Isolation of Glycosidase-Inhibiting Hyacinthacines and Related Alkaloids from *Scilla socialis*

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Received February 25, 2007

An examination of the bulbs of *Scilla socialis* has resulted in the isolation of 11 hyacinthacines, two pyrrolidines, and three piperidines. The structures of the new alkaloids were elucidated by spectroscopic methods as β-1-*C*-ethyldeoxymannojirimycin (**5**), hyacinthacines B_7 (**10**), C_2 (**11**), C_3 (**12**), C_4 (**13**), and C_5 (**14**), and α-5-*C*-(3-hydroxybutyl)-hyacinthacine A_2 (**15**). Although, β-L-homofuconojirimycin (**3**) and α-7-deoxyhomonojirimycin (α-7-deoxy-HNJ, **4**) are previously known alkaloids, this is the first report of their occurrence in the plant family Hyacinthaceae. Alkaloid **11** was found to be a good inhibitor of bacterial β-glucosidase and human placenta α-L-fucosidase, with IC_{50} values of 13 and 17 μM, respectively, while alkaloid **12** showed no inhibitory activity toward α-L-fucosidase but was a more potent inhibitor of bovine liver β-galactosidase (IC_{50} = 52 μM) than **11**. Alkaloids **13** and **14** were shown to be inhibitory toward mammalian α-glucosidase (IC_{50} = 45 and 77 μM, respectively), and alkaloid **14** was demonstrated as a moderate inhibitor of bacterial β-glucosidase (IC_{50} = 48 μM).

Glycosidases are involved in several important anabolic and catabolic process, such as intestinal digestion, lysosomal catabolism, and post-translational modification, which are closely related to the endoplasmic reticulum (ER) quality control and ER-associated degradation of glycoproteins. Thus, glycosidase-inhibiting iminosugars could have enormous potential applications as biochemical tools and therapeutic agents.[1] These iminosugars can inhibit various glycosidases because of a structural resemblance to their sugar moiety to natural substrates. For example, *N*-hydroxyetheyl-1-deoxynojirimycin (Glyset), which corresponds to an α-D-glucose configuration, has been approved as a second-generation α-glucosidase inhibitor to treat type-2 diabetes.[2] *N*-Butyl-1-deoxynojirimycin (Zavesca) is an inhibitor of ceramide-specific glucosyltransferase[3] and has been approved for the oral treatment of substrate reduction therapy in type-1 Gaucher disease. The iminosugar derivatives α-6-*C*-nonylisofagomine,[4] α-1-*C*-nonyl-1,5-dodeoxy-1,5-iminoxylitol,[5] and α-1-*C*-octyl-1-deoxynojirimycin[6] are candidates as oral agents of pharmacological chaperone therapy in type-1 Gaucher disease.

Naturally occurring iminosugars are classified into five structural classes: polyhydroxylated pyrrolidines, piperidines, indolizidines, pyrrolizidines, and nortropans. The distribution of polyhydroxylated piperidine and pyrrolidine alkaloids appears to be widespread in many unrelated families. On the other hand, polyhydroxylated pyrrolizidine alkaloids appear to be restricted to specific families.[7,8] Consequently, the search for lead compounds of this type from natural sources tends to be constrained compared to other compound classes. Pyrrolizidine alkaloids with a hydroxymethyl substituent at C-3 have long been thought to be rare in nature, because almost all classes of pyrrolizidine alkaloids bear a carbon substituent at C-1.[9,10] In 1988, the first polyhydroxylated pyrrolizidine alkaloid with a hydroxymethyl group at C-3 was isolated from pods of *Alexa leiopetala* (Leguminosae) and designated alexine.[11] The Australian legume *Castanospermum australe* is also known to produce the same structural type of pyrrolizidines, such as australine (7a-*epi*-alexine) and its isomers.[12] The alexines and australines have been reported in only two small genera of the Leguminosae, and the highly polyhydroxylated pyrrolizidine casuarine occurs only in related genera in the Casuarinaceae and Myrtaceae.[13] Recently, representatives of a new type of polyhydroxylated pyrrolizidine alkaloids have been isolated from *Hyacinthoides non-scripta* and *Scilla campanulata*, and named hyacinthacines.[14] We later isolated hyacinthacines A_1, A_2, A_3, B_3, and C_1 from *Muscari armeniacum* bulbs.[15] Hyacinthacines are basically characterized as 7a*R*-hydro-1,2-dihydroxy-3-hydroxymethylpyrrolizidines, with a methyl or hydroxymethyl group at C-5 and with hydroxyl substituents present at C-6 and/or C-7 in some cases. Furthermore, the known hyacinthacines have been classified into three groups, A, B, and C, on the basis of the number of hydroxyl and hydroxymethyl groups on the second ring. The total syntheses of hyacinthacines A_2 and A_1 were accomplished in 2001 and 2005, respectively.[16,17] Some novel approaches in this regard have been reported since then.[18,19] However, the syntheses of hyacinthacines would require some alteration because of the lengthy process and/or a lack of selectivity. The distribution of hyacinthacines appears to be restricted to the family Hyacinthaceae, and the genus *Scilla* is an especially rich source of these compounds. Hyacinthacines A_4, A_5, A_6, A_7, B_3, B_4, B_5, and B_6 have been isolated from *Scilla sibirica* bulbs,[20] and hyacinthacine derivatives bearing a long side chain at C-5 have been isolated from *Scilla peruviana* bulbs.[21] The present search for hyacinthacines in the Hyacinthaceae has led to the isolation from *Scilla socialis* bulbs of 11 hyacinthacines, including six new and five known pyrrolizidines. In this paper, we describe the isolation and structural determination of these alkaloids and their inhibitory activities toward glycosidase enzymes.

