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Mechanism of the Cyclopentane Ring Formation of Allosamizoline, an Aminocyclitol Derivative of the Chitinase Inhibitor Allosamidin

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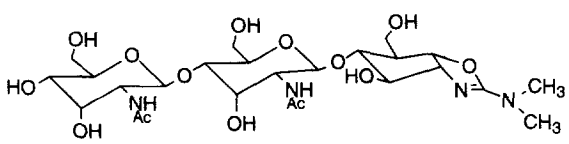
Abstract : Feeding experiments to study the mechanism of cyclopentane ring formation of allosamizoline with [3-^{2}H], [4-^{2}H]-, [5-^{2}H]-, and [6-^{2}H$_2$]-D-glucosamine indicated that the cyclization to form the cyclopentanoid moiety of allosamizoline proceeds *via* an intermediate 6-aldehyde glucosamine derivative. Copyright © 1996 Elsevier Science Ltd

Allosamidin **1** is a chitinase inhibitor produced by *Streptomyces* sp.[1] It shows interesting biological activities against chitin-containing organisms[2] and has a unique pseudotrisaccharide structure consisting of two units of *N*-acetyl-D-allosamine and one unit of an aminocyclitol derivative, termed allosamizoline **2**.[3] Our recent biosynthetic studies on **1** revealed that the carbon skeleton and the nitrogen atom on C-2 of each of allosamine and the cyclopentane moiety of **2** originates from an intact glucosamine molecule.[4] A cyclopentanoid structure of carbohydrate origin is relatively rare in natural products,[5] and the cyclopentane ring of **2** is the first example formed from glucosamne. In this paper, we describe the elucidation of the mechanism of cyclopentane ring formation of **2** by means of feeding experiments with specifically ^{2}H-labeled glucosamines.



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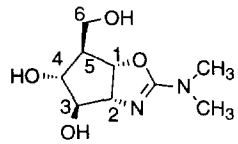


2

Cultivation of *Streptomyces* sp. AJ 9463 was performed in a 500-ml Erlenmeyer flask containing 100 ml Bennet medium. Labeled glucosamine was added in one portion to the culture, and labeled **1** was isolated, as described previously.[4] Acid hydrolysis of labeled **1** afforded labeled allosamine and **2** for ^{2}H NMR

measurement to confirm the position of the incorporated deuterium. The ^2H labeled allosamine and **2** were then converted to labeled allosaminitol peracetate and the triacetate of **2**, respectively, for CI-MS analysis, to evaluate incorporation of deuterium. Since glucosamine is a common precursor of both **2** and allosamine, the comparison of deuterium incorporation into **2** with that into allosamine was very useful to evaluate whether a deuterium loss from labeled glucosamine had specifically occured during the biosynthesis of the cyclopentane ring of **2** or not.

First, [4-^2H]-D-glucosamine[6] (10 mg) was fed to the culture (12 x 100 ml broth). The ^2H NMR[7] and CI-MS analysis of the labeled samples prepared from **1** obtained (9.8 mg) showed that deuterium was incorporated onto each C-4 of allosamine (10.9 %) and **2** (8.4 %). Next, the incorporation experiment of [3-^2H]-D-glucosamine[6] (25 mg, 21 x 100 ml broth) was performed. Deuterium enrichment was observed on C-3 of **2** (14.6 %) and also that of allosamine (12.5 %) from **1** obtained (12.0 mg). This deuterium incorporation onto C-3 of allosamine suggested that the epimerization of a hydroxyl group at C-3 occured with retention of the deuterium on C-3 of glucosamine. [5-^2H]-D-Glucosamine[8] (12 mg) was, next, administered to the culture (10 x 100 ml broth). Deuterium incorporation was observed on C-5 of allosamine (10.9 %) from **1** obtained (6.5 mg), but no incorporation into **2** was detected, indicating that deuterium on C-5 of glucosamine was lost during the formation of the cyclopentane ring.

