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# THE STRUCTURE OF ALLOSAMIDIN, A NOVEL INSECT CHITINASE INHIBITOR, PRODUCED BY STREPTOMYCES SP.

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Abstract: Allosamidin(1), a novel insect chitinase inhibitor, was isolated from the mycelium of Streptomyces sp. and characterized as 1, which was a basic pseudotrisaccharide consisting of 2-acetamido-2-deoxy-D-allose(N-acetyl-D-allosamine) and a novel aminocyclitol derivative(3), termed allosamizoline.

Chitin is a main component of insect cuticle. Since insects' growth is accompanied with ecdysis which associates with synthesis and degradation of chitin[1], the inhibitors against chitinase, which hydrolyze chitin into its monomer, 2-acetamido-2-deoxy-D-glucose(N-acetyl-D-glucosamine), should be the models for designing a new type of insect growth regulators. From this point of view we have been continuing to search the chitinase inhibitors among microbial products and found that the mycelial extract of Streptomyces sp. markedly inhibited the chitinases of the silkworm, Bombyx mori, in vitro and prevented its larval ecdysis in vivo[2]. In this paper we wish to report preliminarily the structural elucidation of the active principle, termed allosamidin(1).

Streptomyces sp. No 1713 was fermented in a Bennet medium at 26.5°C for 5 days. The aqueous methanol extract of the mycelia was adsorbed on a charcoal column and the eluate with 50% ethanol was further purified with ion exchange column chromatography using Dowex-50 and SP-Sephadex C-25, successively, to yield a white powder of 1 termed allosamidin : $C_{25}H_{42}N_4O_{14}$, FABMS m/z 623$(M+H)^+$; IR $\nu_{max}^{nujol}$ $cm^{-1}$:3500, 3350, 3300, 1640~1660, 1560; UV end absorption; $[\alpha]_D$ -24.8°(c=0.5, 0.1M AcOH).

On hydrolysis with 4N HCl(100°C, 4hr), 1 gave two basic products, D-allosamine[3] and an aminocyclitol derivative(3) termed allosamizoline. The FABMS spectrum of 3 showed the $(M+H)^+$ ion at m/z 217 and the signals due to 9 carbons were observed in the $^{13}C$-NMR spectrum[4]. Since 3 gave the triacetate(4), $C_{15}H_{22}N_2O_7$(m/z 342.14063, error -2.0mmu), the molecular formula of 3 was determined as $C_9H_{16}N_2O_4$. The connection from C-1 through C-6, to constitute a cyclopentane ring, was easily revealed with the conventional

proton decoupling experiments and the location of three hydroxyl groups in 3 was determined to be C-3,4 and 6 by acetylation shift in the $^1$H-NMR spectrum of 4. The chemical shifts of the residual three carbons [δ 37.9(q), 38.1(q), 161.2(s)] and six protons [δ 3.08(3H,s), 3.11(3H,s)] suggested the presence of a dimethylamino-oxazoline moiety[5], which was confirmed by spectral comparison with the synthetic analog, 2-dimethylamino-5-methyl-2-oxazoline[6]. Thus the structure of 3 was determined as Figure 1. The relative stereochemistry of cyclopentane ring of 4 was shown as Figure 2, which was suggested by the J values of ring protons and NOE experiments.

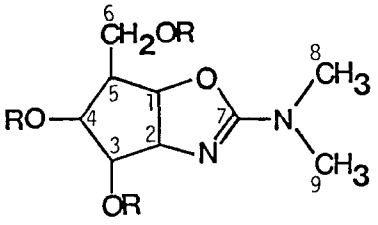



Fig. 1 3 R=H
4 R=Ac

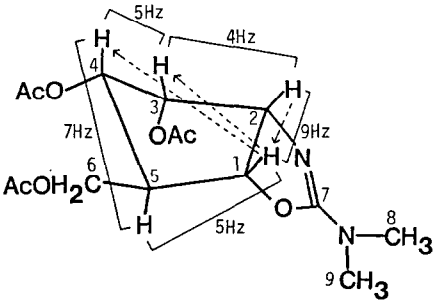



Fig. 2 Relative stereochemistry of cyclopentane ring of 4 (----→ NOE)

