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Structures of Allosamidins, Novel Insect Chitinase Inhibitors, Produced by Actinomycetes

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The structures of allosamidin (**1**) and methylallosamidin (**2**), novel insect chitinase inhibitors, were elucidated as **1** and **2** by acid hydrolysis experiments and analyses of 2D-NMR spectra. They are unique basic pseudotrisaccharides consisting of 2-acetamido-2-deoxy-D-allose (*N*-acetyl-D-allosamine) and a novel aminocyclitol derivative (**3**), termed allosamizoline.

In the course of our screening search for insect chitinase inhibitors as a new type of insect growth regulator, allosamidin (**1**) was isolated from the mycelial extract of *Streptomyces* sp. no. 1713.[1] It showed strong and specific inhibitory activity against insect chitinases that were purified from the silkworm, *Bombyx mori*, *in vitro*,[2] and also insecticidal activity by inhibiting the ecdysis *in vivo*.[1] The structure of allosamidin (**1**) has been preliminarily reported,[3] and is a unique basic pseudotrisaccharide consisting of *N*-acetyl-D-allosamine and a novel aminocyclitol derivative, termed allosamizoline (**3**). The relative stereochemistry around the cyclopentane ring of **3** has also been previously estimated[3] as shown in Fig. 1a, but further work to elucidate its absolute configuration has made it clear that the stereochemistry at C-3 should be revised to that shown in Fig. 1b.

More recently, methylallosamidin (**2**) was isolated from the mycelium of an unidentified actinomycetes, and it showed a little less insecticidal activity than allosamidin (**1**).

In this paper, we report the structural elucidation of allosamidin (**1**) and methylallosamidin (**2**) in detail, and also the revised relative stereochemistry of allosamizoline (**3**).

Allosamidin (**1**) was obtained from an aqueous methanol extract of the mycelium of *Streptomyces* sp. no. 1713 as a white crystalline powder, which was very hygroscopic and not soluble in common solvents, except for an acidic water. The FABMS spectrum of **1** showed the $(M+H)^+$ ion at *m/z* 623, and the signals due to 25 carbons were observed in the ^{13}C-NMR spectrum of **1**. The molecular formula of **1** was determined as $C_{25}H_{42}N_4O_{14}$ by considering the structures of its degradation products and the results of analyses of its ^{1}H- and ^{13}C-NMR spectra, in addition to the aforementioned data.

Allosamidin (**1**) gave two basic products by acid hydrolysis with 4 N HCl (100°C, 4 hr). One was identified as D-allosamine,[4] and the other was a new aminocyclitol derivative named allosamizoline (**3**). **3** showed the $(M+H)^+$ ion at *m/z* 217 in the FABMS spectrum and 9 carbon signals in the ^{13}C-NMR spectrum. By acetylation with pyridine and acetic anhydride,

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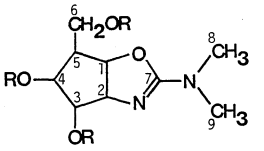
3 gave the triacetate (**4**), whose molecular formula was determined as $C_{15}H_{22}N_2O_7$ by its high resolution mass spectrum (*m/z* 342.14063, error −2.0 mmu). From these data, the molecular formula of **3** was determined as $C_9H_{16}N_2O_4$. The cyclopentane moiety of **3** through C-1 to C-6 was easily revealed by conventional proton decoupling experiments, and the location of three hydroxyl groups in **3** was determined to be C-3,4 and 6 by an acetylation shift in the ^{1}H-NMR spectrum of **4**. After considering of the chemical shifts of the three residual carbons [δ_C37.9 (q), 38.1 (q), 161.2 (s)] and six protons [δ_H 3.08 (3H, s), 3.11 (3H, s)], it was suggested that a dimethylaminooxazoline moiety was present in **3**,[5] which was confirmed by a spectral comparison with the synthetic analog, 2-dimethylamino-5-methyl-2-oxazoline.[6] Thus, the planar structure of allosamizoline was determined as **3**.



3 R=H
4 R=Ac

The relative stereochemistry around the cyclopentane ring of allosamizoline triacetate (**4**) had been previously estimated as that shown in Fig. 1a, which was suggested by the *J* values of the ring protons of **4** and by NOE experiments. But it was observed that the value of $J_{2,3}$ was nearly 2 Hz in the ^{1}H-NMR spectrum of its bis[*p*-(dimethylamino)benzoate] (**5**), which was prepared to elucidate the absolute configuration by the exciton chirality method. Since the small value strongly indicated a *trans* orientation between H-2 and H-3, which had previously been estimated as *cis*, it was suggested that the relative stereochemistry of the cyclopentane ring should be corrected. The NOE enhancements observed among the protons in the ^{1}H-NMR spectrum of **4** were then carefully reinvestigated. The NOE difference spectra are shown in Fig. 2. When H-1 was irradiated (Fig. 2b), NOE enhancements were observed at H-2, H-6 and weakly at H-5. Then, when H-4 was irradiated (Fig. 2c), NOE enhancements were observed at H-6 and weakly at H-5. These data suggested a *cis* orientation between H-1 and H-2, and also a *cis* between the H-1 and C-6 methylene, and the H-4 and C-6 methylene, which could fix the relative stereochemistry among the protons at C-1, 2, 4 and 5, except for C-3. The orientation of H-3 against H-2 was suggested to be *trans* by the value of $J_{2,3}$ that has already been mentioned, and the fact that NOE enhancements were observed at H-5 and weakly at H-2 when H-3 was irradiated (Fig. 2d). The results of these NOE experiments and *J* values suggest that the relative stereochemistry of the cyclopentane ring of **4** should be corrected as shown in Fig. 1b.