Results and Discussion

The bulbs (2.3 kg) of *S. socialis* were extracted with 50% aqueous EtOH. The chromatographic separation of the extract using various ion-exchange resins led to isolation of 16 alkaloids (**1**−**16**). The ^{1}H NMR and ^{13}C NMR spectra of alkaloids **1** and **2** were in accord with those of 2,5-dideoxy-2,5-imino-D-*glycero*-D-mannitol (homoDMDP) and 6-deoxy-6-*C*-(2,5-dihydroxyhexyl)-DMDP, respectively, which have been isolated previously from *Hyacinthus orientalis* and *H. non-scripta*.[14,22] The biosynthesis of the hyacinthacines has not been elucidated yet, but homoDMDP can be speculated as being an important intermediate compound because

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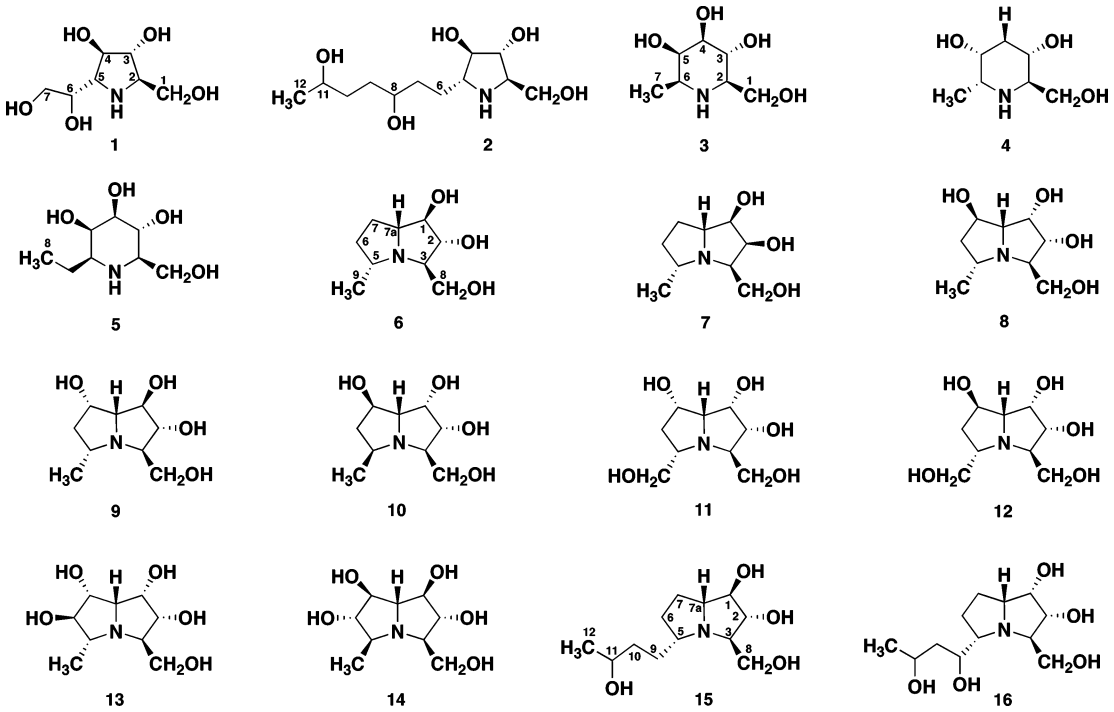
10.1021/np0700826 CCC: $37.00
Published on Web 05/31/2007

