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Polyhydroxylated pyrrolidine and pyrrolizidine alkaloids from *Hyacinthoides non-scripta* and *Scilla campanulata*

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Abstract

Aqueous ethanol extracts from the immature fruits and stalks of bluebell (*Hyacinthoides non-scripta*) were subjected to various ion-exchange column chromatographic steps to give 1,4-dideoxy-1,4-imino-D-arabinitol (**1**), 2(*R*),5(*R*)-bis(hydroxymethyl)-3(*R*),4(*R*)-dihydroxypyrrolidine (DMDP) (**2**), 6-deoxy-6-*C*-(2,5-dihydroxyhexyl)-DMDP (**3**), 2,5-dideoxy-2,5-imino-DL-*glycero*-D-*manno*-heptitol (homoDMDP) (**4**), homoDMDP-7-*O*-apioside (**5**), homoDMDP-7-*O*-β-D-xylopyranoside (**6**), (1*S**,2*R**,3*R**,5*R**,7a*R**)-1,2-dihydroxy-3,5-dihydroxymethylpyrrolizidine (**7**), and (1*S**,2*R**,3*R**,5*R**,6*R**,7*R**,7a*R**)-3-hydroxymethyl-5-methyl-1,2,6,7-tetrahydroxypyrrolizidine (**8**). Bulbs of *Scilla campanulata* (Hyacinthaceae) yielded (1*S**,2*R**,3*R**,5*S**,7a*R**)-1,2-dihydroxy-3,5-dihydroxymethylpyrrolizidine (**9**) in addition to compounds **1**–**7**. Compounds **3**, **6**, **7**, **8**, and **9** are new natural products. Compound **4** is a potent competitive inhibitor with K_i values of 1.5 μM for *Caldocellum saccharolyticum* β-glucosidase and 2.2 μM for bovine liver β-galactosidase. The 7-*O*-β-D-xyloside **6** was a stronger competitive inhibitor than **4** of *C. saccharolyticum* β-glucosidase and rat intestinal lactase, with K_i values of 0.06 and 0.07 μM, respectively, but a weaker inhibitor of bovine liver β-galactosidase. Furthermore, compound **4** is also a competitive inhibitor ($K_i = 1.8$ μM) of porcine kidney trehalase, but **6** was inactive against this enzyme.

Keywords: *Hyacinthoides non-scripta*; *Scilla campanulata*; Hyacinthaceae; 2,5-Dideoxy-2,5-imino-DL-*glycero*-D-*manno*-heptitol (homoDMDP); homoDMDP-7-*O*-β-D-xylopyranoside; β-Galactosidase and β-glucosidase inhibitor

1. Introduction

Glycosidase-inhibiting pyrrolidine alkaloids were recently reported from the leaves of bluebells, *Hyacinthoides non-scripta* (Hyacinthaceae), as the candidate compounds of livestock poisonings by grazing on this species [1]. Those alkaloids are 1,4-dideoxy-1,4-imino-D-arabinitol (DAB) (**1**), 2(*R*),5(*R*)-bis(hydroxymethyl) - 3(*R*),4(*R*) - dihydroxypyrrolidine (DMDP) (**2**), 2,5-dideoxy-2,5-imino-DL-*glycero*-D-*manno*-heptitol (homoDMDP) (**4**), and homoDMDP-7-*O*-apioside (**5**). We more recently reported that **4** also occurred in the

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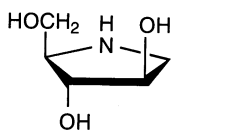
bulbs of *Hyacinthus orientalis* and was a potent inhibitor of *Caldocellum saccharolyticum* β-glucosidase ($IC_{50} = 3.8$ μM), bovine liver β-galactosidase ($IC_{50} = 4.4$ μM), and rat intestinal trehalase ($IC_{50} = 2.2$ μM) [2]. Compound **1** is a good inhibitor with a broad inhibitory spectrum toward mammalian glycosidases, such as endoplasmic reticulum (ER) α-glucosidase II, Golgi α-mannosidases I and II, intestinal isomaltase, and trehalase, and **2** is also a potent inhibitor of mammalian β-galactosidases [3]. In addition to these alkaloids isolated from bluebells, other related alkaloids were also detected in the leaves by GLC–MS and these could also be involved in livestock poisonings. In a search for polyhydroxylated alkaloids in other species of the Hyacinthaceae by GLC–MS, we found the occurrence of similar alkaloids in the bulbs of *Scilla campanulata*. In this paper we report the isolation of alkaloids from the fruits and stalks of bluebells and from the bulbs of *S. campanulata*, their structural determination and glycosidase inhibitory activity.



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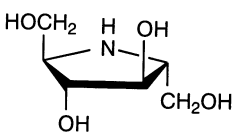

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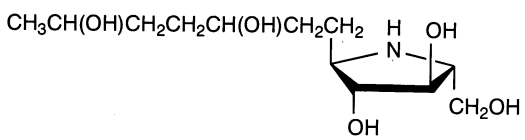

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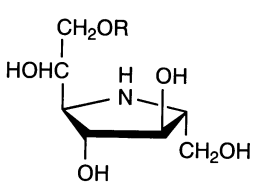
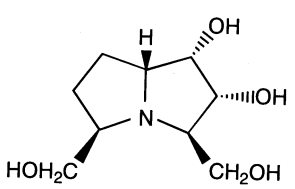


