on the pyrrolizidine ring and H-1 in the side chain indicates that the side chain and H-3 are on opposite sides of the ring. Hence, alkaloid **6** was determined to be α-5-*C*-(1,3-dihydroxybutyl)hyacinthacine A_1. The NOE correlations between H-3 and H-1′, between H-5 and H-2′b, and between H-6α and H-2′b imply the *R* configuration of C-1′.

Alkaloid **7** was determined to have the molecular formula $C_{12}H_{23}NO_6$ by HRFABMS. The ^{13}C NMR spectrum was similar to that of **6**, except for an additional hydroxymethyl signal and disappearance of a methyl signal. The chemical shifts of the pyrrolizidine ring carbon signals were quite consistent with those of alkaloid **6**. The $^1H-^1H$ COSY and HMBC spectra indicated the presence of the 1,3,4-trihydroxybutyl group at C-5. The NOE correlations (Figure S1, Supporting Information) corroborated that the structure of **7** is α-5-*C*-(1,3,4-trihydroxybutyl)hyacinthacine A_1 and suggested that the absolute configuration of C-1′ is *R*.

The IC_{50} values of the alkaloids isolated from *S. peruviana* toward various glycosidases are shown in Table 1. We previously reported that homoDMDP, which can be regarded as α-1-*C*-(1,2-dihydroxyethyl)-D-AB1, is a potent inhibitor of bacterial (*Caldocellum saccharolyticum*) β-glucosidase with an IC_{50} value of 3.2 μM and mammalian β-galactosidase (bovine liver) with an IC_{50} value of 4.4 μM.[9] We also reported that the C-1 OH group in the side chain has a very important role in the inhibition of homoDMDP toward β-glycosidases because its deoxygenation abolished inhibition toward β-glycosidases tested.[8] In the present study, alkaloid **3**, bearing the C-1 OH group in the C_{13} side chain, was a much more potent inhibitor of β-glycosidases than homoDMDP, whereas alkaloids **1** and **2**, without the C-1 OH group in the side chain, were weak inhibitors of β-glycosidases. Alkaloid **1** showed no significant inhibition toward mammalian lysosome β-glucosidase and β-galactosidase but was a moderate inhibitor of rat lysosome β-mannosidase. Broussonetines E, F, G, H, K, and L, all of which have the C-1 OH group in the C_{13} side chain, have been reported to be powerful inhibitors of almond β-glucosidase, with IC_{50} values ranging from 10 to 50 nM, and bovine liver β-galactosidase, with IC_{50} values in the nM range.[12,13,15] As seen in broussonetines C, D, and M, the removal of the C-1 OH group in the C_{13} side chain markedly lowered or abolished their inhbition toward β-glycosidases.[11,17]

Polyhydroxylated pyrrolizidine alkaloids are generally moderate or weak inhibitors of glycosidases.[5,7,8,22] Furthermore, it is not usually easy to predict whether they will inhibit a particular glycosidase from the configuration of the hydroxyl groups and the difference of the side chain

on the pyrrolizidine ring. 7-*epi*-Australine is a weak inhibitor (IC_{50} = 95 μM) of yeast α-glucosidase,[22] whereas alkaloid **4**, α-5-*C*-(3-hydroxybutyl)-7-*epi*-australine, was a good inhibitor of the enzyme with an IC_{50} value of 6.6 μM. Although alkaloid **6** is the α-5-*C*-(1,3-dihydroxybutyl) derivative of hyacinthacine A_1 and is a good inhibitor of yeast α-glucosidase (IC_{50} = 6.3 μM) and bacterial β-glucosidase (IC_{50} = 5.1 μM), alkaloid **7**, having the additional OH group in the side chain of **6**, was a weaker inhibitor of both the enzymes than **6**. Broussonetine N, which can be regarded as the derivative bearing the C_{10} side chain at C-5α of hyacinthacine A_2, has been reported to be a inhibitor of β-glucosidase, β-galactosidase, and β-mannosidase, with IC_{50} values in the μM range[16] and is a much more potent inhibitor than the mother compound, hyacinthacine A_2.

