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New polyhydroxylated pyrrolizidine alkaloids from *Muscari armeniacum*: structural determination and biological activity

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Abstract

Four new polyhydroxypyrrolizidines, hyacinthacines A_1, A_2, A_3 and B_3, have been isolated from the bulbs of *Muscari armeniacum* (Hyacinthaceae) in addition to the known hyacinthacine C_1, which was isolated from *Hyacinthoides non-scripta* (Hyacinthaceae). The structures of hyacinthacines A_1, A_2, A_3 and B_3 were identified on the basis of extensive NMR studies as (1*S*,2*R*,3*R*,7a*R*)-1,2-dihydroxy-3-hydroxymethylpyrrolizidine, (1*R*,2*R*,3*R*,7a*R*)-1,2-dihydroxy-3-hydroxymethylpyrrolizidine, (1*R*,2*R*,3R,5*R*,7a*R*)-1,2-dihydroxy-3-hydroxymethyl-5-methylpyrrolizidine and (1*S*,2*R*,3*R*,5*R*,7*R*,7a*R*)-3-hydroxymethyl-5-methyl-1,2,7-trihydroxypyrrolizidine, respectively, or the corresponding enantiomers. The inhibitory activities of these new hyacinthacines against a variety of glycosidases are described.

1. Introduction

Polyhydroxylated pyrrolizidine alkaloids with a hydroxymethyl substituent at C-3 have long been thought to be of very restricted natural occurrence. The alexines and australines have only been reported in two small genera of the Leguminosae (*Castanospermum* and *Alexa*), and casuarine occurs only in the related genera in the Casuarinaceae and Myrtaceae.[1] We recently isolated three new polyhydroxylated pyrrolizidines, hyacinthacines B_1, B_2 and C_1, from the immature fruits and stalks of bluebells, *Hyacinthoides non-scripta* (Hyacinthaceae).[2] Bluebells co-produce 1,4-dideoxy-1,4-imino-D-arabinitol (DAB), (2*R*,5*R*)-bis(hydroxymethyl)-3(*R*),4(*R*)-dihydroxypyrrolidine (DMDP), 2,5-dideoxy-

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2,5-imino-DL-*glycero*-D-*manno*-heptitol (homoDMDP), homoDMDP-7-*O*-apioside and homoDMDP-7-*O*-β-D-xylopyranoside.

One of the best known approaches to finding novel compounds in plants is to examine close taxonomic relatives. GLCMS analysis of an extract of *Muscari armeniacum*, which is closely related to *H. non-scripta*, showed the existence of new pyrrolizidine alkaloids other than hyacinthacine C_1, homoDMDP, 6-deoxy-homoDMDP, homoDMDP-7-*O*-apioside and DMDP-7-*O*-β-D-xylopyranoside, which have been found in *H. non-scripta* and *Hyacinthus orientalis*.[3] In this paper we report the isolation of new polyhydroxylated pyrrolizidine alkaloids from the bulbs of *M. armeniacum*, their structural determination and glycosidase inhibitory activity (Fig. 1).