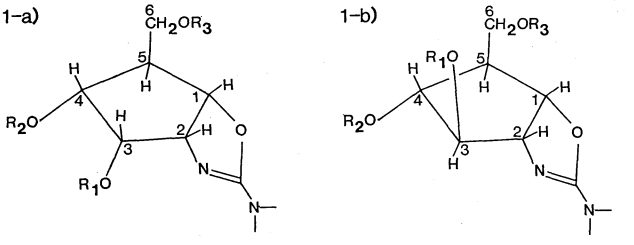


FIG. 1. Relative Stereochemistry of the Cyclopentane Ring of **3**.

a) Formerly reported stereochemistry of **3**.

b) Revised stereochemistry of **3**.

In **3**, $R_1=R_2=R_3=H$, $J_{1,2}=9$Hz, $J_{2,3}=4$Hz, $J_{3,4}=7$Hz, $J_{4,5}=8$Hz, $J_{5,1}=5$Hz.

In **4**, $R_1=R_2=R_3=Ac$, $J_{1,2}=8$Hz, $J_{2,3}=4$Hz, $J_{3,4}=5$Hz, $J_{4,5}=7$Hz, $J_{5,1}=4$Hz.

In **5**, $R_1=H$, $R_2=R_3=p$-(dimethylamino) benzoyl, $J_{1,2}=8$Hz, $J_{2,3}=2$Hz, $J_{3,4}=4$Hz, $J_{4,5}=6$Hz, $J_{5,1}=4$Hz.

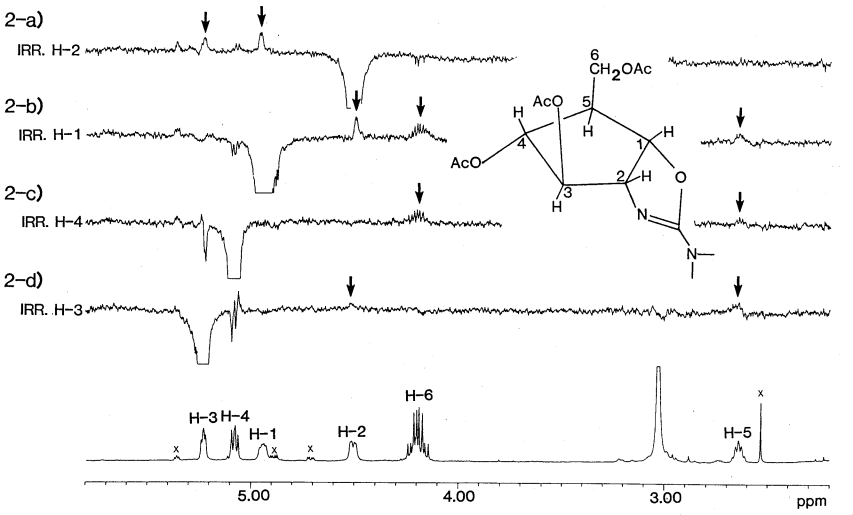


FIG. 2. NOE Difference Spectra of **4** ($CDCl_3$, 400 MHz).
NOE enhancements are shown by arrows.

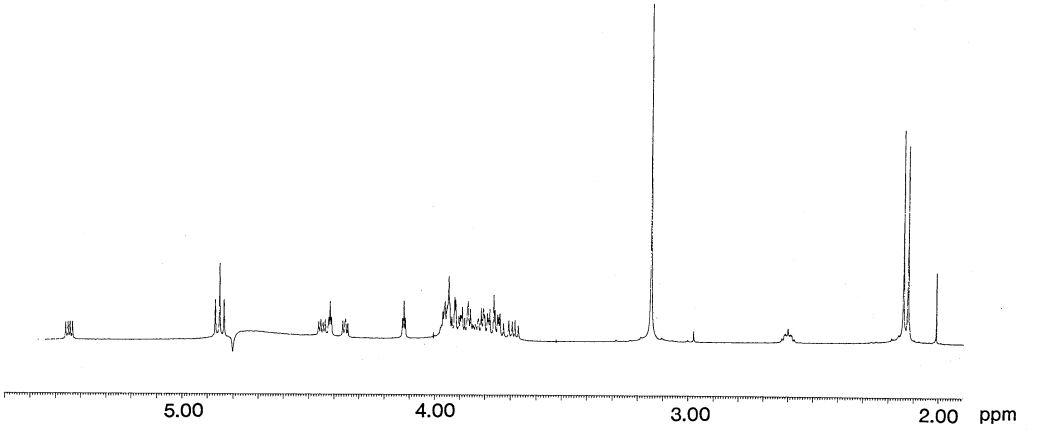


FIG. 3. ^{1}H-NMR Spectrum of **1**($D_2O + CD_3COOD$, 500MHz).