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 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 Materials. The bulbs of *S. peruviana* were purchased in November 1999 from Floribon International (Voorhout, Holland). A voucher specimen (no. NA000602) is deposited in the Herbarium of the Medicinal Plants Garden, Hokuriku University.

Extraction and Isolation. The bulbs (5 kg) of *S. peruviana* were homogenized in 50% aqueous EtOH (6 L) and allowed to stand for 3 days. The filtrate was applied to a column of Amberlite IR-120B (500 mL, H^+ form). The 0.5 M NH_4OH eluate was concentrated to give a brown syrup (9.8 g), which was further chromatographed over a Dowex 1-X2 (100 mL, OH^- form) column with H_2O (1.5 L) as eluant to give a colorless syrup (4.54 g). The syrup was applied to an 83 cm × 2.8 cm Amberlite CG-50 column (NH_4^+ form) with H_2O as eluant (fraction size 15 mL, fractions 1−45). The H_2O eluate was divided into two pools: I (fractions 14−24, 2.18 g) and II (fractions 34−44, 216 mg). The column was then eluted with 0.1 M NH_4OH (fraction size 15 mL, fractions 46−130) and divided into four further pools: III (fractions 48−54, 326 mg), IV (fractions 58−96, 997 mg), V (fractions 97−105, 479 mg), and VI (fractions 106−116, 176 mg). Each pool was further chromatographed with Dowex 1-X2 (H^- 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 alkaloid **3** (160 mg) from pool I, **2** (86 mg) and **4** (70 mg) from pool II, **1** (70 mg) from pool III, **6** (832 mg) from pool IV, **7** (72 mg) from pool V, and **5** (142 mg) from pool VI.

α-1-*C*-(2-Hydroxypropyl)-1,4-dideoxy-1,4-imino-D-arabinitol (1): colorless syrup; $[\alpha]_D$ +40.8° (*c* 0.54, H_2O); ^{1}H NMR (D_2O, 500 MHz) δ 3.98 (1H, dq, *J* = 6.9, 6.4 Hz, H-2′), 3.85 (1H, dd, *J* = 7.3, 6.9 Hz, H-3), 3.75 (1H, dd, *J* = 7.8, 6.9 Hz, H-2), 3.73 (1H, dd, *J* = 11.9, 4.6 Hz, H-5a), 3.66 (1H, dd, *J* = 11.9, 6.4 Hz, H-5b), 3.10 (1H, ddd, *J* = 7.3, 6.4, 4.6 Hz, H-4), 3.06 (1H, ddd, *J* = 8.7, 7.8, 5.0 Hz, H-1), 1.81 (1H, ddd, *J* = 14.2, 6.4, 5.0 Hz, H-1′a), 1.70 (1H, ddd, *J* = 14.2, 8.7, 6.9 Hz, H-1′b), 1.21 (3H, d, *J* = 6.4 Hz, H-3′); ^{13}C NMR (D_2O, 125 MHz) δ 84.3 (CH, C-2), 80.3 (CH, C-3), 68.8 (CH, C-2′), 64.63 (CH_2, C-5), 64.56 (CH, C-4), 61.0 (CH, C-1), 44.4 (CH_2, C-1′), 24.7 (CH_3, C-3′); HRFABMS *m/z* 192.1232 $[M + H]^+$ ($C_8H_{18}NO_4$ requires 192.1236).

α-1-*C*-(1,2-Dihdroxypropyl)-1,4-dideoxy-1,4-imino-D-arabinitol (2): colorless syrup; $[\alpha]_D$ +13.3° (*c* 1.23, H_2O); ^{1}H NMR (D_2O, 500 MHz) δ 4.10 (1H, dd, *J* = 7.3, 6.0 Hz, H-2), 3.91 (1H, q, *J* = 6.4 Hz, H-2′), 3.88 (1H, dd, *J* = 7.3, 6.4 Hz, H-3), 3.73 (1H, dd, *J* = 11.9, 4.6 Hz, H-5a), 3.65 (1H, dd, *J* = 11.9, 6.0 Hz, H-5b), 3.57 (1H, dd, *J* = 6.4, 6.0 Hz, H-1′), 3.15 (1H, dd, *J* = 7.3, 6.0 Hz, H-1), 3.08 (1H, ddd, *J* = 6.4, 6.0, 4.6 Hz, H-4), 1.22 (3H, d, *J* = 6.4 Hz, H-3′); ^{13}C NMR (D_2O, 125 MHz) δ 81.0 (CH, C-2, C-3), 78.1 (CH, C-1′), 71.5 (CH, C-2′), 64.9 (CH, C-4), 64.5 (CH, C-1), 64.4 (CH_2, C-5), 20.3 (CH_3, C-3′); HRFABMS *m/z* 208.1188 $[M + H]^+$ ($C_8H_{18}NO_5$ requires 208.1185).