Chart 1



the distribution of homoDMDP and hyacinthacine is closely related and appears to be restricted to the family Hyacinthaceae. Alkaloids **3** and **4** were identified as β-L-homofuconojirimycin and α-7-deoxyhomonojirimycin (α-7-deoxy-HNJ), respectively, from an analysis of their ^{1}H NMR and ^{13}C NMR spectroscopic data. We have isolated these piperidine alkaloids previously from *Angylocalyx pynaertii*[23] and *S. sibirica*.[20] The optical rotation and ^{1}H and ^{13}C NMR spectroscopic data of alkaloids **6**−**8**, **9**, and **16** were in accord with those of the polyhydroxylated pyrrolizidines hyacinthacines A_3, A_5, B_3, and B_4 and α-5-*C*-(1,3-dihydroxybutyl)hyacinthacine A_1 isolated earlier from *M. armeniacum*,[15] *S. sibirica*,[20] and *S. peruviana*.[21] The structural determination of the new alkaloids **5** and **10**−**15** is described below.

Alkaloid **5** was determined to have the molecular formula $C_8H_{17}NO_4$ by HRFABMS. The ^{13}C NMR spectroscopic data revealed the presence of a single methyl (δ 13.4), two methylenes (δ 24.3, 63.4), and five methine (δ 57.8, 62.1, 71.2, 73.7, 74.1) carbon atoms. The connectivity of the carbon and hydrogen atoms was defined from COSY and HMBC spectroscopic data. Two methine signals (δ 57.8 and 62.1) with relatively high-field chemical shifts were suggestive of being bonded to the nitrogen of the piperidine ring. The methylene signal at δ 63.4 (C-6) was attributed to the hydroxymethyl carbon. The HMBC spectrum indicated the presence of the ethyl group at C-1. These results suggested that alkaloid **5** is the *C*-alkyl derivative of 1-deoxynojirimycin or its epimer bearing the ethyl group at C-1. In the ^{1}H NMR spectrum, the coupling patterns of H-4 (t, $J_{3,4} = J_{4,5} = 9.5$ Hz) and H-2 (t, $J_{1,2} = J_{2,3} = 2.5$ Hz) indicated axial orientations of H-3, H-4, and H-5 and the equatorial orientation of H-2. The NOE correlation between H-1 and H-3 suggested the axial orientation of H-1. Hence, alkaloid **5** was established as β-1-*C*-ethyl-1-deoxymannojirimycin.

Alkaloid **10** was determined to have the molecular formula $C_9H_{17}NO_4$ by HRFABMS (*m*/*z* 204.1233 $[M + H]^+$). This alkaloid gave a tetra-*O*-$SiMe_3$ derivative, and its GC-MS analysis showed a characteristic fragment ion at *m*/*z* 476 $[M - CH_3]^+$ and a base peak at *m*/*z* 388 $[M - CH_2OSiMe_3]^+$. The ^{1}H and ^{13}C NMR spectroscopic features of **10** were similar to those of hyacinthacine B_3 (**8**). The ^{13}C NMR spectroscopic data revealed the presence of a single methyl, two methylenes, and six methine carbon atoms, as shown in Table 1. The signals in the ^{1}H and ^{13}C NMR spectra were assigned from the HMQC, COSY, and HMBC spectroscopic data. The relative configurations of the stereogenic centers in **10** were corroborated by the NOE effects (Figure 1a) and $^3J_{H,H}$ coupling constants (Table 2). Coupling patterns of H-1 ($J_{7a,1} = J_{1,2} = 4.4$ Hz) and H-2 ($J_{1,2} = 4.4$ Hz, $J_{2,3} = 7.6$ Hz) and the NOE correlations between H-7a and H-1 and between H-7a and H-2 indicated that H-7a, H-1, and H-2 are on the same side of the ring, and H-2 and H-3 are in a pseudo-*trans*-axial position. The large coupling constant (7.6 Hz) observed between H-7a and H-7 suggested that they are in a pseudo-*trans*-axial position. The NOE correlations observed between the C-9 (CH_3) proton and the C-8 (CH_2OH) proton and between H-2 and the C-8 (CH_2OH) proton indicated that these side chains are on the same side of the molecule. Thus, alkaloid **10** was determined to be (1*S*,2*R*,3*R*,5*S*,7*R*,7a*R*)-3-hydroxymethyl-5-methyl-1,2,7-trihydroxypyrrolizidine, or its enantiomer, and has been designated hyacinthacine B_7.