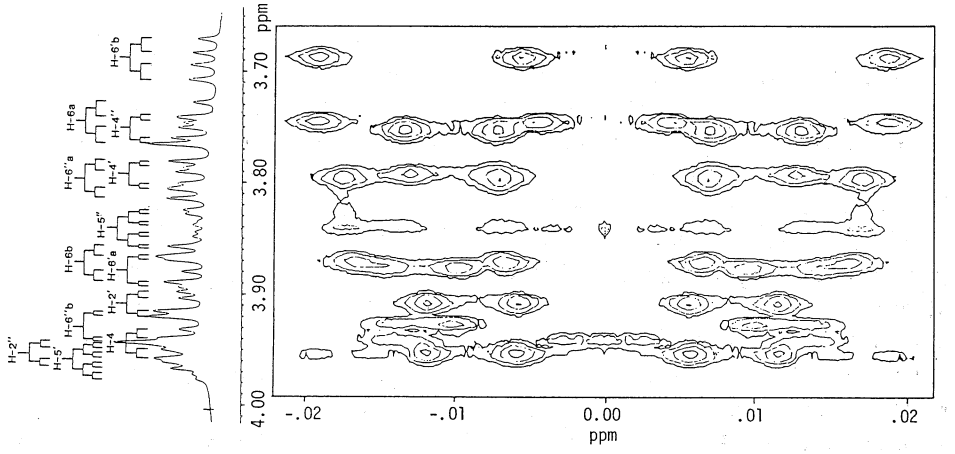


FIG. 4. *J*-Resolved 2D-NMR Spectrum of the Complicated Region of **1**.

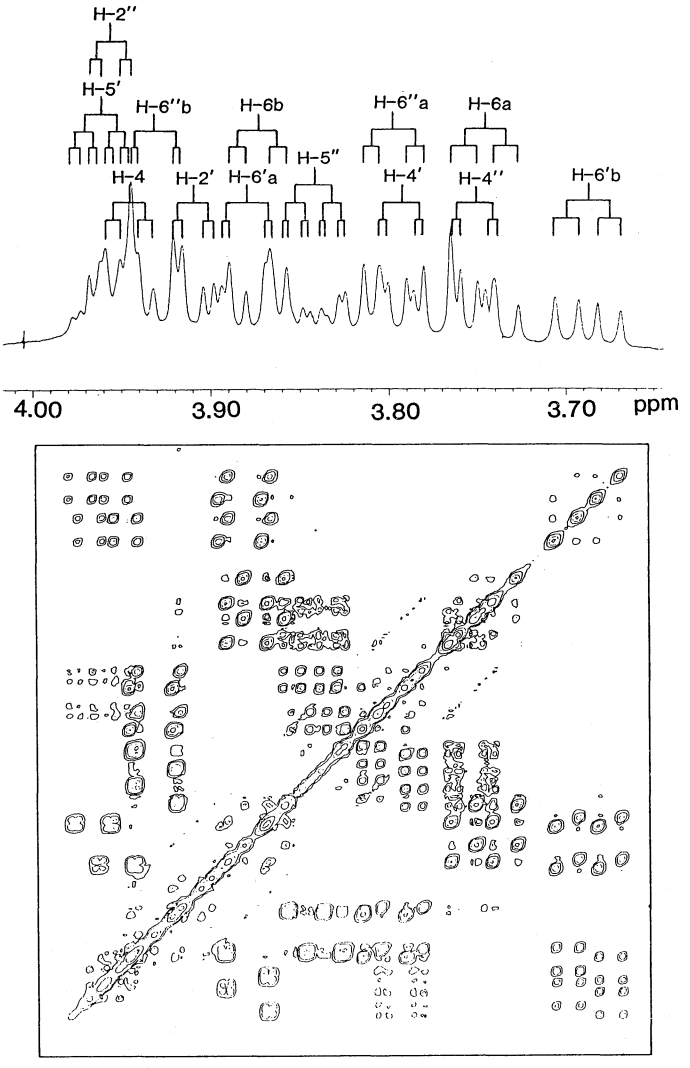


FIG. 5. COSY Spectrum of the Complicated Region of **1**.

An application of the exciton chirality method was attempted against the 4,6-bis-(**5**), 3,6-bis-(**6**) and tri[*p*-(dimethylamino)benzoate] (**7**) of **3** to determine its absolute configuration, but in all cases could not be applied because the dominant conformation of *p*-(dimethylamino)benzoyloxymethyl could not be deduced.