α-1-*C*-(1,10,13-Trihydroxytridecyl)-1,4-dideoxy-1,4-imino-D-arabinitol (3): colorless syrup; $[\alpha]_D$ +20.5° (*c* 0.65, H_2O); ^{1}H NMR (D_2O, 500 MHz) δ 4.05 (1H, dd, *J* = 7.3, 6.8 Hz, H-2), 3.86 (1H, dd, *J* = 7.7, 6.8 Hz, H-3), 3.84 (2H, m, H-5′, H-7′), 3.72 (1H, dd, *J* = 11.7, 4.4 Hz, H-5a), 3.70 (2H, m, H-1′, H-12′), 3.64 (1H, dd, *J* = 11.7, 6.3 Hz, H-5b), 3.60 (1H, dd, *J* = 11.7, 4.4 Hz, H-13′a), 3.48 (1H, dd, *J* = 11.7, 6.7 Hz, H-13′b), 3.06 (1H, ddd, *J* = 7.7, 6.3, 4.4 Hz, H-4), 2.95 (1H, dd, *J* = 7.3, 5.2 Hz, H-1), 1.68 (1H, m, H-6′a), 1.63 (1H, m, H-6′b), 1.62−1.40 (14H, m, H-2′a, H-2′b, H-3′a, H-3′b, H-4′a, H-4′b, H-8′a, H-8′b, H-9′a, H-9′b, H-10′a, H-10′b, H-11′a, H-11′b); ^{13}C NMR (D_2O, 125 MHz) δ 80.9 (CH, C-3), 80.6 (CH, C-2), 75.2 (CH, C-1′), 74.6 (CH, C-12′), 72,7 (CH, C-7′ or C-5′), 72.6 (CH, C-5′ or C-7′), 68.3 (CH_2, C-13′), 66.9 (CH, C-1), 64.6 (CH, C-4), 64.5 (CH_2, C-5), 45.7 (CH_2, C-6′), 38.9 (CH_2, C-8′ or C-4′), 38.8 (CH_2, C-4′ or C-8′), 35.8 (CH_2, C-2′), 35.0 (CH_2, C-11′), 27.5 (CH_2, C-9′), 27.3 (CH_2, C-10′), 23.8 (CH_2, C-3′); HRFABMS *m/z* 396.2590 $[M + H]^+$ ($C_{18}H_{38}NO_8$ requires 396.2597).

α-5-*C*-(3-Hydroxybutyl)-7-*epi*-australine (4): colorless powder; $[\alpha]_D$ −20.3° (*c* 1.35, H_2O); ^{1}H NMR (D_2O, 500 MHz) δ 4.33 (1H, dt, *J* = 5.5, 1.8 Hz, H-7), 3.97 (1H, t, *J* = 6.4 Hz, H-2), 3.87 (1H, m, H-3′), 3.82 (1H, dd, *J* = 6.4, 6.0 Hz, H-1), 3.71 (1H, dd, *J* = 11.5, 4.1 Hz, H-8), 3.65 (1H, dd, *J* = 11.5, 6.0 Hz, H-8), 3.35 (1H, m, H-5), 3.22 (1H, dd, *J* = 6.0, 1.8 Hz, H-7a), 3.10 (1H, ddd, *J* = 6.4, 6.0, 4.1 Hz, H-3), 1.89 (1H, ddd, *J* = 13.7, 5.0, 1.8 Hz, H-6β), 1.82 (1H, m, H-2′a), 1.79 (1H, ddd, *J* = 13.7, 11.4, 5.5, H-6α), 1.62−1.46 (3H, m, H-1′a, H-1′b, H-2′b), 1.20 (3H, d, *J* = 6.4 Hz, H-4′); ^{13}C NMR (D_2O, 125 MHz) δ 80.8 (CH, C-1), 80.6 (CH, C-2), 80.0 (CH, C-7a), 76.7 (CH, C-7), 70.5 (CH, C-3′), 65.5 (CH, C-3), 65.5 (CH_2, C-8), 64.4 (CH, C-5), 40.0 (CH_2, C-6), 39.0 (CH_2, C-2′), 28.6 (CH_2, C-1′), 24.7 (CH_3, C-4′); HRFABMS *m/z* 262.1656 $[M + H]^+$ ($C_{12}H_{24}NO_5$ requires 262.1654).