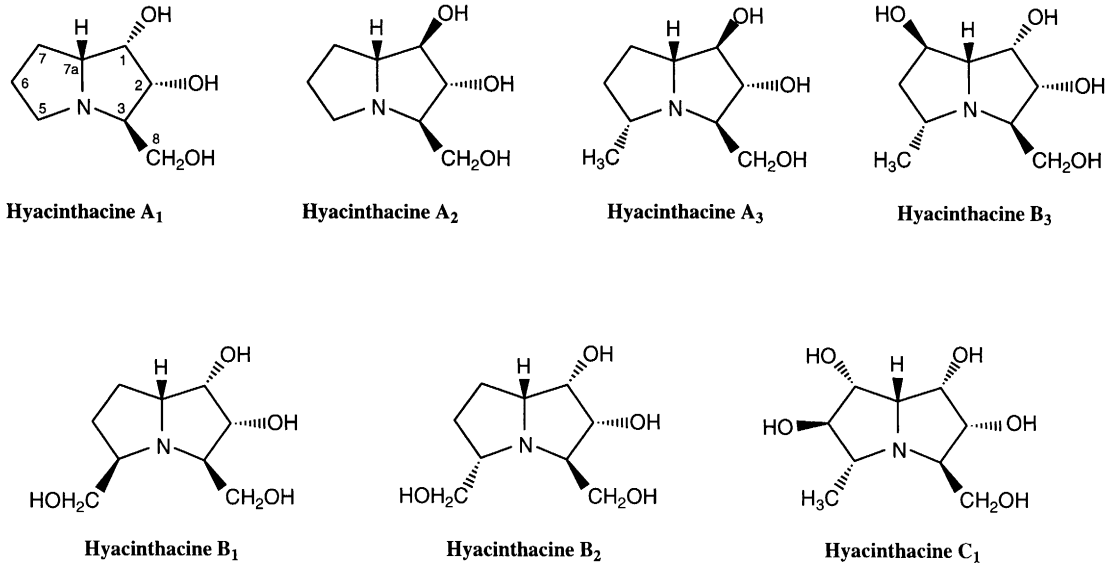

Fig. 1. Structures of polyhydroxylated pyrrolizidines isolated from the plants in the Hyacinthaceae

2. Results and discussion

A 60% EtOH extract of the bulbs (4 kg) of *M. armeniacum* was chromatographed with various ion-exchange resins to give hyacinthacines A_1 (21 mg), A_2 (5 mg), A_3 (6 mg) and B_3 (72 mg), in addition to the known alkaloids, hyacinthacine C_1 (7 mg), homoDMDP (75 mg), 6-deoxy-homoDMDP (70 mg), homoDMDP-7-*O*-apioside (120 mg) and homoDMDP-7-*O*-β-D-xylopyranoside (210 mg).

The ^{13}C NMR spectral analysis of hyacinthacine A_1 revealed the presence of four methylene and four methine carbon atoms (Table 1). This result and HRFABMS (*m/z* 174.1129 $[M+H]^+$) established that the molecular formula was $C_8H_{15}NO_3$. Hyacinthacine A_1 gave a tri-*O*-$SiMe_3$ derivative and its GLCMS analysis showed a characteristic fragment ion at *m/z* 374 $[M-CH_3]^+$ and a base peak at *m/z* 286 $[M-CH_2OSiMe_3]^+$. 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. The ^{13}C NMR data of polyhydroxylated pyrrolizidine alkaloids isolated are summarized in Table 1. From these NMR results in CD_3OD, the methylene triplet at δ 65.2 (C-8) was attributed to the hydroxymethyl carbon. The methine doublets at δ 73.7 and 77.5 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 3.87, $J_{1,2}=J_{1,7a}=4.0$ Hz) and H-2 (δ 3.88, $J_{1,2}=4.0$, $J_{2,3}=9.0$ Hz). The methines at δ 71.9 (C-3) indicated that it must be bonded to the

nitrogen of the heterocyclic ring, with the hydroxymethyl group, and the last one at δ 67.9, with the doublet of double doublets at δ 3.47 in the ^{1}H NMR spectrum, was identified as the bridgehead C-7a. These data suggest that this compound has a pyrrolizidine ring system. The relative configurations at the stereogenic centers in hyacinthacine A_1 were determined by extensive NOE experiments. The NOE interactions are shown in Fig. 2. The definite NOE effects between H-3 and H-5α and between H-5α and H-7α indicate that H-3, H-5α and H-7α are on the same side of the ring. Irradiation of H-7a at δ 3.47 enhanced the NOE intensity of H-1, H-2 and H-7β. Thus, hyacinthacine A_1 was determined to be (1*S*,2*R*,3*R*,7a*R*)-1,2-dihydroxy-3-hydroxymethylpyrrolizidine or its enantiomer.