Analysis of the 500MHz COSY and J-resolved as well as the C-H shift correlated 2D-NMR of 1 revealed the presence of two moles of N-acetyl-allosamine and one mole of 3, which was in accordance with the molecular formula. Assignment of carbons and protons in the NMR spectra are summarized in Table 1. When 1 was acetylated with pyridine and acetic anhydride, the heptaacetate(2) was obtained, and the positions of acetylated hydroxyl groups were determined as Figure 3 by analysis of COSY and J-resolved 2D-NMR of 2. These data indicated 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. The possibility of the ether linkage 4'-4 was eliminated by the facts that a pseudodisaccharide(5) having one mole each of N-acetyl-allosamine and 3 was obtained by mild acid hydrolysis of 1 with 0.5N HCl(80°C, 4hr) and the presence of the newly appeared hydroxyl group at C-4' was confirmed by the acetylation shift of H-4' in pentaacetate of 5(6)[7]. Thus the sequence of the pseudotrisaccharide was determined as Figure 4, which gave the total structure of 1. The J values of anomeric protons(H-1" and H-1') showed both glycosidic linkage were β.

Allosamidin showed strong inhibitory activity against the chitinases of the silkworm, Bombyx mori, at a concentration of 0.35μM in vitro. Furthermore when 1 was injected to the peneltimate instar larvae of the silkworm at the dose of 10μg per larva, all test insects failed in larval ecdysis to the next stage and were followed by death.

Table 1. $^{13}C$ and $^{1}H$-NMR Spectra of 1

| C-No. | | $C^{a)}$ | $H^{b)}$ |
|---|---|---|---|
| 1 | | 87.6 | 5.45 (dd,J=5,9) |
| 2 | | 65.4 | 4.45 (dd,J=4,9) |
| 3 | | 81.4 | 4.35 (dd,J=4,5) |
| 4 | | 85.9 | 3.94 (dd,J=5,7) |
| 5 | | 52.4 | 2.60 (m,J=5,5,7,7) |
| 6 | | 60.2 | 3.74 (dd,J=7,12), 3.88 (dd,J=5,12) |
| 7 | | 161.5 | |
| 8 | | 38.4 | 3.14 (s) |
| 9 | | 38.4 | 3.14 (s) |
| 1' | | 100.7 | 4.84 (d,J=9) |
| 2' | | 53.4 | 3.91 (dd,J=3,9) |
| 3' | | 69.8 | 4.41 (t,J=3) |
| 4' | | 77.7 | 3.79 (dd,J=3,10) |
| 5' | | 74.4 | 3.84 (ddd,J=2,5,10) |
| 6' | | 61.8 | 3.80 (dd,J=5,12), 3.93 (dd,J=2,12) |
| NAc | (C=O) | 174.8 | |
| | ($CH_3$) | 22.9 | 2.14[c)] (s) |
| 1" | | 101.4 | 4.86 (d,J=9) |
| 2" | | 53.7 | 3.95 (dd,J=3,9) |
| 3" | | 70.9 | 4.12 (t,J=3) |
| 4" | | 67.3 | 3.75 (dd,J=3,10) |
| 5" | | 73.4 | 3.96 (ddd,J=2,7,10) |
| 6" | | 61.8 | 3.69 (dd,J=7,12), 3.87 (dd,J=2,12) |
| NAc | (C=O) | 174.8 | |
| | ($CH_3$) | 22.9 | 2.12[c)] (s) |

a) ppm (25MHz in $D_2O$+$CD_3COOD$)
b) ppm (500MHz in $D_2O$+$CD_3COOD$)
c) May be interchanged.