α-5-*C*-(3-Hydroxybutyl)hyacinthacine A_1 (5): colorless syrup; $[\alpha]_D$ −20.3° (*c* 1.35, H_2O); ^{1}H NMR (D_2O, 500 MHz) δ 4.17 (1H, dd, *J* = 4.6, 3.9 Hz, H-1), 4.12 (1H, dd, *J* = 8.5, 4.6 Hz, H-2), 3.84 (1H, m, H-3′), 3.83 (1H, m, H-7a), 3.78 (1H, dd, *J* = 11.9, 5.0 Hz, H-8), 3.75 (1H, dd, *J* = 11.9, 4.5 Hz, H-8), 3.33 (1H, ddd, *J* = 8.5, 5.0, 4.5 Hz, H-3), 3.28 (1H, m, H-5), 2.06 (1H, m, H-7α), 1.99 (1H, m, H-6α), 1.88 (1H, m, H-1′a), 1.81−1.73 (2H, m, H-6β, H-7β), 1.54 (1H, m, H-2′a), 1.52−1.44 (2H, m, H-1′b, H-2′b), 1.19 (3H, d, *J* = 6.4 Hz, H-4′); ^{13}C NMR (D_2O, 125 MHz) δ 76.6 (CH, C-2), 73.1 (CH, C-1), 70.6 (CH, C-7a), 70.5 (CH, C-3′), 66.5 (CH, C-5), 64.7 (CH, C-3), 63.4 (CH_2, C-8), 38.6 (CH_2, C-2′), 32.7 (CH_2, C-6), 27.6 (CH_2, C-1′), 25.5 (CH_2, C-7), 24.6 (CH_3, C-4′); HRFABMS *m/z* 246.1714 $[M + H]^+$ ($C_{12}H_{24}NO_4$ requires 246.1705).

α-5-*C*-(1,3-Dihydroxybutyl)hyacinthacine A_1 (6): colorless powder; $[\alpha]_D$ +42.0° (*c* 1.02, H_2O); ^{1}H NMR (D_2O, 500 MHz) δ 4.38 (1H, ddd, *J* = 9.6, 3.2, 1.8 Hz, H-1′), 4.10 (1H, dd, *J* = 9.1, 3.7 Hz, H-2), 3.99 (1H, t, *J* = 3.7, H-1), 3.98 (1H, ddq, *J* = 9.6, 6.4, 3.7 Hz, H-3′), 3.85 (1H, dd, *J* = 11.9, 3.7 Hz, H-8), 3.70 (1H, dt, *J* = 7.8, 3.7 Hz, H-7a), 3.68 (1H, dd, *J* = 11.9, 6.4 Hz, H-8), 3.39 (1H, ddd, *J* = 9.1, 6.4, 3.7 Hz, H-3), 3.16 (1H, m, H-5), 2.12 (1H, m, H-6α), 2.05−1.89 (2H, m, H-6β, H-7α), 1.76 (1H, m, H-7β), 1.67 (1H, ddd, *J* = 14.7, 9.6, 3.7 Hz, H-2′a), 1.49 (1H, ddd, *J* = 14.7, 9.6, 3.2 Hz, H-2′b), 1.22 (3H, d, *J* = 6.4 Hz, H-4′); ^{13}C NMR (D_2O, 125 MHz) δ 78.7 (CH, C-2), 74.6 (CH, C-1), 68.8 (CH, C-7a), 68.7 (CH, C-1′), 67.2 (CH, C-3′), 67.0 (CH, C-5), 65.7 (CH_2, C-8), 63.7 (CH, C-3), 46.0 (CH_2, C-2′), 29.4 (CH_2, C-6), 25.6 (CH_3, C-4′), 24.9 (CH_2, C-7); HRFABMS *m/z* 262.1660 $[M + H]^+$ ($C_{12}H_{24}NO_5$ requires 262.1654).