Table 1. ^{13}C NMR Spectroscopic Data of Hyacinthacines **10**−**15** at 500 MHz in D_2O[a]

C	10	11	12	13	14	15
1	77.9	75.4	72.2	75.2	78.2	82.6
2	74.9	78.3	75.4	77.3	81.0	80.8
3	66.2	66.0	65.5	65.1	65.1	65.4
5	57.7	64.0	67.5	62.0	61.4	66.6
6	45.2	40.8	39.4	82.9	81.7	31.4
7	76.5	75.1	71.7	80.0	77.8	30.5
7a	69.9	70.4	79.9	67.3	69.2	70.7
8	66.8	66.6	61.7	66.4	65.7	65.1
9	18.4	64.2	61.8	16.0	15.7	28.5
10						39.0
11						70.9
12						24.6

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

The HRFABMS of alkaloids **11**−**14** showed the same molecular formula, $C_9H_{17}NO_5$. Alkaloids **11**−**14** gave a penta-*O*-$SiMe_3$ derivative, and the GC-MS analysis showed a characteristic fragment ion at *m*/*z* 564 $[M - CH_3]^+$ and a base peak at *m*/*z* 476 $[M - CH_2OSiMe_3]^+$. The ^{1}H and ^{13}C NMR spectroscopic features of **11** were similar to those of **12**. The ^{13}C NMR spectra of **11** and **12** revealed the presence of three methylenes and six methine carbon

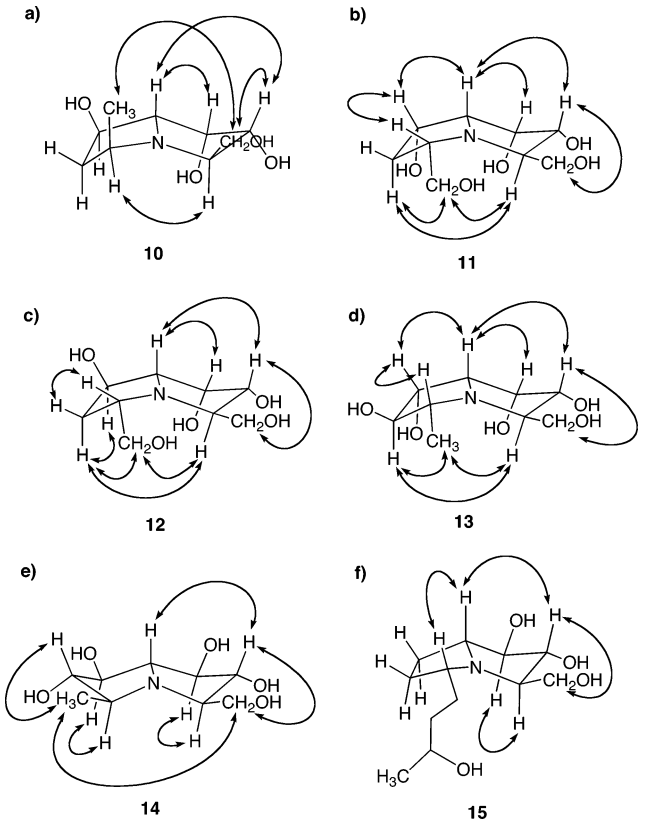


Figure 1. NOE interactions of hyacinthacines B_7 (**10**) (a), C_2 (**11**) (b), C_3 (**12**) (c), C_4 (**13**) (d), and C_5 (**14**) (e) and α-5-*C*-(3-hydroxybutyl)hyacinthacine A_2 (**15**) (f).

Table 2. ^{1}H NMR Spectroscopic Data of Hyacinthacine B_7 (**10**) and α-5-*C*-(3-Hydroxybutyl)hyacinthacine A_2 (**15**) at 500 MHz in D_2O[a]

position	**10**	**15**
1	4.35 t (4.4)[b]	3.76 t (6.9)
2	3.97 dd (4.4, 7.6)	3.96 t (6.9)
3	3.29 ddd (7.6, 5.5, 3.5)	2.18 ddd (5.0, 5.7, 6.9)
5	3.22 m	3.09 m
6α	1.68 m	1.62 m
6*β*	2.16 m	1.93 m
7α	4.50 m	1.87 m
7*β*		1.95 m
7α	3.45 dd (4.4, 7.6)	3.40 m
8	3.57 dd (3.5, 11.5)	3.73 dd (5.0, 12.0)
8′	3.63 dd (5.5, 11.5)	3.75 dd (5.7, 12.0)
9	1.25 d (7.0)	1.46 m
9′		1.95 m
10		1.46 m
10′		1.62 m
11		3.86 m
12		1.19 d (6.0)