Table 1
^{13}C NMR chemical shifts[a] for hyacinthacines A_1 (CD_3OD), A_2 (D_2O), A_3 (D_2O) and B_3 (CD_3OD)

C	A_1	A_2	A_3	B_3
1	73.7 *d*	82.9 *d*	82.6 *d*	72.2 *d*
2	77.5 *d*	79.8 *d*	80.4 *d*	77.4 *d*
3	71.9 *d*	72.1 *d*	64.8 *d*	63.8 *d*
5	57.5 *t*	57.7 *t*	61.6 *d*	57.1 *d*
6	28.8 *t*	27.3 *t*	34.3 *t*	44.4 *t*
7	26.0 *t*	32.5 *t*	30.4 *t*	71.5 *d*
7a	67.9 *d*	69.2 *d*	70.8 *d*	77.0 *d*
8	65.2 *t*	65.3 *t*	64.6 *t*	65.0 *t*
9			17.9 q	17.5 *q*

[a] 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.

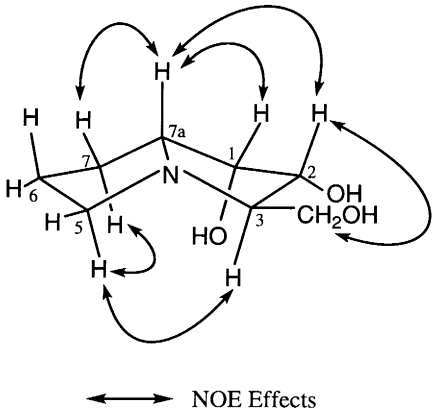

Fig. 2. NOE interactions for hyacinthacine A_1

Hyacinthacine A_2, as well as hyacinthacine A_1, gave a tri-*O*-$SiMe_3$ derivative and a characteristic fragment ion at *m/z* 374 $[M-CH_3]^+$ and a base peak at *m/z* 286 $[M-CH_2OSiMe_3]^+$. The results of ^{13}C NMR (Table 1) and HRFABMS (*m/z* 174.1129 $[M+H]^+$) analyses, together with GLCMS analysis, suggest that hyacinthacine A_2 is an epimer of A_1. The ^{1}H NMR spectral data, combined with extensive decoupling experiments and 2D ^{1}H–^{13}C COSY spectral data, defined the complete connectivity of the carbon and hydrogen atoms. In the ^{1}H NMR spectrum in D_2O, the large coupling constants ($J_{1,2}$=$J_{2,3}$=8.8 Hz) seen in H-2 signal indicate the all *trans*-axial configuration of H-1, H-2 and H-3. The relative

configurations at the stereogenic centers in hyacinthacine A_2 were also corroborated by the definite NOE effects between H-1 and H-3 and between H-2 and H-7a (Fig. 3). Thus, hyacinthacine A_2 was determined to be (1*R*,2*R*,3*R*,7a*R*)-1,2-dihydroxy-3-hydroxymethylpyrrolizidine or its enantiomer.