The complete structure of allosamidin (**1**) was elucidated mainly by analyzing various 2D-NMR spectra of **1**. Especially, the complex signals around $\delta 3.8 \sim 4.0$ in the ^{1}H-NMR spectrum of **1** (Fig. 3) were analyzed by a *J*-resolved 2D-NMR spectrum (Fig. 4), which revealed the chemical shift and coupling constant of each proton, and a COSY spectrum (Fig. 5), which elucidated the mutural connection of those protons. These analyses of the COSY and *J*-resolved spectra as well as the C–H shift-correlated 2D-NMR spectrum of **1**, revealed the presence of two moles of *N*-acetylallosamine and one mole of **3**, which is in accordance with the molecular formula of **1**. The assignments of carbons and protons in the NMR spectra are summarized in Tables I and II. When **1** was acetylated

TABLE I. ^{1}H-NMR ASSIGNMENTS OF **1**, **2**, **8**, **9**, **10** AND **11**[a]

Assignment	**1**	**2**	**8**	**11**	**9**	**10**
H-1[b]	5.45 dd (5, 9)	5.44 dd (5, 9)	5.27 dd (5, 9)	5.57 dd (5, 9)	5.20 dd (5, 9)	5.30 dd (5, 9)
H-2[b]	4.45 dd (4, 9)	4.44 dd (4, 9)	4.48 dd (2, 9)	4.66 dd (2, 9)	4.28 dd (4, 9)	4.29 dd (3, 9)
H-3[b]	4.35 dd (4, 5)	4.35 dd (4, 5)	5.18 dd (2, 4)	5.27 dd (2, 4)	4.18 dd (4, 5)	5.25 dd (3, 4)
H-4[b]	3.94 dd (5, 7)	3.93 dd (5, 7)	4.25 dd (4, 6)	4.35 dd (4, 6)	3.84 dd (5, 7)	4.50 dd (4, 7)
H-5[b]	2.60 m (5, 5, 7, 7)	2.60 m (5, 5, 7, 7)	2.81 m (5, 6, 6, 7)	2.96 m (5, 6, 6, 7)	2.47 m (5, 5, 7, 7)	2.84 m (5, 5, 7, 7)
H-6	3.74 dd (7, 12)	3.74 dd (7, 12)	4.26 dd (7, 12)	4.26 dd (7, 12)	3.72 dd (7, 12)	4.25 dd (7, 12)
	3.87 dd (5, 12)	3.87 dd (5, 12)	4.38 dd (6, 12)	4.37 dd (6, 12)	3.85 dd (5, 12)	4.39 dd (5, 12)
NCH_3[b]	3.14 s	3.14 s	3.04 s	3.15 s	3.04 s	3.06 s
H-1′	4.84 d (9)	4.84 d (9)	4.96 d (9)	4.99 d (9)	4.79 d (9)	5.03 d (9)
H-2′	3.91 dd (3, 9)	3.90 dd (3, 9)	3.97 dd (3, 9)	3.94 dd (3, 9)	3.89 dd (3, 9)	4.14 dd (3, 9)
H-3′	4.41 t (3)	4.39 t (3)	5.81 t (3)	5.80 t (3)	4.10 t (3)	5.61 t (3)
H-4′	3.79 dd (3, 10)	3.78 dd (3, 10)	4.05 dd (3, 10)	4.03 dd (3, 10)	3.69 dd (3, 10)	5.05 dd (3, 10)
H-5′	3.96 ddd (2, 7, 10)	3.93 ddd (2, 6, 10)	4.23 ddd (2, 5, 10)	4.23 ddd (2, 5, 10)	3.88 ddd (2, 7, 10)	4.34 ddd (2, 4, 10)
H-6′	3.69 dd (7, 12)	3.70 dd (6, 12)	4.17 dd (5, 12)	4.16 dd (5, 12)	3.71 dd (7, 12)	4.27 dd (7, 12)
	3.88 dd (2, 12)	3.80 dd (2, 12)	4.40 dd (2, 12)	4.41 dd (2, 12)	3.85 dd (2, 12)	4.44 dd (4, 12)
H-1″	4.86 d (9)	4.84 d (9)	5.05 d (9)	4.99 d (9)		
H-2″	3.95 dd (3, 9)	3.94 dd (3, 9)	4.19 dd (3, 9)	4.14 dd (3, 9)		
H-3″	4.12 t (3)	4.11 t (3)	5.58 t (3)	5.56 t (3)		
H-4″	3.75 dd (3, 10)	3.74 dd (3, 10)	4.99 dd (3, 10)	4.97 dd (3, 10)		
H-5″	3.84 ddd (2, 5, 10)	3.87 ddd (2, 6, 10)	4.33 ddd (3, 7, 10)	4.15 ddd (2, 4, 10)		
H-6″	3.80 dd (5, 12)	3.68 dd (6, 12)	4.22 dd (7, 12)	3.57 dd (4, 11)		
	3.93 dd (2, 12)	3.94 dd (2, 12)	4.31 dd (3, 12)	3.66 dd (2, 11)		
OCH_3		3.46 s		3.41 s		
Ac	2.12 s, 2.14 s	2.12 s, 2.13 s	2.02 s, 2.05 s	2.02 s, 2.04 s	2.10 s	2.05 s, 2.10 s
			2.10 s, 2.19 s	2.09 s, 2.22 s		2.20 s, 2.23 s
			2.23 s, 2.23 s	2.24 s, 2.24 s		2.23 s, 2.30 s
			2.25 s, 2.26 s	2.27 s, 2.30 s		
			2.31 s			