4 R = H
5 R = apiosyl
6 R = β-D-xylosyl



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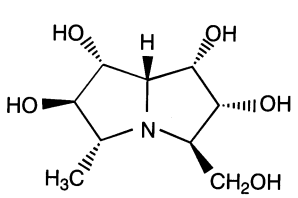

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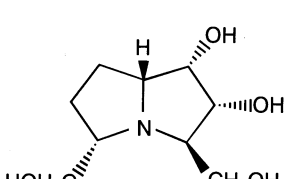

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2. Results

GLC–MS analysis of the extract of bluebells.—50% EtOH extracts of the immature fruits and stalks of bluebells and the bulbs of *S. campanulata* were silylated with Sigma Sil-A and analyzed by GLC–MS after preliminary purification using ion-exchange resins of Amberlite IR-120B (H^+) and Dowex 1-X2 (OH^-). GLC analysis of the trimethylsilylated resin-treated extract of the bulbs of *S. campanulata* is shown in Fig. 1. Two major components **2** and **4** in Fig. 1, with retention times of 4.89 and 7.85 min, were identified as DMDP and homoDMDP, respectively. Compound **2** gave a tetra-*O*-trimethylsilyl (tetra-*O*-$SiMe_3$) derivative and characteristic fragment ions at *m/z* 436 and 348 caused by loss of CH_3 and CH_2O–$SiMe_3$, respectively, while **4** gave a penta-*O*-$SiMe_3$ derivative and characteristic fragment ions at *m/z* 538 $[M - CH_3]^+$ and 450 $[M - CH_2OSiMe_3]^+$. GLC analysis of the trimethylsilylated resin-treated extract of the bluebell material also showed the presence of DMDP and homoDMDP as the major components.

Isolation and purification of alkaloids.—A 50% EtOH extract of the fruiting stalks (2 kg) of bluebells was chromatographed with various ion-exchange resins to give compounds **1** (57 mg), **2** (567 mg), **3** (20 mg), **4** (242 mg), **5** (15 mg), **6** (32 mg), **7** (23 mg), and **8** (22 mg). A 50% EtOH extract of the bulbs (1.3 kg) of *S. campanulata* was also chromatographed in a similar manner to give compounds **1** (29 mg), **2** (1 g), **3** (38 mg), **4** (239 mg), **5** (80 mg), **6** (129 mg), **7** (29 mg), and **9** (6 mg). The ^1H and ^{13}C NMR spectra of compounds **1**, **2**, **4**, and **5** were completely in accord with those of DAB, DMDP, homoDMDP, and homoDMDP-7-*O*-apioside, respectively.

Structural determination of compound **3**.—The ^{13}C NMR spectral analysis of compound **3** revealed the presence of five methylene and seven methine carbon atoms. This result and HRFABMS (*m/z* 264.1802 $[M + H]^+$) established that the molecular formula was $C_{12}H_{25}NO_5$. Compound **3** gave a penta-*O*-$SiMe_3$ derivative and characteristic fragment ions at *m/z* 608 $[M - CH_3]^+$ and 520 [M –

$CH_2OSiMe_3]^+$. The ^1H NMR spectral data, combined with extensive decoupling experiments and 2D $^1H-^{13}C$ COSY spectral data, defined the complete connectivity of the carbon and hydrogen atoms. From these NMR spectral data in D_2O, the methylene triplet at δ 64.6 (C-1) was attributed to the hydroxymethyl carbon, with the methine doublets at δ 70.8, 74.1, 80.4, and 84.0 assigned to C-11, C-8, C-3, and C-4 bearing the OH groups, respectively. The relatively high-field methine doublets at δ 63.1 (C-5) and 64.4 (C-2) indicated that they must be bonded to the nitrogen of the heterocyclic ring. The relatively large coupling constants ($J_{2,3} = J_{3,4} = 7.1$ Hz, $J_{4,5} = 7.8$ Hz) of the pyrrolidine ring protons suggested the all-trans configuration of H-2, H-3, H-4, and H-5, consistent with those reported for the all-trans configuration of 6-deoxyhomoDMDP [4]. The relative configuration at the stereogenic centers on the pyrrolidine ring was also corroborated by NOEs between H-2 and H-4 and between H-3 and H-5. Thus, compound **3** was determined to be 6-deoxy-6-*C*-(2,5-dihydroxyhexyl)-DMDP or its enantiomer. It was not possible to determine the relative configuration of **3** at C-8 and C-11 from the NMR data.

Structural determination of compound **6**.— The HRFABMS (*m*/*z* 326.1464 $[M+H]^+$) and 12 resonances in the ^{13}C NMR spectrum (in D_2O) of **6** established that the molecular formula was $C_{12}H_{23}NO_9$. The response to the naphthoresorcinol–sulfuric acid reagent and the characteristic anomeric proton (H-1′, δ 4.45, $J_{1',2'}$ 7.8 Hz) and carbon (C-1′, δ 106.3) signals in the NMR spectrum suggested that **6** was a glycoside of an alkaloid. After acid hydrolysis of this glycoside using Dowex 50W-X2 (H^+), the aglycone part was displaced from the resin with 0.5 M NH_4OH, concentrated to dryness, and confirmed as homoD-