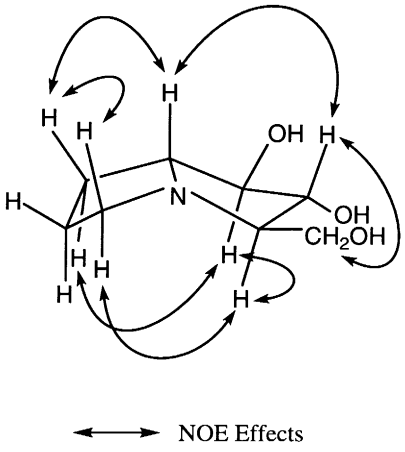

Fig. 3. NOE interactions for hyacinthacine A_2

The ^{13}C NMR spectral analysis of hyacinthacine A_3 revealed the presence of one methyl, three methylene and five methine carbon atoms (Table 1). This result and HRFABMS (*m/z* 188.1286 $[M+H]^+$) established that the molecular formula was $C_9H_{17}NO_3$. Hyacinthacine A_3 gave a tri-*O*-$SiMe_3$ derivative and its GLCMS analysis showed a characteristic fragment ion at *m/z* 388 $[M-CH_3]^+$ and a base peak at *m/z* 300 $[M-CH_2OSiMe_3]^+$. 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. Irradiation of H-3 enhanced the NOE signal intensity of H-1 and the methyl group at C-5 (Fig. 4). This indicates that H-1, H-3 and the methyl group are on the same side of the ring. The relative configurations at the stereogenic centers in hyacinthacine A_3 were also corroborated by the definite NOE effect between H-2 and H-7a, and the large coupling constants ($J_{1,2}$=7.3 Hz, $J_{2,3}$=8.1 Hz) seen in H-2 signal. Thus, hyacinthacine A_3 was determined to be (1*R*,2*R*,3*R*,5*R*,7a*R*)-1,2-dihydroxy-3-hydroxymethyl-5-methylpyrrolizidine or its enantiomer.

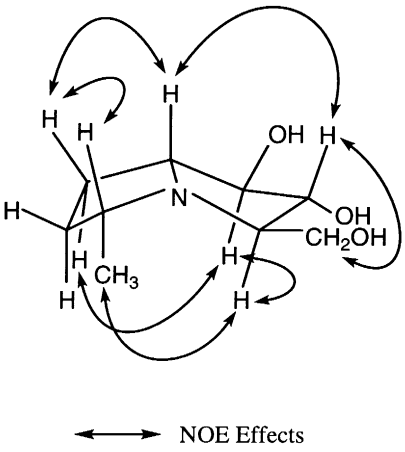

Fig. 4. NOE interactions for hyacinthacine A_3

The ^{13}C NMR spectral analysis of hyacinthacine B_3 revealed the presence of one methyl, two methylene and six methine carbon atoms (Table 1). This result and HRFABMS (*m/z* 204.1236 $[M+H]^+$)

established that the molecular formula was $C_9H_{17}NO_4$. Hyacinthacine B_3 gave a tetra-*O*-$SiMe_3$ derivative and its GLCMS 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 ^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 triplet at δ 65.0 (C-8) was attributed to the hydroxymethyl carbon. Two methine carbons at δ 57.1 and 63.8 were assigned to C-5 and C-3, which must be bonded to the nitrogen of the heterocyclic ring, with the methyl and hydroxymethyl groups, respectively. The methine doublets at δ 71.5, 72.2 and 77.4 were assigned to C-7, C-1 and C-2 bearing the OH groups, respectively, and the remaining methine carbon at δ 77.0, with the doublet of doublets ($J_{1,7a}$=4.6 Hz, $J_{7,7a}$=4.2 Hz) at δ 3.30 in the ^1H NMR spectrum, was identified as the bridgehead C-7a. The relative configurations at the stereogenic centers were determined by extensive NOE experiments (Fig. 5). Irradiation of H-7a enhanced the NOE signal intensity of H-1 and H-2, and a strong NOE between the methyl group at C-5 and H-3 or H-7 was also observed. These NOE interactions indicate that H-1, H-2 and H-7a are on the same side of the ring and the methyl group, H-3 and H-7 on the opposite side. Thus, hyacinthacine B_3 was determined to be (1*S*,2*R*,3*R*,5*R*,7*R*,7a*R*)-3-hydroxymethyl-5-methyl-1,2,7-trihydroxypyrrolizidine or its enantiomer.