The feeding experiment of [6-$^2H_2$]-D-glucosamine[8] (10 mg, 19 x 100 ml broth) was carried out finally. In the ^2H NMR spectrum of labeled allosamine prepared from **1** obtained (11 mg), two deuterium signals, whose chemical shifts corresponded to those of the methylene protons on C-6 of allosamine, were observed (Fig. 1a). On the other hand, only one deuterium signal, which had the same chemical shift as that of one of the two protons on C-6 of **2**, was observed in the spectrum of labeled **2** (Fig. 1c). The CI-MS spectra of labeled allosaminitol peracetate and the triacetate of **2** indicated that the mono- and di-labeled molecules increased by 2.7 and 14.0 %, and 3.7[9] and -0.14 %, respectively. These data suggested that one of the two deuterium atoms on C-6 of glucosamine was lost stereospecifically during the formation of the cyclopentane ring.

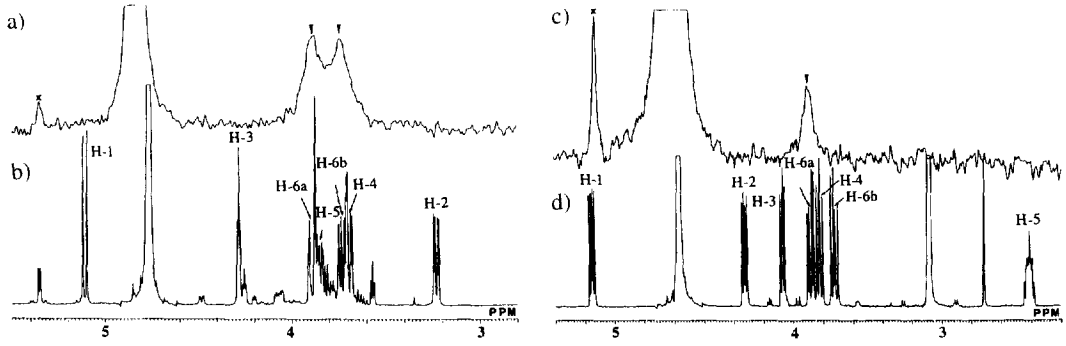
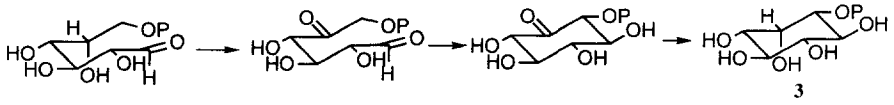
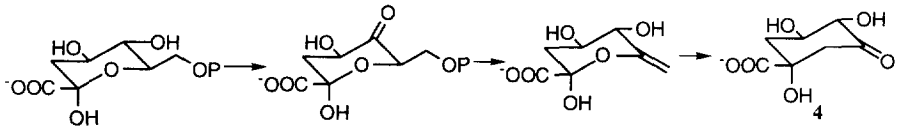


Figure 1. (a) ^2H NMR spectrum of allosamine derived from [6-$^2H_2$]-D-glucosamine (61MHz, 5 mg in 0.6 ml of D_2O). (b) ^1H NMR spectrum of natural allosamine (400MHz, 10 mg in 0.6ml of D_2O). Signals of the β-anomer are mainly observed. (c) ^2H NMR spectrum of **2** derived from [6-$^2H_2$]-D-glucosamine (61MHz, 3 mg, in 0.6ml of D_2O). (d) ^1H NMR spectrum of natural **2** (400MHz, 10 mg in 0.6 ml of D_2O).
X: not identified, whose chemical shift doesn't correspond to that of H-1 of α-anomer of allosamine or H-1 of **2**.

The cyclization to form the cyclopentane ring of **2** is presumed to proceed *via* a 4-keto or 6-aldehyde (or their enol equivalent) glucosamine derivative, which would undergo an aldol condensation of C-5 with C-1. By analogy with the mechanisms of formation of cyclohexane rings observed in the biosynthetic pathways of inositol or shikimic acid (Scheme 1 and 2), there are three possible pathways to form the cyclopentane ring of **2** (Fig. 2).[10] The results obtained here strongly suggest the presence of a pathway *via* a 6-aldehyde intermediate (pathway B). Work to investigate the stereochemistry of the cyclization mechanism is now in progress.