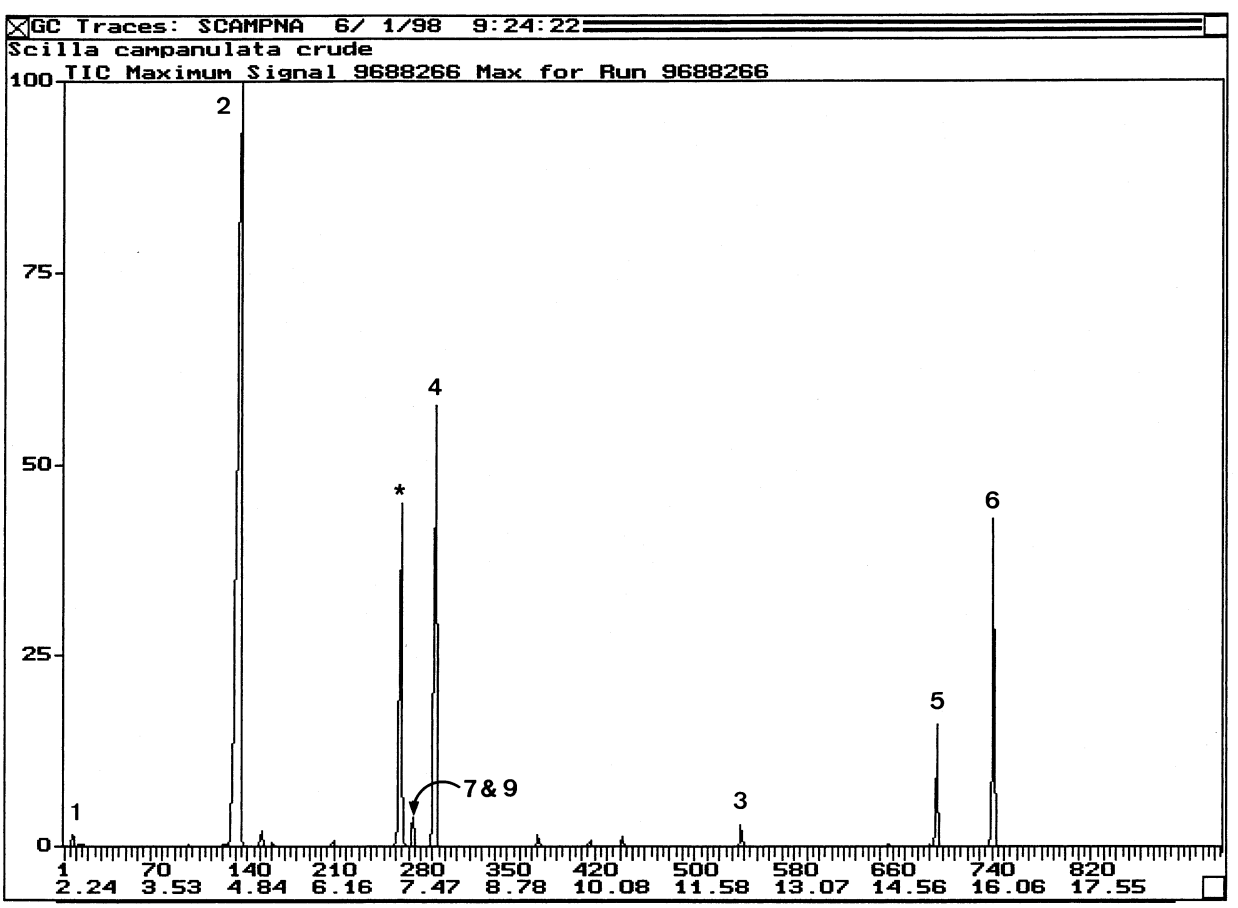

Fig. 1. GLC analysis of the *O*-trimethylsilylated resin-treated extract of *S. campanulata*. Peak (*) represents a component of unknown composition.

MDP (**4**) by direct comparison of its ^{13}C NMR spectrum with that of an authentic sample. The various 1D and 2D NMR spectral data in D_2O defined the complete connectivity of the carbon and hydrogen atoms. From the chemical shift and the coupling constant of the anomeric proton, the type of glycosidic linkage was determined to be β. Irradiation of H-1′ enhanced the NOE signal intensity of H-7a, H-3′, and H-5′ax. The NOE between H-1′ and H-7a and an 8.4 ppm downfield shift for C-7 determined that the position of a glycosidic linkage was at C-7. The NOEs mentioned above and the large vicinal coupling constants ($J_{2',3'} = J_{3',4'} = 9.3$ Hz, $J_{4',5'ax} = 10.5$ Hz) were indicative of the xylopyranoside. Thus, the structure of **6** was shown to be homoDMDP-7-*O*-β-D-xylopyranoside.