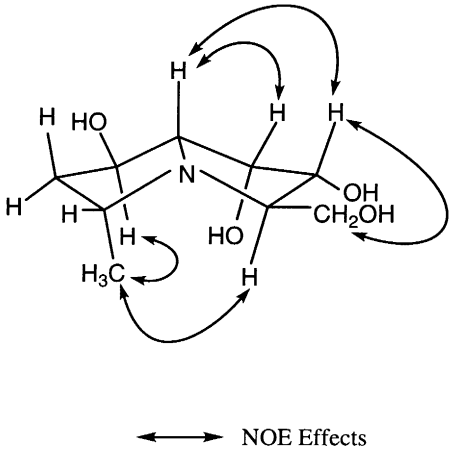

Fig. 5. NOE interactions for hyacinthacine B_3

The IC_{50} values of new pyrrolizidine alkaloids against various glycosidases are shown in Table 2. We have reported that hyacinthacine B_2 is a potent inhibitor of rat intestinal lactase.[2] The present work revealed that hyacinthacine A_1, which loses the hydroxymethyl group at C-5 in hyacinthacine B_2, was also a potent inhibitor of the same enzyme, with an IC_{50} value of 4.4 μM. Hyacinthacine A_1 was, furthermore, a moderate inhibitor of α-L-fucosidase and amyloglucosidase, with IC_{50} values of 46 and 25 μM, respectively. The inversion of the hydroxyl group at C-1 in hyacinthacine A_1 to give hyacinthacine A_2 enhanced its inhibitory potential toward amyloglucosidase but abolished its inhibition toward α-L-fucosidase. Hyacinthacine A_3, which is the α-5-*C*-methyl derivative of hyacinthacine A_2, was a two-fold less effective inhibitor of rat intestinal lactase and amyloglucosidase than hyacinthacine A_2. Hyacinthacine B_3 proved to be a moderate inhibitor of lactase and amyloglucosidase, but had no significant activity toward other glycosidases. The introduction of a hydroxyl group to C-7α or C-7β in hyacinthacine A_2 gives 7a-*epi*alexine (australine) or 7,7a-di*epi*alexine, respectively, and these two compounds have been known to also be potent inhibitors of amyloglucosidase.[4,5] 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 substituent on the pyrrolizidine ring. It would be of value to have a variety of highly

oxygenated or substituted pyrrolizidine alkaloids in order to understand the structural requirements for glycosidase inhibition.

Table 2
Concentration of alkaloids giving 50% inhibition of glycosidase activities

Enzyme	IC_{50} (μM)			
	A_1	A_2	A_3	B_3
α-Glucosidase				
Rice	240	NI[a]	NI	NI
Rat intestinal maltase	NI	NI	NI	NI
β-Glucosidase				
Almond	250	150	NI	NI
α-Mannosidase				
Jack bean	NI	NI	NI	NI
β-Mannosidase				
Rat epididymis	390	1000	NI	NI
β-Galactosidase				
Rat intestinal lactase	4.4	73	160	18
α-L-Fucosidase				
Rat epididymis	46	NI	NI	850
Trehalase				
Porcine kidney	NI	260	NI	NI
Amyloglucosidase				
Aspergillus niger	25	8.6	17	51

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

3. Experimental

3.1. General

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*-tolidine 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. MS were measured on a Jeol JMS-SX 102A spectrometer. In GLCMS 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 25m×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.

3.2. Extraction and isolation

A 60% EtOH extract of the bulbs (4 kg) of *Muscari armeniacum* 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 oil (14 g), which was chromatographed over a Dowex 1-X2 column (2.5×95 cm, OH^- form) with H_2O as eluant (fraction size 11 mL). The H_2O eluate was divided into four pools: A (fractions 48–57, 310 mg), B (fractions 58–66, 230 mg), C (fractions 67–80, 110 mg) and D (fractions 101–130, 225 mg). The MeOH eluate from the same column was designated pool E (160 mg). Pool A was further chromatographed on a CM-Sephadex C-25 column (2.2×60 cm, NH_4^+ form) with 0.01 M NH_4OH as eluant to give hyacinthacine A_1 (21 mg) and hyacinthacine A_2 (5 mg), and the elution with 0.02 M NH_4OH gave hyacinthacine A_3 (6 mg) and hyacinthacine B_3 (72 mg). Further chromatography of pool B with an Amberlite CG-50 column (2.2×60 cm, NH_4^+ form) (H_2O elution) gave 6-deoxy-homoDMDP (70 mg), and that of pool C with the same column (0.05 M NH_4OH elution) gave homoDMDP (75 mg) and hyacinthacine C_1 (7 mg). Pools D and E were chromatographed on a Dowex 1-X2 (1.5×40 cm, OH^- form) with 50% MeOH as eluant to give homoDMDP-7-*O*-β-D-xylopyranoside (210 mg) and homoDMDP-7-*O*-apioside (120 mg), respectively.