[a] Chemical shifts are in ppm ($D_2O + CD_3COOD$, 500 MHz). Coupling constants in Hertz are given in parentheses.
[b] Chemical shifts of these protons were very variable depending on the pH of the solution.

TABLE II. ^{13}C-NMR ASSIGNMENTS OF **1** AND **2**[a]

Assignment	1	2	Assignment	1	2
C-1	87.6	87.4	C-5′	73.4	73.0[c]
C-2	65.4	64.8	C-6′	61.8	61.4
C-3	81.4	80.9	C-1″	101.4	101.1
C-4	85.9	85.6	C-2″	53.7	53.3[b]
C-5	52.4	52.1	C-3″	70.9	70.5
C-6	60.2	59.7	C-4″	67.3	67.1
C-7	161.5	161.0	C-5″	74.4	72.6[c]
C-8	38.4	38.0	C-6″	61.8	71.9[c]
C-9	38.4	38.0	OCH_3		59.2
C-1′	100.7	100.3	NAc (C=O)	174.8	174.1
C-2′	53.4	53.1[b]	(CH_3)	22.9	22.5
C-3′	69.8	69.5		22.9	23.0
C-4′	77.7	77.4			

[a] Chemical shifts are in ppm (D_2O+CD_3COOD, 25 MHz).
[b,c] May be interchanged.

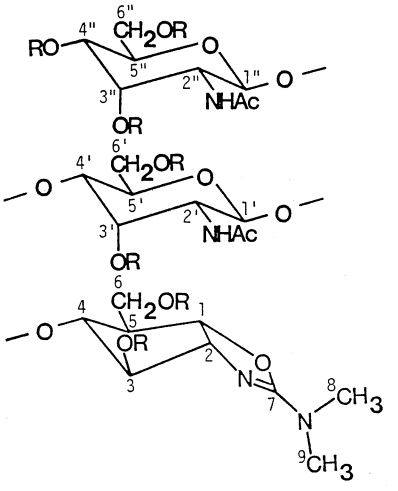


FIG. 6.
1 R=H **8** R=Ac

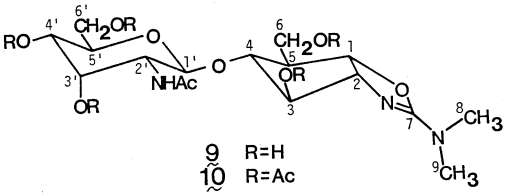


FIG. 7. Structures of **9** and **10**.
The absolute configuration of the aglycon was not determined.

with pyridine and acetic anhydride, the heptaacetate (**8**) was obtained. The COSY and *J*-resolved 2D-NMR spectra of **8** were analyzed, and then the positions of the acetylated hydroxyl groups were determined as those in Fig. 6 by the acetylation shift in the ^{1}H-NMR spectrum of **8** (Table I). These data indicate that **1** was a pseudotrisaccharide composed of two moles of *N*-acetyl-D-allosamine and one mole of **3**, containing two ether bonds 1′-4 and 1″-4′, or 1″-1′ and 4′-4. To determine which combination of ether linkage was present in **1**, a mild acid hydrolysis experiment was performed with 0.5 N HCl (80°C, 4 hr), which produced a pseudodisaccharide (**9**) that had one mole each of *N*-acetylallosamine and **3**. Since analyses of the COSY spectra of **9** and its pentaacetate (**10**) revealed the presence of the newly appeared hydroxyl group at C-4′ in **9**, which was shown by the acetylation shift of H-4′ in **10** (Table I), the possibility of the ether linkage 4′-4 was eliminated. Thus, the ether linkage in **1** was determined as 1′-4 and 1″-4′, which gave the total structure of **1** as that shown in Fig. 8. The *J* values of the anomeric protons (H-1′ and H-1″) were 9 Hz, showing that both glycosidic linkages were *β*.