Structural determination of compound **7**.— The ^{13}C NMR spectral data of compound **7** revealed the presence of four methylene and five methine carbon atoms. This result and HRFABMS (*m/z* 204.1235 $[M + H]^+$) established that the molecular formula was $C_9H_{17}NO_4$. Compound **7** gave a tetra-*O*-SiMe_3 derivative and a characteristic fragment ion at *m/z* 476 $[M - CH_3]^+$ and a base peak at *m/z* 388 $[M - CH_2OSiMe_3]^+$. The ^1H NMR spectral data, combined with extensive decoupling experiments and 2D ^1H–^{13}C COSY spectral data, defined the complete connectivity of the carbon and hydrogen atoms. From these NMR results in CD_3OD, the methylene triplets at δ 64.7 (C-8) and 66.5 (C-9) were attributed to the hydroxymethyl carbons. The methine doublets at δ 73.8 and 76.8 were assigned to C-1 and C-2 bearing the OH groups, respectively, due to the appearance in the low-field region of H-1 (δ 3.81, $J_{1,2} = J_{1,7a} = 3.9$ Hz) and H-2 (δ 3.91, $J_{1,2}$ 3.9, $J_{2,3}$ 8.8 Hz). The two methines at δ 70.7 (C-5) and 71.7 (C-3) of the remaining three methine carbons indicated that they must be bonded to the nitrogen of the heterocyclic ring, with the hydroxymethyl group, and the last one at δ 68.5, with the doublet of double doublets at δ 3.55 in the ^1H NMR spectrum, was identified as the bridgehead C-7a. These data suggest that **7** has a pyrrolizidine ring system. The relative configurations at the stereogenic centers in **7** were determined by extensive NOE experiments. The NOE interactions are shown in Fig. 2. The definite NOEs between H-3 and H-5 and between H-5 and H-7α indicate that H-3, H-5, and H-7α are on the same side of the ring. Irradiation of the lowest field H-2 at δ 3.91 enhanced the NOE intensity of H-7a and H-8, and strong NOEs between H-1 and H-7a, between H-7a and H-6β, and between H-6β and H-9 were also observed. From these NOE interactions, H-1, H-2, H-7a, and the hydroxymethyl groups on C-3 and C-5 were found to be on the same side of the ring. Thus, compound **7** was determined to be (1*S*,2*R*,3*R*,5*R*,7a*R*)-1,2-dihydroxy-3,5-dihydroxymethylpyrrolizidine or its enantiomer, and it was named hyacinthacine B_1.

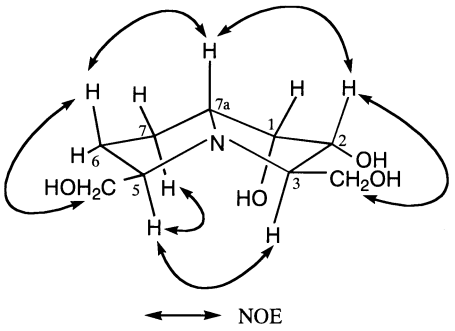

Fig. 2. NOE interactions for hyacinthacine B_1 (**7**).

Structural determination of compound **8**.— The ^{13}C NMR spectral data of compound **8** revealed the presence of one methyl, one methylene and seven methine carbon atoms. This result and HRFABMS (*m/z* 220.1186 $[M + H]^+$) established that the molecular formula was $C_9H_{17}NO_5$. Compound **8** gave a penta-*O*-SiMe_3 derivative and a characteristic fragment ion at *m/z* 565 $[M - CH_3]^+$ and a base peak at *m/z* 477 $[M - CH_2OSiMe_3]^+$. The ^1H NMR spectral data, combined with extensive decoupling experiments and 2D ^1H–^{13}C COSY spectral data, defined the complete connectivity of the carbon and hydrogen atoms. From these NMR spectral data in D_2O, the four methine carbons at δ 74.6, 75.7, 77.1, and 77.7 were assigned to C-1, C-7, C-6, and C-2 bearing the OH groups, respectively, and the three methines at δ 60.4, 64.7, and 68.3 were attributed to C-5, C-3, and C-7a, respectively, which must be bonded to the nitrogen of the heterocyclic ring. The

methylene carbon at δ 65.9 and the methyl carbon at δ 12.8 were attributed to C-8 and C-9 bonded to C-3 and C-5, respectively. Thus, compound **8** was found to be a fully substituted pyrrolizidine alkaloid. As shown in Fig. 3, the relative configurations at the stereogenic center in **8** were determined by extensive NOE experiments at 70 °C. The enhancement of the NOE signal intensity of H-3 and H-6 by irradiation of the methyl protons indicates that H-3, H-6, and the methyl group at C-5 are on the same side of the ring. From the result of NOE interactions shown in Fig. 3, it was found that H-1, H-2, the hydroxymethyl group at C-3, H-5, H-7, and H-7a are on the same side of the ring. Thus, compound **8** 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 it was named hyacinthacine C_1.

Structural determination of compound **9**.— Compound **9** was found to be an isomer of **7** from its HRFABMS, GLC–MS, and ^{13}C NMR spectral data. The *O*-trimethylsilyl derivatives of **7** and **9** were not separated by GLC, with the same retention time of 7.51 min (Fig. 1). The NOE interactions for **9** are shown in Fig. 4. From the definite NOE between H-3 and the hydroxymethyl group on C-5, **9** was determined to be an epimer at C-5 of **7**, (1*S*,2*R*,3*R*,5*S*,7a*R*)-1,2-dihydroxy-3,5-dihydroxymethylpyrrolizidine or its enantiomer, and it was named hyacinthacine B_2.

Glycosidase inhibitory activities of alkaloids.—The IC_{50} values of the natural alkaloids against various glycosidases are shown in Table 1. We have reported that homoDMDP (**4**) is a potent inhibitor of β-glucosidase, β-galactosidase, and trehalase [1,2]. The present work revealed that **4** was an inhibitor of the competitive type for these enzymes, with K_i values of 1.5 μM (*C. saccharolyticum* β-glucosidase), 2.2 μM (bovine liver β-galactosidase), and 1.8 μM (porcine kidney trehalase). The introduction of the apiosyl group to C-7 in **4** abolished its inhibition toward β-glucosidase and trehalase, although it slightly enhanced inhibitory potential toward rat intestinal lactase and *Aspergillus niger* amyloglucosidase. The introduction of the β-xylosyl group to the same position of **4** also abolished its inhibition of trehalase, but activities toward *C. saccharolyticum* β-glucosidase and rat intestinal lactase were significantly enhanced (Table 1). Compound **6** inhibited *C. saccharolyticum* β-glucosidase and rat intestinal lactase in a competitive manner, with K_i values of 0.06 and 0.07 μM, respectively (Fig. 5). The new pyrrolidine **3** was a weak inhibitor of α-L-fucosidase and amyloglucosidase.