3.3. Glycosidase inhibitory activities

The enzymes α-glucosidase (from rice), β-glucosidase (from almond), α-mannosidase (Jack bean), trehalase (from porcine kidney), amyloglucosidase (from *Aspergillus niger*), *p*-nitrophenyl glycosides and disaccharides were purchased from Sigma Chemical Co. Brush border membranes, prepared from the intestine of male Wistar rats using the method of Kessler et al.,[6] were used as the source of rat intestinal glycosidases. The rat epididymal fluid was purified from epididymis according to the method of Skudlarek et al.[7]

The activities of rice α-glucosidase, rat intestinal glycosidases, trehalase and amyloglucosidase 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 spectrometrically at 400 nm.

3.4. Hyacinthacine A_1, (1S*,2R*,3R*,7aR*)-1,2-dihydroxy-3-hydroxymethylpyrrolizidine

$[\alpha]_D$ +38.2 (*c* 0.23, H_2O); HRFABMS: *m/z* 174.1129 $[M+H]^+$ ($C_8H_{16}NO_3$ requires 174.1130); δ_H (400 MHz, CD_3OD) 1.68 (1H, m, H-7β), 1.74 (1H, m, H-6β), 1.93 (1H, m, H-6α), 2.08 (1H, m, H-7α), 2.65 (1H, ddd, $J_{5\alpha,6\alpha}$=6.1 Hz, $J_{5\alpha,6\beta}$=8.1 Hz, $J_{5\alpha,5\beta}$=10.0 Hz, H-5α), 2.76 (1H, ddd, $J_{2,3}$=9.0 Hz, $J_{3,8}$=6.4 Hz, $J_{3,8'}$=3.4 Hz, H-3), 3.05 (1H, ddd, $J_{5\beta,6\alpha}$=4.9 Hz, $J_{5\beta,6\beta}$=6.4 Hz, $J_{5\alpha,5\beta}$=10.0 Hz, H-5β), 3.47 (1H, ddd, $J_{1,7a}$=4.0 Hz, $J_{7a,7\alpha}$=6.6 Hz, $J_{7a,7\beta}$=8.1 Hz, H-7a), 3.58 (1H, dd, $J_{3,8}$=6.4 Hz, $J_{8,8'}$=11.3 Hz, H-8), 3.78 (1H, dd, $J_{3,8'}$=3.4 Hz, $J_{8,8'}$=11.3 Hz, H-8′), 3.87 (1H, t, $J_{1,7a}$=$J_{1,2}$=4.0 Hz, H-1), 3.88 (1H, dd, $J_{1,2}$=4.0 Hz, $J_{2,3}$=9.0 Hz, H-2); ^{13}C NMR data, see Table 1.

3.5. Hyacinthacine A_2, (1R*,2R*,3R*,7aR*)-1,2-dihydroxy-3-hydroxymethylpyrrolizidine

$[\alpha]_D$ +20.1 (*c* 0.44, H_2O); HRFABMS: *m/z* 174.1127 $[M+H]^+$ ($C_8H_{16}NO_3$ requires 174.1130); δ_H (400 MHz, D_2O) 1.77 (1H, m, H-7α), 1.82 (1H, m, H-6β), 1.90 (1H, m, H-6α), 1.97 (1H, m, H-7β), 2.77