Methylallosamidin (**2**) was isolated from an aqueous methanol extract of the mycelium of an unidentified actinomycetes, **2** showing very

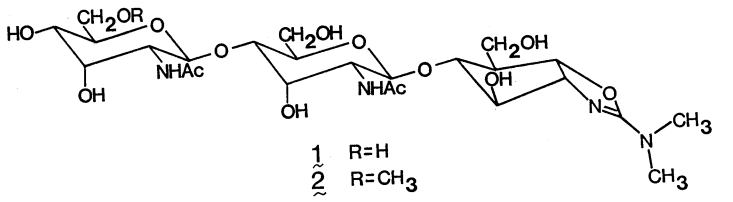


FIG. 8. Structures of **1** and **2**.
The absolute configuration of the aglycon was not determined.

similar physico-chemical properties to that of allosamidin. The FABMS spectrum of **2** showed the $(M+H)^+$ ion at *m/z* 637, which was larger by a 14 mass number than that of **1**. Since a sharp singlet at δ_H 3.46 and a methyl carbon at δ_C 59.2 were observed in the ^{1}H- and ^{13}C-NMR spectra of **2**, respectively, this additional mass number was accounted for by the presence of one methoxyl group in **2**, which was formed by methylation of one of the seven hydroxyl groups in **1**. The carbon bearing the methylated hydroxyl group was assigned to C-6′′ by the fact that the methylene protons at C-6′′ were not shifted by acetylation, this being confirmed by analyses of COSY spectra of **2** and its hexaacetate (**11**). Thus, the structure of **2** was determined to be that shown in Fig. 8. The assignments of carbons and protons in the NMR spectra of **2** are shown in Tables I and II.

Allosamidins consist of two unique compounds. *N*-acetyl-D-allosamine is the C-3 epimer of *N*-acetyl-D-glucosamine and has hitherto been unknown in nature. The relative stereochemistry of C-2 and -3 of allosamizoline (**3**) is identical with those of glucosamine. Further studies to investigate the absolute configuration of allosamizoline (**3**) are now in progress.

EXPERIMENTAL

General procedure. ^{13}C-NMR spectra were recorded at 25 MHz on a JEOL FX-100 spectrometer, using dioxane δ_C 67.4 as an external reference for D_2O solutions. ^{1}H-NMR spectra were recorded at 500 MHz and 300K on a Brucker AM500 spectrometer, or at 100 MHz on a JEOL FX-100 spectrometer. DHO δ_H 4.8 and TMS δ_H 0.0 were used as internal references for the D_2O and $CDCl_3$ solutions, respectively. NOE experiments were carried out on a JEOL GX-400 spectrometer, and mass spectra were obtained on a JEOL JMS DX-303 spectrometer. CD spectra were recorded on a Jasco J-20-C spectropolarimeter, and optical rotation values were measured on a Jasco DIP-140 polarimeter.

Isolation of allosamidin (**1**) *and methylallosamidin* (**2**). The production and isolation of allosamidin (**1**) have been reported elsewhere in detail.[1] **1** was obtained as a crystalline powder, mp 228~238°C (dec.). UV (0.1 M AcOH) end absorption; IR ν_{max}(Nujol) cm^{-1}: 3500, 3350, 3300, 1640~1660, 1560; $[\alpha]_D^{22}$ −24.8° (*c*=0.5, 0.1 M AcOH); ^{1}H- and ^{13}C-NMR (see Tables I and II); FABMS *m/z* 623 $(M+H)^+$(glycerol matrix).

The methylallosamidin-producing microorganism, an unidentified actinomycetes, was fermented in Bennet medium at 26.5°C for 7 days. This strain produced about the same amount of allosamidin as that of methylallosamidin at 4 days' fermentation, but after 7 days' culture, only methylallosamidin was produced. The culture broth (200 l) was filtered and the mycelial cake was extracted with aqueous methanol. After concentrating, the residual aqueous solution was adsorbed on a charcoal column and then eluted with 50% ethanol. The active solution was applied to a SP-Sephadex C-25 column that had been pre-equilibrated with 50 mM $AcONH_4$–AcOH (pH 5.0), the column being eluted with the same buffer. The active eluate was rechromatographed with the same column, and the active fraction afforded 106 mg of methylallosamidin (**2**) as a white powder, mp 205~215°C. $[\alpha]_D^{23}$ −30.2° (*c*=0.5, 0.1 M AcOH); ^{1}H- and ^{13}C-NMR (see Tables I and II); FABMS *m/z* 637 $(M+H)^+$ (glycerol matrix).

Acid hydrolysis of allosamidin (**1**). Allosamidin (**1**, 23 mg) was dissolved in 5.75 ml of 4 N HCl, and the solution was heated at 100°C for 4 hr in a sealed tube. After being concentrated and lyophilized, the obtained solid was dissolved in 0.5 ml of 0.3 N HCl, which was then applied to a Dowex-50 (H^+ type, 200~400 mesh) column (1×23 cm). The column was developed with 0.3 N HCl, and then D-allosamine and allosamizoline (**3**) were separately eluted in this order. After being lyophilized, 13.2 mg of D-allosamine hydrochloride and 7.5 mg of

allosamizoline hydrochloride were obtained.