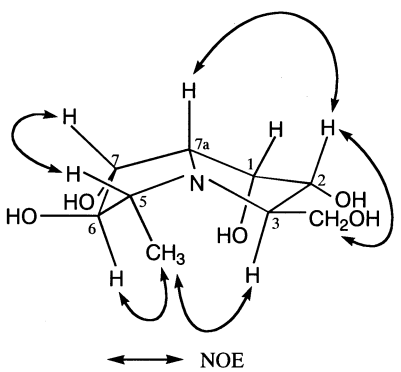

Fig. 3. NOE interactions for hyacinthacine C_1 (**8**).

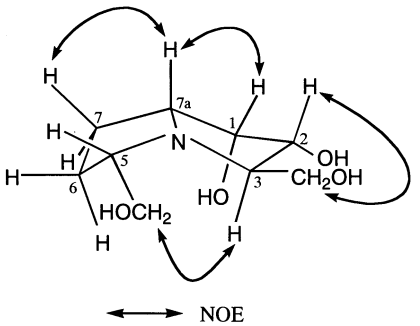

Fig. 4. NOE interactions for hyacinthacine B_2 (**9**).

Polyhydroxylated pyrrolizidines **7** and **8** were weak inhibitors of β-glucosidase and β-galactosidase, and amyloglucosidase, respectively. Interestingly, the inversion of the hydroxymethyl group at C-5 in **7** markedly enhanced its inhibition only toward rat intestinal lactase, with an IC_{50} value of 3.6 μM, and its inhibition was the competitive type, with a K_i value of 0.97 μM (Fig. 6).

3. Discussion

Bluebell and *S. campanulata* contain high concentrations of DMDP (**2**), which is the major alkaloid, and homoDMDP (**4**) is the

second most abundant alkaloid in both plants. Compound **2** is a potent inhibitor of mammalian β-galactosidase with an IC_{50} value in the micromolar range, while **4** is a potent inhibitor of β-glucosidase, β-galactosidase, and trehalase [1,2]. In both plants **4** also occurs as an apioside and a xyloside, which exhibit potent inhibitory activity against β-galactosidase and/or β-glucosidase. Compound **1**, which lacks one hydroxymethyl group from **2**, is a good inhibitor of α-glucosidases, trehalase, and α-mannosidases because of its good superimposition on the glucosyl and mannosyl cations [3]. However, it is not usually easy to predict whether it will inhibit a particular glycosidase from the configuration of the hydroxyl groups and the difference of the side chain on the pyrrolidine and pyrrolizidine rings. The potencies of inhibition for baker's yeast α-glucosidase in **1** and **2** are the same, with the IC_{50} value of 0.7 μM, whereas **4** is a poor inhibitor ($IC_{50} = 290$ μM) of this enzyme.

According to a general mechanism proposed for glycosidase action, the surrounding environment of the two carboxylic acid groups in the active site of the enzyme differs: one group holds the proton at catalytic pH before it is transferred to the glycosidic oxygen: the other group is ionized at catalytic pH, consistent with its role in electrostatic stabilization of the glycosyl cation intermediate. It is attractive to speculate that the strong affinity of **4** and **6** for β-glucosidase and β-galactosidase is due to the protonation of the OH group at C-6 by an acidic group (AH) in the active site of the enzyme, in place of the β-glycosidic oxygen (Fig. 7). In fact, the deoxygenation at C-6 in **4** to give 6-deoxy-homoDMDP markedly decreases its inhibitory activity toward *C. saccharolyticum* β-glucosidase ($IC_{50} = 380$ μM) and rat intestinal lactase ($IC_{50} = 320$ μM), but it generates a potent inhibitory activity toward α-glucosidases [2]. Thus, enzyme inhibitors can often provide information about the mechanism of action and chemical topography of the active sites of enzymes.

4. Experimental

General methods.—The purity of samples was checked by HPTLC on Silica Gel $60F_{254}$ (E. Merck) using the solvent system 4:1:1 PrOH–AcOH–H_2O, and a chlorine–*o*-tol-

Table 1
Concentration of alkaloids giving 50% inhibition of glycosidase activities

Enzyme	IC_{50} (μM)						
	3	**4**	**5**	**6**	**7**	**8**	**9**
α-Glucosidase							
Rice	NI[a]	130	NI	NI	NI	NI	NI
Rat intestinal maltase	NI	400	NI	NI	NI	NI	NI
β-Glucosidase							
Almond	NI	23	NI	4.6	320	NI	100
C. saccharolyticum	NI	3.8	NI	0.34	520	NI	490
β-Galactosidase							
Bovine liver	NI	4.4	40	24	110	NI	160
Rat intestinal lactase	NI	4.0	1.6	0.18	270	NI	3.6
β-Mannosidase							
Snail	NI	400	NI	140	NI	NI	NI
Trehalase							
Porcine kidney	NI	5.0	NI	NI	NI	NI	NI
α-L-Fucosidase							
Bovine epididymis	110	NI	NI	NI	NI	NI	NI
Amyloglucosidase							
A. niger	40	180	60	100	NI	84	NI

[a] No inhibition (less than 50% inhibition at 1000 μM).