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

atoms, as shown in Table 1. The signals in the ^{1}H and ^{13}C NMR spectra were assigned by HMQC, COSY, and HMBC NMR spectroscopic analyses. The methylene signals at *δ* 66.6 (C-8) and 64.2 (C-9) in **11** and those at *δ* 61.7 (C-8) and 61.8 (C-9) in **12** were attributed to the hydroxymethyl carbons attached to C-3 and C-5, respectively. The relative configurations at the stereogenic centers in **11** and **12** were corroborated by the NOE effects (Figures 1b, 1c) and $^3J_{H,H}$ coupling constants (Table 3). In alkaloid **11**, the coupling pattern (dd, $J_{1,2} = 4.4$, $J_{2,3} = 7.6$ Hz) of H-2 in the ^{1}H NMR spectrum suggested that H-1 and H-2 are in a *cis* arrangement, and H-2 and H-3 are a pseudo-*trans*-axial pair. The definite NOE interactions between H-7a and H-2, between H-7 and H-7a, and between H-5 and H-7 indicated that H-1, H-2, H-5, H-7, and H-7a are on the same side of the ring. Thus, alkaloid **11** was determined to be (1*S*,2*R*,3*R*,5*R*,7*S*,7a*R*)-3,5-dihydroxymethyl-1,2,7-trihydroxypyrrolizidine or its enantiomer and was designated hyacinthacine C_2. NOE correlations were observed between H-7 and H-5 in **11** but were absent in **12**. The NOE interaction between H-7 and H-6α in **12** suggests that H-7 is in the α-orientation. Consequently, alkaloid **12** was determined to be (1*S*,2*R*,3*R*,5*S*,7*R*,7a*R*)-3,5-dihydroxymethyl-1,2,7-trihydroxypyrrolizidine, or its enantiomer, and was designated hyacinthacine C_3.

The ^{1}H and ^{13}C NMR spectroscopic features of **13** and **14** were similar to those of hyacinthacine C_1, which has been found in *H. non-scripta*.[14] The signals in the ^{1}H and ^{13}C NMR spectra were assigned from analysis of the HMQC, COSY, and HMBC spectra. The relative configurations at the various stereogenic centers in **13** and **14** were corroborated by observed NOE effects (Figures 1d, 1e) and $^3J_{H,H}$ coupling constants (Table 3). In alkaloid **13**, the coupling patterns of H-1 (t, $J_{1,2} = J_{7a,1} = 4.0$ Hz) and H-2 (dd, $J_{1,2} = 4.0$, $J_{2,3} = 8.7$ Hz) and the strong NOE correlations between H-7a and H-1 and between H-7a and H-2 suggested that the orientations of H-7a, H-1, H-2, and H-3 are *β*, *β*, *β*, and α, respectively. Irradiation of H-3 at *δ* 3.38 enhanced the NOE intensity of the C-9 (CH_3) proton and H-6, and a strong NOE between H-5 and H-7 was also observed. From these results, it was found that H-1, H-2, H-5, H-7, H-7a, and the hydroxymethyl group on C-3 are on the same side of the ring, and H-3, H-6, and the methyl group on C-5 are on the opposite side. A large coupling constant (8.2 Hz) observed between H-7a and H-7 could be attributed to the dihedral angle between them being close to 0°. Thus, alkaloid **13** was determined to be (1*S*,2*R*,3*R*,5*R*,6*R*,7*R*,7a*R*)-3-hydroxymethyl-5-methyl-1,2,6,7-tetrahydroxypyrrolizidine, or its enantiomer, and was designated hyacinthacine C_4. In alkaloid **14**, the large coupling patterns of H-1 (t, $J = 7.6$ Hz), H-2 (t, $J = 7.6$ Hz), and H-6 (t, $J = 7.6$ Hz) indicated that H-1, H-2, H-3, H-5, H-6, H-7, and H-7a are all in pseudo-*trans*-axial positions. These configurations were also supported by the NOE correlations between H-7a and H-2, between H-1 and H-3, and between H-5 and H-7. Thus, alkaloid **14** was determined to be (1*R*,2*R*,3*R*,5*S*,6*S*,7*S*,7a*R*)-3-hydroxymethyl-5-methyl-1,2,6,7-tetrahydroxypyrrolizidine, or its enantiomer, and was designated hyacinthacine C_5.

Alkaloid **15** was determined to have the molecular formula $C_{12}H_{23}NO_4$ by HRFABMS. The ^{13}C NMR spectrum showed the presence of a single methyl (*δ* 24.6), five methylenes (*δ* 28.5, 30.5, 31.4, 39.0, 65.1), and six methine (*δ* 65.4, 66.6, 70.7, 70.9, 80.8, 82.6) carbon atoms (Table 1), which were very similar to those of α-5-*C*-(3-hydroxybutyl)hyacinthacine A_1 isolated from *S. peruviana*.[21] These results suggested that alkaloid **15** is an epimer of α-5-*C*-(3-hydroxybutyl)hyacinthacine A_1. The connectivity of the carbon and hydrogen atoms was defined from the COSY and HMBC NMR spectra. The ^{1}H−^{1}H COSY and HMBC spectra indicated that the presence of the hydroxymethyl carbon (*δ* 65.1) at C-3 and a 3-hydroxybutyl group at C-5. The relative configurations at the stereogenic centers in **15** were determined from NOE correlations and from the $^3J_{H,H}$ coupling constants (Table 2). Irradiation of H-7a enhanced the NOE signal intensity of H-2 and H-5. These results indicated that H-7a, H-2, and H-5 are on the same side of the ring, and H-1 and H-3, on the basis of an NOE between H-2 and the C-8 (CH_2OH) proton, are on the opposite side. Hence, alkaloid **15** was determined to be α-5-*C*-(3-hydroxybutyl)hyacinthacine A_2.