α-5-*C*-(1,3,4-Trihydroxybutyl)hyacinthacine A_1 (7): colorless powder; $[\alpha]_D$ +33.4° (*c* 0.67, H_2O); ^1H NMR (D_2O, 500 MHz) δ 4.42 (1H, ddd, *J* = 9.7, 2.8, 2.3 Hz, H-1′), 4.10 (1H, dd, *J* = 9.2, 3.7 Hz, H-2), 3.99 (1H, t, *J* = 3.7, H-1), 3.87 (1H, dddd, *J* = 10.1, 6.9, 4.2, 3.2 Hz, H-3′), 3.84 (1H, dd, *J* = 11.4, 4.1 Hz, H-8), 3.67 (1H, m, H-7a), 3.67 (1H, dd, *J* = 11.4, 6.9 Hz, H-8), 3.61 (1H, dd, *J* = 11.4, 4.2 Hz, H-4′a), 3.49 (1H, dd, *J* = 11.4, 6.9 Hz, H-4′b), 3.37 (1H, ddd, *J* = 9.2, 6.9, 3.7 Hz, H-3), 3.15 (1H, m, H-5), 2.11 (1H, m, H-6α), 1.96 (1H, m, H-6*β*), 1.93 (1H, m, H-7α), 1.75 (1H, m, H-7*β*), 1.66 (1H, ddd, *J* = 14.6, 9.7, 3.2 Hz, H-2′a), 1.45 (1H, ddd, *J* = 14.6, 10.1, 2.8 Hz, H-2′b); ^{13}C NMR (D_2O, 125 MHz) δ 78.9 (CH, C-2), 74.7 (CH, C-1), 71.2 (CH, C-3′), 68.8 (CH_2, C-4′), 68.7 (CH, C-7a), 68.4 (CH, C-1′), 66.9 (CH, C-5), 66.0 (CH_2, C-8), 63.6 (CH, C-3), 40.5 (CH_2, C-2′), 29.6 (CH_2, C-6), 24.9 (CH_2, C-7); HRFABMS *m*/*z* 278.1607 $[M + H]^+$ ($C_{12}H_{24}NO_6$ requires 278.1604).

Glycosidase Inhibitory Activities. The enzymes α-glucosidase (from rice, assayed at pH 5.0; from yeast, assayed at pH 6.8), *β*-glucosidase (from *Caldocellum saccharolyticum*, pH 5.0; from almond, pH 5.0), α-galactosidase (from coffee bean, pH 6.5), *β*-galactosidase (from bovine liver, pH 6.8), α-L-fucosidase (from bovine epididymis, pH 5.5), α-L-rhamnosidase (from *Penicillium decumbens*, 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.[23] and assayed at pH 5.2 for *β*-galactosidase, α-mannosidase, and *β*-mannosidase using *p*-nitrophenyl-glycosides. Brush border membranes prepared from rat small intestine according to the method of Kessler et al.[24] were assayed at pH 5.8 for rat intestinal maltase using maltose. Human placenta *β*-glucocerebrosidase (Ceredase) was purchased from Genzyme (Boston, MA) and assayed at pH 5.5. For the activity of rice α-glucosidase and rat intestinal maltase, the reaction mixture (0.2 mL) contained 25 mM maltose and the appropriate amount of enzyme, and the incubations were performed for 10−30 min at 37 °C. The reaction was stopped by heating at 100 °C for 3 min. After centrifugation (600 g; 10 min), 0.05 mL of resulting reaction mixture was added to 3 mL of Glucose B-test Wako (Wako Pure Chemical Ind.). The absorbance at 505 nm was measured to determined the amount of the released D-glucose. Other glycosidase activities were determined using an appropriate *p*-nitrophenyl glycoside as substrate at the optimum pH of each enzyme. The reaction mixture (1 mL) contained 2 mM of the substrate and the appropriate amount of enzyme. The reaction was stopped by adding 2 mL of 400 mM Na_2CO_3. The released *p*-nitrophenol was measured spectrometrically at 400 nm.