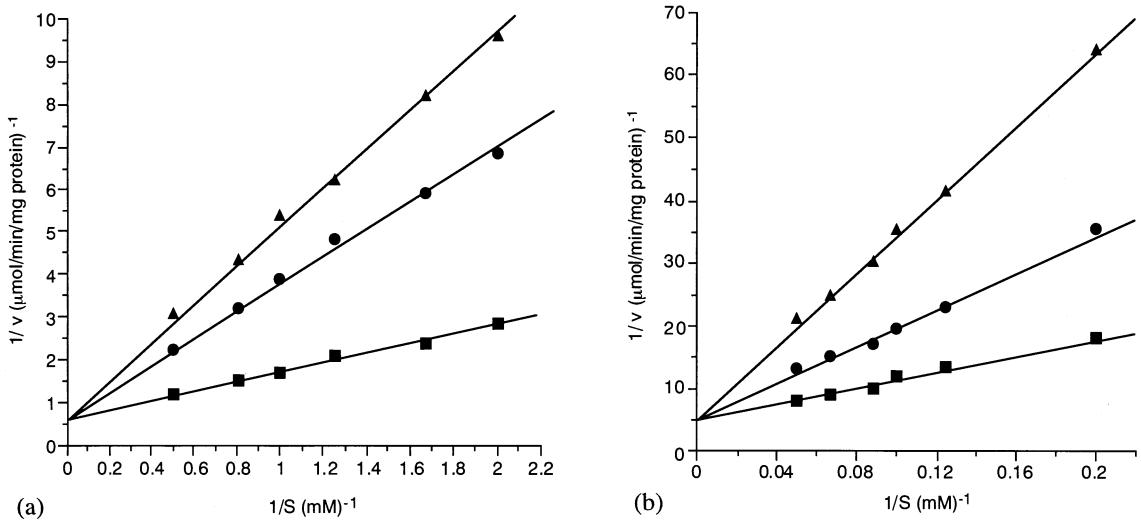

Fig. 5. Lineweaver–Burk plots of homoDMDP-7-*O*-β-D-xylopyranoside (**6**) inhibition of *C. saccharolyticum* β-glucosidase (a) and rat intestinal lactase (b). The increasing concentrations of substrate were used to determine the K_m and K_i values and the data were plotted as $1/V$ vs. $1/S$. (a) Concentrations of **6** were 0 (■), 0.1 μM (●), and 0.2 μM (▲). The calculated K_m and K_i values were 1.6 mM and 0.06 μM, respectively. (b) Concentrations of **6** were 0 (■), 0.1 μM (●), and 0.25 μM (▲). The calculated K_m and K_i values were 13.0 mM and 0.07 μM, respectively.

idine reagent or iodine vapor was used for detection. Optical rotations were measured with a Jasco DIP-370 digital polarimeter. ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were recorded on a Jeol JNM-GX 400 spectrometer. Chemical shifts are expressed in ppm downfield from sodium 3-(trimethylsilyl)propionate (TSP) in D_2O and Me_4Si (TMS) in CD_3OD as internal standards. MSs were measured on a Jeol JMS-SX 102A spectrometer.

GLC–MS analyses.—Samples were dried and silylated at 20 °C for 60 min using 100 μL of Sigma Sil-A (Sigma Chemical Co.) per milligram of material. The column was a 25-m × 0.25 mm BPX5 (film thickness, 0.25 μm) capillary column (SGE), and the 25-min temperature program ran from 180 to 300 °C with an initial rate of increase of 10 °C/min and then held at 300 °C. The mass spectrometer was a Perkin–Elmer QMASS 910 set at 70 eV and a mass range of 100–650 amu.

Extraction and isolation.—A 50% EtOH extract of the immature fruits and stalks (2 kg) of bluebells (*H. non-scripta*) was applied to a column of Dowex 50W-X2 (500 mL, H^+ form). The 0.5 M NH_4OH eluate was concentrated to give a brown oil (13.2 g), which was chromatographed over a Dowex 1-X2 (2.5 × 86 cm, OH^- form) with H_2O as eluant (fraction size 15 mL). The H_2O eluate was divided into three pools: A (Fractions 29–33), B (Fractions 34–45), and C (Fractions 53–100). The MeOH eluate from the same column was designated Pool D. Each pool was further chromatographed on an Amberlite CG-50 (1.9 × 92 cm, NH_4^+ form) column with H_2O as eluant to give **3** (20 mg) from Pool A, **1** (57 mg), **2** (567 mg), **4** (242 mg), and **7** (23 mg)

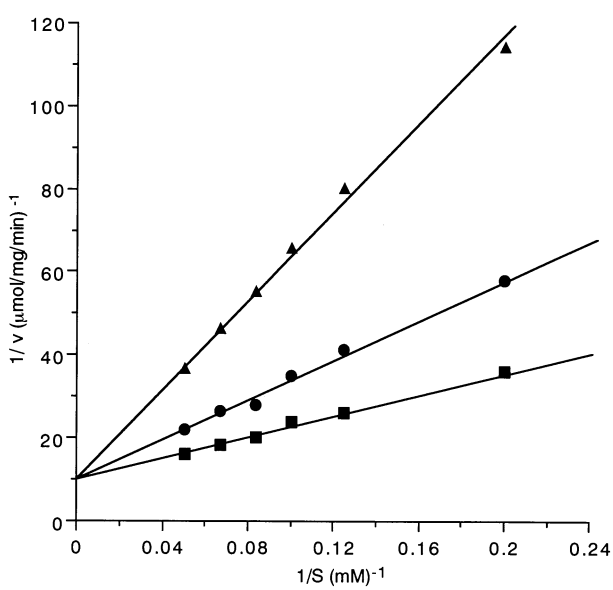

Fig. 6. Lineweaver–Burk plots of hyacinthacine B_2 (**9**) inhibition of rat intestinal lactase. Concentrations of **9** were 0 (■), 1 μM (●), and 5 μM (▲). The calculated K_m and K_i values were 13.0 mM and 0.97 μM, respectively.