D-Allosamine hydrochloride was identified by an authentic sample (obtained from Sigma) by GC-MS analysis of its alditol acetates using a column packed with OV-1 (3 mm × 2 m) and by the following physico-chemical properties: FABMS *m/z* 180 $(M+H)^+$; $[\alpha]_D^{22}$ 15.4° ($c=1.0$, H_2O, after 30 min) [lit.[4] 26→17°, 15 min ($c=0.8$, H_2O)]; in the ^{13}C- and ^1H-NMR spectra, the β-anomer was mainly observed. ^{13}C-NMR δ (D_2O, 25 MHz): 54.9 (d), 61.5 (t), 66.9 (d), 68.3 (d), 74.6 (d), 91.3 (d); ^1H-NMR δ (D_2O, 500 MHz): 3.28 (1H, dd, $J=3$, 9, H-2), 3.74 (1H, dd, $J=3$, 10, H-4), 3.77 (1H, dd, $J=5$, 12, H-6a), 3.90 (1H, ddd, $J=2$, 5, 10, H-5), 3.94 (1H, dd, $J=2$, 12, H-6b), 4.33 (1H, t, $J=3$, H-3), 5.16 (1H, d, $J=9$, H-1).

Allosamizoline hydrochloride was a hygroscopic solid. FABMS *m/z* 217 $(M+H)^+$; $[\alpha]_D^{22}$ −22.2° ($c=0.5$, H_2O); ^{13}C-NMR δ (D_2O, 25 MHz): 37.9 (q), 38.1 (q), 51.9 (d), 59.9 (t), 64.2 (d), 75.4 (d), 82.2 (d), 87.2 (d), 161.2 (s). ^1H-NMR δ (D_2O, 100 MHz): 2.43 (1H, m, $J=5$, 5, 7, 8, H-5), 3.08 (3H, s, H-8 or H-9), 3.11 (3H, s, H-8 or H-9), 3.72 (1H, dd, $J=7$, 12, H-6a), 3.83 (1H, dd, $J=7$, 8, H-4), 3.92 (1H, dd, $J=5$, 12, H-6b), 4.14 (1H, dd, $J=4$, 7, H-3), 4.34 (1H, dd, $J=4$, 9, H-2), 5.37 (1H, dd, $J=5$, 9, H-1).

Allosamizoline triacetate (**4**) was prepared by following the same method as that with which **8** was prepared. EIMS 342 $(M)^+$, 282, 223, 113; ^1H-NMR δ ($CDCl_3$, 400 MHz): 2.03 (3H, s), 2.06 (3H, s), 2.11 (3H, s). 2.63 (1H, m, $J=4$, 5, 6, 7, H-5), 3.03 (6H, s, H-8 and H-9), 4.17 (1H, dd, $J=6$, 11, H-6), 4.21 (1H, dd, $J=5$, 11, H-6), 4.51 (1H, dd, $J=8$, 4, H-2), 4.94 (1H, dd, $J=4$, 8, H-1), 5.08 (1H, dd, $J=5$, 7, H-4), 5.22 (1H, dd, $J=4$, 5, H-3).

Synthesis of 2-dimethylamino-5-methyl-2-oxazoline. A mixture of 0.8 g of *N,N*-dimethyl-*N'*-allylurea and 20 ml of 48% hydrobromic acid in a sealed tube was heated at 100°C for 4 hr. After cooling, the reaction mixture was made alkaline with 10 N NaOH aq. and then extracted with $CHCl_3$. The organic layer was washed with brine and dried over anhydrous Na_2SO_4. The solution was evaporated under reduced pressure and distilled to give 0.6 g of colorless oil, bp 70°C/20 mmHg. ^{13}C-NMR δ (D_2O+DCl, 25 MHz): 19.4 (q), 37.8 (q), 37.9 (q), 49.9 (t), 82.7 (d), 161.5 (s), ^1H-NMR δ (D_2O+DCl, 100 MHz): 1.39 (3H, d, $J=6$, 5-CH_3), 2.92 (3H, s, N-CH_3), 2.95 (3H, s, N-CH_3), 3.37 (1H, dd, $J=8$, 10, H-4a), 3.88 (1H, dd, $J=8$, 10, H-4b), 5.13 (1H, m, $J=6$, 8, 8, H-5).

Preparation of the p-(dimethylamino)benzoyl derivatives of **3**. The *p*-(dimethylamino)benzoyl derivatives of **3** were prepared from *p*-(dimethylamino)benzoyl cyanide, which itself was prepared by phase transfer catalysis using the method of Koenig *et al.*[7] 0.2 g of NaCN in 2 ml of water was added to a solution of 0.64 g of *p*-(dimethylamino)benzoyl chloride, 10 mg of tetraammonium bromide and 30 ml of CH_2Cl_2 at 0°C under N_2 gas. The reaction mixture was stirred for 1 hr and filtered. After washing the solids with CH_2Cl_2, the organic layer was dried over anhydrous Na_2SO_4 and evaporated under reduced pressure to produce solids, which gave 0.33 g (54% yield) of a bright yellow product by recrystallization from EtOH, mp 167-168°C (lit.[8] 172°C); EIMS *m/z* 174 $(M)^+$.