Supporting Information Available: Figure S1, NOE correlations of alkaloids **4**−**7**. This material is available free of charge via the Internet at http://pubs.acs.org.

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) Asano, N. *Glycobiology* **2003**, *13*, 93R-104R.
(4) Watson, A. A.; Nash, R. J.; Wormald, M. R.; Harvey, D. J.; Dealler, S.; Lees, E.; Asano, N.; Kato, A.; Griffiths, R. C.; Cairns, A.; Fleet, G. W. J. *Phytochemistry* **1997**, *46*, 255−259.
(5) Kato, A.; Adachi, I.; Miyauchi, M.; Ikeda, K.; Komae, T.; Kizu, H.; Kameda, Y.; Watson, A. A.; Nash, R. J.; Wormald, M. R.; Fleet, G. W. J.; Asano, N. *Carbohydr. Res.* **1999**, *316*, 95−103.
(6) 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.
(7) Asano, N.; Kuroi, H.; Ikeda, K.; Kizu, H.; Kameda, Y.; Kato, A.; Adachi, I.; Watson, A. A.; Nash, R. J.; Fleet, G. W. J. *Tetrahedron Asymmetry* **2000**, *11*, 1−8.
(8) 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.
(9) Takebayashi, M.; Hiranuma, S.; Kanie, Y.; Kajimoto, T.; Kanie, O.; Wong, C. H. *J. Org. Chem.* **1999**, *64*, 5280−5291.
(10) 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.
(11) Shibano, M.; Kitagawa, S.; Kusano, G. *Chem Pharm. Bull.* **1997**, *45*, 505−508.
(12) Shibano, M.; Kitagawa, S.; Nakamura, S.; Akazawa, N.; Kusano, G. *Chem. Pharm. Bull.* **1997**, *45*, 700−705.
(13) Shibano, M.; Nakamura, S.; Akazawa, N.; Kusano, G. *Chem. Pharm. Bull.* **1998**, *46*, 1048−1050.
(14) Shibano, M.; Nakamura, S.; Kubori, M.; Minoura, K.; Kusano, G. *Chem. Pharm. Bull.* **1998**, *46*, 1416−1420.
(15) Shibano, M.; Nakamura, S.; Motoya, N.; Kusano, G. *Chem Phar. Bull.* **1999**, *47*, 472−476.
(16) Shibano, M.; Tsukamoto, D.; Kusano, G. *Chem. Pharm. Bull.* **1999**, *47*, 907−908.
(17) Shibano, M.; Tsukamoto, D.; Fujimoto, R.; Masui, Y.; Sugimoto, H.; Kusano, G. *Chem. Pharm. Bull.* **2000**, *48*, 1281−1285.
(18) Tsukamoto, D.; Shibano, M.; Okamoto, R.; Kusano, G. *Chem. Pharm. Bull.* **2001**, *49*, 492−496.
(19) Tsukamoto, D.; Shibano, M.; Kusano, G. *Chem. Pharm. Bull.* **2001**, *49*, 1487−1491.
(20) Denmark, S. E.; Herbert, B. *J. Am. Chem. Soc.* **1998**, *120*, 7357−7358.
(21) Denmark, S. E.; Herbert, B. *J. Org. Chem.* **2000**, *65*, 2887−2896.
(22) Kato, A.; Kano, E.: Adachi, I.; Molyneux, R. J.; Alison, A. A.; Nash, R. J.; Fleet, G. W. J.; Wormald, M. R.; Kizu, H.; Ikeda, K.; Asano, N. *Tetrahedron: Asymmetry* **2003**, *14*, 325−331.
(23) Skudlarek, M. D.; Tulsiani, D. R. P.; Orgebin-Crist, M.-C. *Biochem. J.* **1992**, *286*, 907−914.
(24) Kessler, M.; Acuto, O.; Strelli, C.; Murer, H.; Semenza, G. A. *Biochim. Biophys. Acta* **1978**, *506*, 136−154.

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