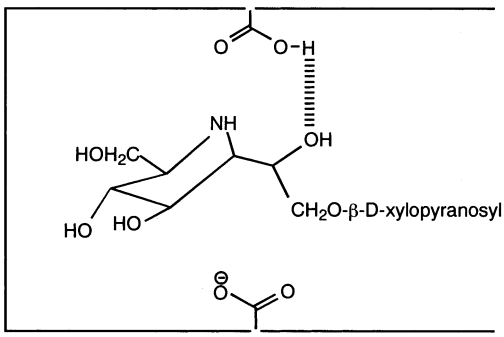

Fig. 7. Proposed mechanism of reversible binding of homoDMDP-7-*O*-β-D-xylopyranoside (**6**) on the active center of β-glucosidase or β-galactosidase.

from Pool B, **6** (32 mg) and **8** (22 mg) from Pool C, and **5** (15 mg) from Pool D. A 50% EtOH extract of the bulbs (1.3 kg) of *S. campanulata* was also chromatographed in a similar manner to give compounds **1** (29 mg), **2** (1 g), **3** (38 mg), **4** (239 mg), **5** (90 mg), **6** (144 mg), **7** (29 mg), and **9** (6 mg).

Glycosidase inhibitory activities.—The enzymes α-glucosidase (from rice), β-glucosidase (from almond and *C. saccharolyticum*), β-galactosidase (from bovine liver), β-mannosidase (from snail), trehalase (from porcine kidney), α-L-fucosidase (from bovine epididymis), amyloglucosidase (from *A. niger*), *p*-nitrophenyl glycosides, and disaccharides were purchased from Sigma Chemical Co. Brush border membranes, prepared from the intestines of male Wister rats by the method of Kessler et al. [4], were used as the source of rat intestinal glycosidases.

The activities of rice α-glucosidase, rat intestinal glycosidases, and trehalase were determined using the appropriate disaccharides as substrates at the optimum pH of each enzyme. The released D-glucose was determined colorimetrically using 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 spectrophotometrically at 400 nm. Kinetic parameters were determined by the Lineweaver–Burk double-reciprocal-plot method at increasing concentrations of substrate.

*6-Deoxy-6-*C*-(2,5-dihydroxyhexyl)-DMDP* (**3**).—$[\alpha]_D$ +42.8° (*c* 0.60, H_2O); HRFABMS: *m/z* 264.1802 $[M+H]^+$ ($C_{12}H_{26}NO_5$ requires 264.1811); ^{1}H NMR (400 MHz, D_2O) δ 1.18 (d, 3 H, CH_3), 1.44–1.76 (8 H), 2.95 (ddd, 1 H, *J* 4.5, 7.8, 8.1 Hz, H-5), 3.10 (ddd, 1 H, *J* 4.6, 6,4, 7.1 Hz, H-2), 3.66 (dd, 1 H, *J* 6.4, 11.7 Hz, H-1a), 3.68 (m, 1 H, H-8), 3.73 (dd, 1 H, *J* 4.6, 11.7 Hz, H-1b), 3.74 (dd, 1 H, *J* 7.1, 7.8 Hz, H-4), 3.84 (m, 1 H, H-11), 3.86 (t, 1 H, *J* 7.1 Hz, H-3); ^{13}C NMR (100 MHz, D_2O) δ 24.6 (C-12), 31.6 (C-6), 35.0 (C-9), 35.6 (C-7), 36.8 (C-10), 63.1 (C-5), 64.4 (C-2), 64.6 (C-1), 70.8 (C-11), 74.1 (C-8), 80.4 (C-3), 84.0 (C-4).

*HomoDMDP-7-*O*-β-*D*-xylopyranoside* (**6**).—$[\alpha]_D$ −8.7° (*c* 0.94, H_2O); HRFABMS: *m/z* 326.1448 $[M+H]^+$ ($C_{12}H_{24}NO_9$ requires 326.1451); ^{1}H NMR (400 MHz, D_2O) δ 3.04–3.08 (2 H, H-2, H-5), 3.33 (dd, 1 H, *J* 10.5, 11.7 Hz, H-5′ax), 3.32 (dd, 1 H, *J* 7.8, 9.3 Hz, H-2′), 3.46 (t, 1 H, *J* 9.3 Hz, H-3′), 3.63 (ddd, 1 H, *J* 5.4, 9.3, 10.5 Hz, H-4′), 3.65 (dd, 1 H, *J* 5.9, 11.7 Hz, H-1a), 3.71 (dd, 1 H, *J* 7.1, 11.0 Hz, H-7a), 3.73 (dd, 1 H, *J* 4.4, 11.7 Hz, H-1b), 3.86 (dd, 1 H, *J* 6.8, 7.8 Hz, H-3), 3.93 (ddd, 1 H, *J* 3.2, 5.6, 6.8 Hz, H-6), 3.97 (dd, 1 H, *J* 5.4, 11.7 Hz, H-5′eq), 4.02 (dd, 1 H, *J* 3.2, 11.0 Hz, H-7b), 4.10 (dd, 1 H, *J* 6.8, 7.4 Hz, H-4), 4.45 (d, 1 H, *J* 7.8 Hz, H-1′); ^{13}C NMR (100 MHz, D_2O) δ 64.4 (C-1, C-5), 64.7 (C-2), 68.0 (C-5′), 72.0 (C-4′), 74.2 (C-6), 74.6 (C-7), 75.9 (C-2′), 78.5 (C-3′), 80.5 (C-4), 80.6 (C-3), 106.3 (C-1′).