Scheme 1. Mechanism of the *myo*-inositol-1-phosphate (**3**) biosynthesis.



Scheme 2. Mechanism of the dehydroquinate (**4**) biosynthesis involved in the biosynthetic pathway of shikimic acid.

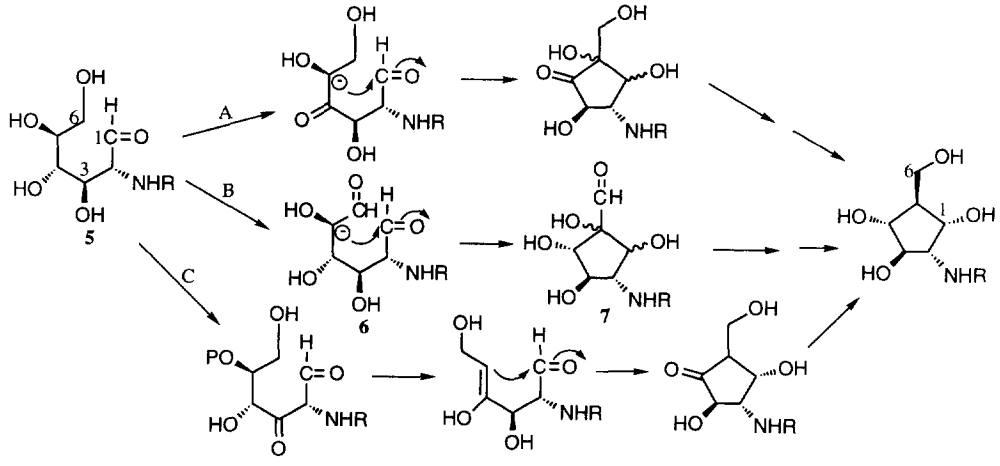


Figure 2. Plausible mechanism of formation of the cyclopentane ring of **2**.
Pathway A and B: Analogous mechanism of cyclization during inositol biosynthesis.
Pathway C: Analogous mechanism of cyclization during shikimic acid biosynthesis.

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6. [3-^{2}H]-D-Glucosamine (98 atom % ^{2}H) and [4-^{2}H]-D-glucosamine (72 atom % ^{2}H) were prepared with NaB^2H_4 (98 atom % ^{2}H) as described in the following: Bundle, D. R.; Jennings, H. J.; Smith, I. C. R. *Can. J. Chem.*, **1973**, *51*, 3812-3819.
7. Spectra were taken on a JEOL JNM-GSX-400 spectrometer in an unlocked mode with ^{1}H broad-band decoupling (pulse width = 45° , acquisition time = 4.096 s).
8. [5-^{2}H]-D-Glucosamine (70 atom % ^{2}H) and [6-$^2H_2$]-D-glucosamine (98 atom % ^{2}H) were prepared from [5-^{2}H]- and [6-$^2H_2$]-D-glucose, respectively, as described in the followings: (a) Nishida, Y.; Hori, H.; Ohrui, H.; Meguro, H. *Carbohydr. Res.*, **1987**, *170*, 106-111. (b) Mackie, W.; Perlin, A. S. *Can. J. Chem.*, **1965**, *43*, 2645-2651. (c) Moss, G. *Arch. Biochem. Biophys.*, **1960**, *90*, 111-113.
9. It's not clear why this increased value is much lower than that of di-labeled molecules of the labeled allosaminitol peracetate. If a non-stereospecific interconversion of **5** and **6**, or an interconversion of **6** and its 6-carboxylic acid derivative or **7** and its 6-carboxylic acid derivative occurs during the formation of the cyclopentane ring in the pathway B, such a low deuterium incorporation onto C-6 of **2** might be observed.
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