The IC_{50} values of the alkaloids isolated from *S. socialis* toward various glycosidases are shown in Table 4. *β*-1-*C*-Hydroxymethyl-1-deoxymannojirimycin (*β*-homomannojirimycin) is known to be a fairly potent inhibitor of α-glucosidases and α-L-fucosidases with IC_{50} values in the *μ*M range.[22] The new alkaloid, *β*-1-*C*-ethyl-1-deoxymannojirimycin (**5**), showed abolished or lowered inhibition toward these enzymes, but exhibited inhibitory activity toward bacterial *β*-glucosidase ($IC_{50} = 51$ *μ*M) and bovine liver *β*-galactosidase ($IC_{50} = 86$ *μ*M). We reported previously that most of

Table 3. ^{1}H NMR Spectroscopic Data of Hyacinthacines C_2 (**11**), C_3 (**12**), C_4 (**13**), and C_5 (**14**) at 500 MHz in D_2O[a]

position	11	12	13	14
1	4.16 t (4.4)[b]	4.32 t (4.4)	4.18 t (4.0)	4.01 t (7.6)
2	3.85 dd (4.4, 7.6)	4.04 dd (4.4, 9.5)	4.00 dd (4.0, 8.7)	3.80 t (7.6)
3	3.33 ddd (5.0, 5.7, 7.6)	3.50 m	3.38 dt (4.6, 8.7)	3.01 m
5	3.24 m	3.84 ol[c]	2.96 dq (6.9, 9.2)	2.81 dq (6.9, 7.6)
6a	1.78 m	1.93 m	3.90 dd (8.2, 9.2)	3.60 t (7.6)
6b	2.03 m	2.07 m		
7	4.40 m	4.56 ddd (2.5, 4.4, 2.5)	4.15 t (8.2)	3.99 t (7.6)
7a	3.39 dd (4.4, 6.9)	3.85 ol	3.57 dd (3.7, 8.2)	3.24 t (7.6)
8	3.54 dd (5.7, 12.0)	3.69 dd (3.2, 12.6)	3.65 d (4.6)	3.50 dd (5.3, 12.0)
8′	3.61 dd (5.0, 12.0)	3.85 dd (3.2, 12.6)	3.65 d (4.6)	3.55 dd (4.1, 12.0)
9	3.66 dd (5.0, 12.0)	3.79 dd (6.2, 12.0)	1.29 d (6.9)	1.13 d (6.9)
9′	3.85 dd (6.9, 12.0)	3.84 ol		

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

Table 4. Concentration of β-1-*C*-Ethyldeoxynojirimycin (**5**) and Hyacinthacines (**10**−**15**) Giving 50% Inhibition of Glycosidases

	IC_{50} (μM)						
enzyme	5	10	11	12	13	14	15
α-glucosidase							
rice	NI	NI	NI	NI	110	NI	NI
rat intestinal maltase	NI	NI	NI	NI	45	77	NI
β-glucosidase							
C. saccharolyticum	51	NI	13	25	660	48	NI
α-galactosidase							
coffee beans	NI	NI	NI	NI	NI	NI	NI
β-galactosidase							
bovine liver	86	NI	370	52	890	900	NI
α-mannosidase							
jack beans	NI	NI	NI	NI	NI	NI	NI
α-L-fucosidase							
human placenta	83	NI	17	NI	NI	NI	NI
amyloglucosidase							
Aspergillus niger	94	270	550	870	57	57	180

[a] NI: no inhibition (less than 50% inhibition at 1000 μM).

hyacinthacines are weak or moderate inhibitors of glycosidases.[14,15,20] Hyacinthacine B_2 is a weak inhibitor of bacterial β-glucosidase and bovine liver β-galactosidase, with IC_{50} values of 490 and 160 μM, respectively.[14] Introduction of an OH group to C-7α in hyacinthacine B_2 to give hyacinthacine C_2 (**11**) and to C-7β to give hyacinthacine C_3 (**12**) enhanced their inhibitory potential toward bacterial β-glucosidase, which showed IC_{50} values of 13 and 84 μM, respectively. Furthermore, hyacinthacine C_3 is also a good inhibitor of bovine liver β-galactosidase (IC_{50} = 52 μM). Introduction of an OH group at C-6β in hyacinthacine B_5[20] to give hyacinthacine C_4 (**13**) enhanced its inhibitory potential toward intestinal maltase and amyloglucosidase, while hyacinthacine C_5 (**14**) was found to be a better inhibitor of bacterial β-glucosidase than hyacinthacine C_4.