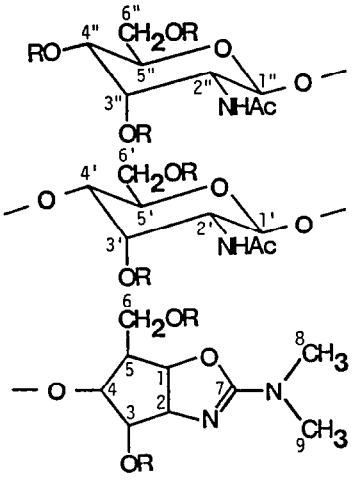
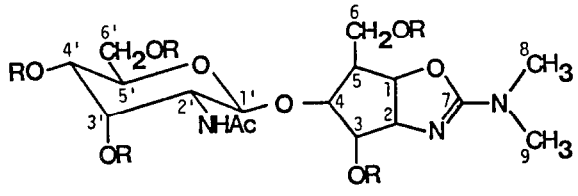


Fig. 3 1 R=H
2 R=Ac



5 R=H
6 R=Ac

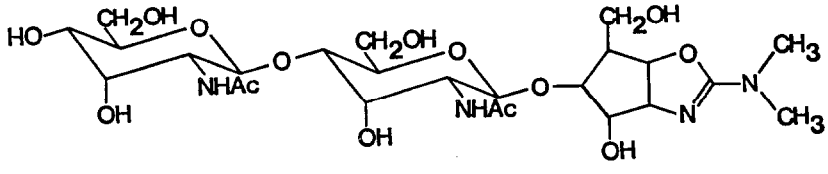


Fig. 4 Structure of allosamidin(1)

Allosamidin consists of two unique compounds. N-acetyl-D-allosamine is the C-3 epimer of N-acetyl-D-glucosamine and hitherto unknown in nature. The relative configurations of C-2,3 and 4 of allosamizoline(3) were identical with those of allosamine, suggesting that 3 might be biosynthesized from allosamine.

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References and notes

1) K. J. Kramer, C. Dziadik-Turner and D. Koga, "Comprehensive Insect Physiology Biochemistry and Parmacology," Vol. 3, ed. by G. A. Kerkut and L. I. Gilbert, Pergamon Press, Inc., New York, 1985 p. 75.

2) Chitinase assay method and biological activity of allosamidin will be published in detail elsewhere.

3) (a) R. W. Jeanloz, "Methods in Carbohydrate Chemistry," Vol. 1, Academic Press, Inc., New York, 1962 p. 212.
(b) D-allosamine hydrochloride was identified with the authentic sample by GC-MS analysis of alditol acetates and following physico-chemical properties : FABMS m/z 180 $(M+H)^+$, $[\alpha]_D$ 15.4°(c=1.0, $H_2O$, after 30 minutes), in $^{13}C$ and $^1H$-NMR spectra β- anomer was mainly observed. $^{13}C$-NMR δ ($D_2O$, 25MHz) : 54.9(d), 61.5(t), 66.9(d), 68.3(d), 74.6(d) 91.3(d), $^1H$-NMR δ ($D_2O$, 500MHz) : 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).

4) 3 hydrochloride was a hygroscopic solid, $[\alpha]_D$ -22.2°(c=0.5, $H_2O$), $^{13}C$-NMR δ($D_2O$, 25MHz) : 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 ($D_2O$, 100MHz) : 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).

5) V. S. Bogdanov, M. A. Aitzhanova, I. A. Abronin and L. B. Medvedskaya, Izv. Akad. Nauk SSSR, Ser. Khim., 305(1980).

6) (a) L. A. Tsoi, A. D. Salimbaeva and B. D. Abiyurov, Zh. Org. Khim., 19, 2605(1983).
(b) 2-dimethylamino-5-methyl-2-oxazoline ; $^{13}C$-NMR δ ($D_2O$+DCl, 25MHz) : 19.4(q), 37.8(q), 37.9(q), 49.9(t), 82.7(d), 161.5(s), $^1H$-NMR δ($D_2O$+DCl,100MHz) : 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).

7) $\delta_H$ of H-4' in 5 : 3.69, $\delta_H$ of H-4' in 6 : 5.05.

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