A reaction mixture of 5 mg of allosamizoline hydrochloride, 16 mg of *p*-(dimethylamino)benzoyl cyanide, 8 mg of tri-*n*-butylamine and 1.5 ml of dried CH_3CN was stirred overnight at room temperature.[9] The reaction solution was purified by HPLC using a Senshu Pak SSC-C_8 column (4.6 × 250 mm) with a mobile phase of 10 mM $AcONH_4$: CH_3CN (27:73) to give the 4, 6-bis[*p*-(dimethylamino)benzoate] (**5**, 1000 μg), 3, 6-bis[*p*-(dimethylamino)benzoate] (**6**, 450 μg), and tri[*p*-(dimethylamino)benzoate] (**7**, 230 μg) of allosamizoline (**3**). **5**: FABMS *m/z* 511 $(M+H)^+$ (diethanolamine matrix); ^1H-NMR δ ($CDCl_3$, 500 MHz): 2.95 (1H, m, H-5), 4.45 (1H, dd, $J=8$, 11, H-6), 4.50 (1H, dd, $J=2$, 4, H-3), 4.55 (1H, dd, $J=2$, 8, H-2), 4.58 (1H, dd, $J=5$, 11, H-6), 5.17 (1H, dd, $J=4$, 6, H-4), 5.22 (1H, dd, $J=4$, 8, H-1); UV λ_{max} ($CHCl_3$) nm: 315.5; CD($CHCl_3$): $\Delta\varepsilon_{324}=+27$, $\Delta\varepsilon_{312}=0$, $\Delta\varepsilon_{301}=-11$. **6**: FABMS *m/z* 511 $(M+H)^+$ (diethanolamine matrix); ^1H-NMR δ ($CDCl_3$, 500 MHz): 2.90 (1H, m, H-5), 4.34 (1H, t, $J=4$, H-4), 4.42 (1H, dd, $J=5$, 11, H-6), 4.47 (1H, dd, $J=9$, 11, H-6), 4.84 (1H, dd, $J=2$, 8, H-2), 5.50 (1H, dd, $J=3$, 8, H-1), 5.68 (1H, dd, $J=2$, 4, H-3); UV λ_{max}($CHCl_3$) nm: 315.5; CD($CHCl_3$): $\Delta\varepsilon_{324}=+11$, $\Delta\varepsilon_{312}=0$, $\Delta\varepsilon_{301}=-5$. **7**: FABMS *m/z*658 $(M+H)^+$ (diethanolamine matrix); ^1H-NMR δ ($CDCl_3$, 500 MHz): 2.95 (1H, m, H-5), 4.45 (1H, dd, $J=6$, 11, H-6), 4.56 (1H, dd, $J=9$, 11, H-6), 5.07 (1H, dd, $J=2$, 8, H-2), 5.41 (1H, br.s, $J=4<$, H-4), 5.61 (1H, dd, $J=3$, 8, H-1), 5.77 (1H, br.s, $J=4<$, H-3); UV λ_{max} ($CHCl_3$) nm: 316; CD($CHCl_3$): $\Delta\varepsilon_{324}=-24$, $\Delta\varepsilon_{311}=0$, $\Delta\varepsilon_{301}=+9$.

Preparation of allosamidin heptaacetate (**8**) *and methylallosamidin hexaacetate* (**11**). Allosamidin (**1**, 23.5 mg) was mixed with pyridine (4 ml) and acetic anhydride (2 ml), and the mixture was left at 26.5°C for 40 hr. The reaction solution was put into ice-cold water, and the solution was applied to a SEP-PAK C_{18} cartridge (Waters Associates). After washing with water, the cartridge was eluted with 50% CH_3CN. The eluate was further purified by HPLC using an Asahipak ES-502C column (7.6 × 100 mm) with a mobile phase of 10 mM $AcONH_4$ (pH 7.0): CH_3CN (80:20) to give a white powder (14.5 mg) of allosamidin heptaacetate (**8**); FABMS *m/z* 917 $(M+H)^+$ (diethanolamine matrix); ^1H-NMR (see Table I).

Methylallosamidin hexaacetate (**11**) was prepared by the same method as that just mentioned; FABMS *m/z* 889 $(M+H)^+$ (diethanolamine matrix); ^1H-NMR (see Table I).

Mild acid hydrolysis of allosamidin (**1**). Allosamidin (**1**, 39 mg) was dissolved in 3.75 ml of 0.5 N HCl, and the solution was heated at 80°C for 4 hr. After being lyophi-

lized, the obtained powder was purified by HPLC using an Asahipak ES-502C column (7.6 × 100 mm) with a mobile phase of 10 mM $AcONH_4$–NH_4OH (pH 9.1) to give 3.4 mg of the pseudodisaccharide (**9**) as a white powder; FABMS *m/z* 420 $(M+H)^+$ (glycerol matrix); ^{1}H-NMR (see Table I).

The pentaacetate (**10**) of **9** was prepared by following the same method as that with which **8** was prepared; FABMS *m/z* 630 $(M+H)^+$ (diethanolamine matrix); ^{1}H-NMR (see Table I).

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