We have reported previously that α-5-*C*-(1,3-dihydroxybutyl)-hyacinthacine A_1 (**16**) and α-5-*C*-(1,3,4-trihydroxybutyl)hyacinthacine A_1 both show a potent inhibitory activity toward bacterial β-glucosidase (IC_{50} = 5.1 and 11.4 μM, respectively).[21] Furthermore, broussonetine N, which has a C_{10} side chain at C-5α of hyacinthacine A_2, is well known as a potent inhibitor of β-glucosidase and β-galactosidase.[24] Although alkaloid **15** also can be regarded as the α-5-*C*-(3-hydroxybutyl) derivative of A_2, it lost inhibitory activity completely. These results reveal that not only the presence of a long side chain at C-5α but also the number of OH groups in the side chain play very important roles in the inhibition of these compounds toward β-glucosidase.

Experimental Section

General Experimental Procedures. 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 Bruker DRX500 spectrometer. Chemical shifts are expressed in ppm downfield from sodium 3-(trimethylsilyl)propionate (TSP) in D_2O as internal standard. The assignment of proton and carbon NMR signals was determined from extensive homonuclear decoupling experiments and the DEPT, ^{1}H−^{1}H COSY, HMQC, and HMBC spectroscopic data. FABMS were measured using glycerol as a matrix on a JEOL JMS-700 spectrometer. For GC-MS analyses, samples were dried and prepared as pertrimethylsilyl ethers at 60 °C for 20 min using Tri-Sil reagent (Pierce Biotechnology Inc., Rockford, IL). The column was a 25 m × 0.25 mm VF-5ms "Factor Four" (film thickness, 0.25 μm) capillary column (Varian Inc.), and the 25 min temperature program ran from 160 to 300 °C with an initial rate of increase of 10 °C/min and then held at 300 °C. The mass spectrometer was a Perkin-Elmer TurboMass Gold, with a quadrupole ion filter system, which was run at 250 °C constantly during analysis, and the mass range was set to 100 to 650 amu. 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.

Plant Material. Plants of *Scilla socialis* Baker (syn. *Ledebouria socialis* Jessop), Hyacinthaceae, were purchased in July 2003 from Wyevale Limited (Hereford, U.K.) and grown in heated greenhouses. A voucher specimen (no. RJN200401) is deposited at the herbarium of the Institute of Grassland and Environmental Research, Aberystwyth, U.K.

Extraction and Isolation. The bulbs (2.3 kg) of *S. socialis* were homogenized in 50% aqueous EtOH. The filtrate was applied to a column of Amberlite IR-120B (2000 mL, H^+ form). The 0.5 M NH_4-OH eluate was concentrated to give a brown oil (43.3 g), which was applied to Dowex 1-X2 (OH^- form) to remove amino acids and pigments, and eluted with H_2O. This eluate was concentrated and chromatographed over an Amberlite CG-50 column (2.0 × 55 cm, NH_4^+ form) with H_2O as eluant (fraction size 8 mL). Fractions were divided into three pools: I (fractions 22−46, 13.3 g), II (fractions 47−84, 5.3 g), and III (fractions 85−128, 3.7 g). Each pool was further chromatographed with Dowex 1-X2 (OH^- form) with H_2O as eluant and/or aluminum oxide with 100−60% acetone as eluants to give alkaloids **1** (1014 mg), **4** (224 mg), **11** (259 mg), and **15** (154 mg) from pool I, **2** (526 mg), **3** (4 mg), **5** (50 mg), **12** (5 mg), **13** (151 mg), **14** (7 mg), and **16** (140 mg) from pool II, and **6** (148 mg), **7** (27 mg), **8** (30 mg), **9** (31 mg), and **10** (130 mg) from pool III.