(1H, ddd, $J_{2,3}$=8.8 Hz, $J_{3,8}$=6.5 Hz, $J_{3,8'}$=3.9 Hz, H-3), 2.81 (1H, dt, $J_{5\alpha,6\alpha}=J_{5\alpha,6\beta}$=5.6 Hz, $J_{5\alpha,5\beta}$=11.0 Hz, H-5α), 2.96 (1H, ddd, $J_{5\beta,6\alpha}$=7.3 Hz, $J_{5\beta,6\beta}$=5.9 Hz, $J_{5\alpha,5\beta}$=11.0 Hz, H-5β), 3.32 (1H, m, H-7a), 3.67 (1H, dd, $J_{3,8}$=6.5 Hz, $J_{8,8'}$=11.8 Hz, H-8), 3.76 (1H, dd, $J_{1,2}$=8.8 Hz, $J_{1,7a}$=7.1 Hz, H-1), 3.80 (1H, dd, $J_{3,8'}$=3.9 Hz, $J_{8,8'}$=11.8 Hz, H-8′), 3.81 (1H, t, $J_{1,2}=J_{2,3}$=8.8 Hz, H-2); ^{13}C NMR data, see Table 1.

*3.6. Hyacinthacine A$_3$, (1*R*,*2*R*,*3*R*,*5*R*,*7a*R*)-1,2-dihydroxy-3-hydroxymethyl-5-methylpyrrolizidine*

$[\alpha]_D$ +19.2 (*c* 0.43, H_2O); HRFABMS: *m/z* 188.1286 $[M+H]^+$ ($C_9H_{18}NO_3$ requires 188.1287); δ_H (400 MHz, D_2O) 1.26 (3H, d, *J*=6.8 Hz, CH_3), 1.68 (1H, m, H-6α), 1.85 (1H, m, H-7α), 1.95 (1H, m, H-6β), 2.00 (1H, m, H-7β), 3.19 (1H, dt, $J_{2,3}$=8.1 Hz, $J_{3,8}=J_{3,8'}$=4.9 Hz, H-3), 3.36 (1H, m, H-5), 3.44 (1H, m, H-7a), 3.74 (2H, d, *J*=4.9 Hz, H-8, H-8′), 3.77 (1H, t, $J_{1,2}=J_{1,7a}$=7.3 Hz, H-1), 3.95 (1H, dd, $J_{1,2}$=7.3 Hz, $J_{2,3}$=8.8 Hz, H-2); ^{13}C NMR data, see Table 1.

*3.7. Hyacinthacine B$_3$, (1*R*,*2*R*,*3*R*,*5*R*,*7*R*,*7a*R*)-3-hydroxymethyl-5-methyl-1,2,7-trihydroxy-pyrrolizidine*

$[\alpha]_D$ +3.1 (*c* 0.33, H_2O); HRFABMS: *m/z* 204.1236 $[M+H]^+$ ($C_9H_{18}NO_4$ requires 204.1236); δ_H (400 MHz, CD_3OD) 1.17 (3H, d, *J*=6.8 Hz, CH_3), 1.82 (2H, m, H-6, H-6′), 3.08 (1H, ddd, $J_{2,3}$=7.3 Hz, $J_{3,8}$=4.4 Hz, $J_{3,8'}$=4.9 Hz, H-3), 3.30 (1H, t, $J_{1,7a}=J_{7,7a}$=4.6 Hz, H-7a), 3.50 (1H, m, H-5), 3.53 (1H, dd, $J_{3,8}$=4.4 Hz, $J_{8,8'}$=11.0 Hz, H-8), 3.57 (1H, dd, $J_{3,8'}$=4.9 Hz, $J_{8,8'}$=11.0 Hz, H-8′), 3.91 (1H, dd, $J_{1,2}$=4.2 Hz, $J_{2,3}$=7.3 Hz, H-2), 4.03 (1H, dd, $J_{1,2}$=4.2 Hz, $J_{1,7a}$=4.6 Hz, H-1), 4.52 (1H, ddd, $J_{6,7}$=3.9 Hz, $J_{6',7}$=6.8 Hz, $J_{7,7a}$=4.6 Hz, H-7); ^{13}C NMR data, see Table 1.

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