*Hyacinthacine B_1 ((1*S**,2*R**,3*R**,5*R**,-7a*R**)-1,2-dihydroxy-3,5-dihydroxymethyl-pyrrolizidine)* (**7**).—$[\alpha]_D$ +41.3° (*c* 1.04, H_2O); HRFABMS: *m/z* 204.1235 $[M+H]^+$ ($C_9H_{18}NO_4$ requires 204.1236); ^{1}H NMR (400 MHz, D_2O) δ 1.54 (m, 1 H, H-6β), 1.75 (m, 1 H, H-7β), 2.02 (m, 1 H, H-6α), 2.12 (m, 1 H, H-7α), 2.90 (ddd, 1 H, *J* 3.2, 6.1, 8.8 Hz, H-3), 3.04 (m, 1 H, H-5), 3.42 (dd, 1 H, *J* 6.6, 11.0 Hz, H-9a), 3.46 (dd, 1 H, *J* 5.1, 11.0 Hz, H-9b), 3.55 (m, 1 H, H-7a), 3.58 (dd, 1 H, *J* 6.1, 11.0 Hz, H-8a), 3.76 (dd, 1 H, *J* 3.2, 11.0 Hz, H-8b), 3.81 (t, 1 H, *J* 3.9 HZ, H-1), 3.91 (dd, 1 H, *J* 3.9, 8.8 Hz, H-2); ^{13}C NMR (100 MHz, D_2O) δ 25.3 (C-7), 32.2 (C-6), 65.9 (C-8), 67.4 (C-9), 69.5 (C-7a), 72.0 (C-5), 72.2 (C-3), 74.9 (C-1), 78.1 (C-2).

*Hyacinthacine C_1 ((1*S**,2*R**,3*R**,5*R**,-6*R**,7*R**,-7a*R**)-3-hydroxymethyl-5-methyl-1,2,6,7-tetrahydroxypyrrolizidine)* (**8**).—$[\alpha]_D$ +14.7° (*c* 0.28, H_2O); HRFABMS: *m/z* 220.1186 $[M+H]^+$ ($C_9H_{18}NO_5$ requires 220.1185); ^1H NMR (400 MHz, D_2O) δ 1.27 (d, 3 H, *J* 7.0 Hz, CH_3), 3.29 (dt, 1 H, *J* 3.9, 7.0 Hz, H-5), 3.44 (dt, 1 H, *J* 4.4, 9.0 Hz, H-3), 3.66 (dd, 1 H, *J* 3.9, 8.6 Hz, H-7a), 3.67 (m, 2 H, H-8a, H-8b), 3.89 (dd, 1 H, *J* 3.9, 4.6 Hz, H-6), 4.00 (dd, 1 H, *J* 3.9, 9.0 Hz, H-2), 4.15 (t, 1 H, *J* 3.9 Hz, H-1), 4.50 (dd, 1 H, *J* 4.6, 8.6 Hz, H-7); ^{13}C NMR (100 MHz, D_2O) δ 12.8 (C-9), 60.4 (C-5), 64.7 (C-3), 65.9 (C-8), 68.3 (C-7a), 74.6 (C-1), 75.7 (C-7), 77.1 (C-6), 77.7 (C-2).

*Hyacinthacine B_2 ((1*S**,2*R**,3*R**,5*S**,-7a*R**)-1,2-dihydroxy-3,5-dihydroxymethyl-pyrrolizidine)* (**9**).—$[\alpha]_D$ +41.3° (*c* 0.36, H_2O); HRFABMS: *m/z* 204.1233 $[M+H]^+$ ($C_9H_{18}NO_4$ requires 204.1236); ^1H NMR (400 MHz, D_2O) δ 1.81 (m, 1 H, H-7β), 1.91–2.08 (3 H, H-6α, H-6β, H-7α), 2.02 (m, 1 H, H-6α), 3.37 (ddd, 1 H, *J* 3.9, 5.1, 8.6 Hz, H-3), 3.43 (m, 1 H, H-5), 3.73 (dd, 1 H, *J* 5.1, 11.9 Hz, H-8a), 3.78–3.85 (3 H, H-7a, H-9a, H-9b), 3.89 (dd, 1 H, *J* 3.9, 11.9 Hz, H-8b), 4.09 (dd, 1 H, *J* 4.2, 6.3 Hz, H-1), 4.11(dd, 1 H, *J* 4.2, 8.6 Hz, H-2); ^{13}C NMR (100 MHz, D_2O) δ 25.0 (C-7), 32.0 (C-6), 63.1 (C-9), 64.0 (C-8), 64.4 (C-3), 69.9 (C-5), 70.4 (C-7a), 73.6 (C-1), 77.5 (C-2).

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