β-1-*C*-Ethyl-1-deoxymannojirimycin (5): colorless powder; $[\alpha]_D$ +5.0 (*c* 0.19, H_2O); ^{1}H NMR (500 MHz, D_2O) δ 0.97 (3H, t, J = 7.6 Hz, C-8), 1.60 (2H, m, H-7a, H-7b), 2.79 (1H, m, H-2), 2.98 (1H, m, H-6), 3.66 (1H, t, J = 9.5 Hz, H-3), 3.76 (1H, dd, J = 3.1, 9.5 Hz, H-4), 3.79 (2H, d, J = 3.5 Hz, H-1a, H-1b), 3.98 (1H, t, J = 2.5 Hz, H-5); ^{13}C NMR (500 MHz, D_2O) δ 74.1 (C-4), 73.7 (C-5), 71.2 (C-3), 63.4 (C-1), 62.1 (C-6), 57.8 (C-2), 24.3 (C-7), 13.4 (C-8); HRFABMS *m*/*z* 192.1237 $[M + H]^+$ ($C_8H_{18}NO_4$ requires 192.1236).

Hyacinthacine B_7 [(1*S,2*R**,3*R**,5*S**,7*R**,7a**R*)-3-hydroxymethyl-5-methyl-1,2,7-trihydroxypyrrolizidine] (10):** colorless powder; $[\alpha]_D$ −4.4 (*c* 0.20, H_2O); ^{1}H NMR, see Table 2; ^{13}C NMR, see Table 1; HRFABMS *m*/*z* 204.1233 $[M + H]^+$ ($C_9H_{18}NO_4$ requires 204.1236).

Hyacinthacine C_2 [(1*S,2*R**,3*R**,5*R**,7*S**,7a**R*)-3,5-dihydroxymethyl-1,2,7-trihydroxypyrrolizidine] (11):** colorless powder; $[\alpha]_D$ +12.9 (*c* 0.22, H_2O); ^{1}H NMR, see Table 3; ^{13}C NMR, see Table 1; HRFABMS *m*/*z* 220.1187 $[M + H]^+$ ($C_9H_{18}NO_5$ requires 220.1185).

Hyacinthacine C_3 [(1*S,2*R**,3*R**,5*S**,7*R**,7a**R*)-3,5-dihydroxymethyl-1,2,7-trihydroxypyrrolizidine] (12):** colorless powder; $[\alpha]_D$

+3.5 (*c* 0.23, H_2O); ^1H NMR, see Table 3; ^{13}C NMR, see Table 1; HRFABMS *m*/*z* 220.1186 $[M + H]^+$ ($C_9H_{18}NO_5$ requires 220.1185).

Hyacinthacine C_4 [(1*S,2*R**,3*R**,5*R**,6*R**,7*R**,7a**R*)-3-hydroxymethyl-5-methyl-1,2,6,7-tetrahydroxypyrrolizidine] (13):** colorless powder; $[\alpha]_D$ −37.9 (*c* 0.44, H_2O); ^1H NMR, see Table 3; ^{13}C NMR, see Table 1; HRFABMS *m*/*z* 220.1186 $[M + H]^+$ ($C_9H_{18}NO_5$ requires 220.1185).

Hyacinthacine C_5 [(1*R,2*R**,3*R**,5*S**,6*S**,7*S**,7a**R*)-3-hydroxymethyl-5-methyl-1,2,6,7-tetrahydroxypyrrolizidine] (14):** colorless powder; $[\alpha]_D$ +1.5 (*c* 0.22, H_2O); ^1H NMR, see Table 3; ^{13}C NMR, see Table 1; HRFABMS *m*/*z* 220.1183 $[M + H]^+$ ($C_9H_{18}NO_5$ requires 220.1185).

α-5-*C*-(3-Hydroxybutyl)hyacinthacine A_2 (15): colorless syrup; $[\alpha]_D$ +1.2 (*c* 0.25, H_2O); ^1H NMR, see Table 2; ^{13}C NMR, see Table 1; HRFABMS *m*/*z* 246.1707 $[M + H]^+$ ($C_{12}H_{24}NO_4$ requires 246.1705).

Glycosidase Inhibitory Activities. The enzymes α-glucosidase (from rice, assayed at pH 5.0; from yeast, assayed at pH 6.8), *β*-glucosidase (from *C. saccharolyticum*, pH 5.0), α-galactosidase (from coffee bean, pH 6.5), *β*-galactosidase (from bovine liver, pH 6.8), α-mannosidase (from jack beans, pH 4.5), α-L-fucosidase (from human placenta, pH 5.5), amyloglucosidase (from *Aspergillus niger*, pH 4.5), *p*-nitrophenyl glycosides, and various disaccharides were purchased from Sigma Chemical Co. Brush border membranes were prepared from the rat small intestine according to the method of Kessler et al.[25] and were assayed at pH 5.8 for rat intestinal maltase using maltose. For rice α-glucosidase and rat intestinal maltase activities, 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 the resulting reaction mixture was added to 3 mL of the Wako glucose CII-test (Wako Pure Chemical Ind., Osaka, Japan). The absorbance at 505 nm was measured to determine 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.

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NP0700826