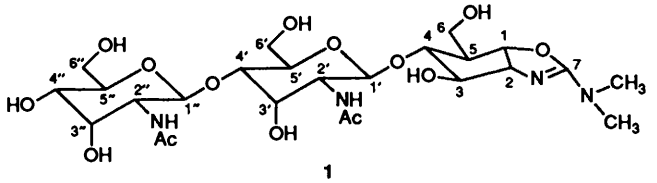
# Biosynthetic Studies on the Chitinase Inhibitor, Allosamidin. Origin of the Carbon and Nitrogen Atoms

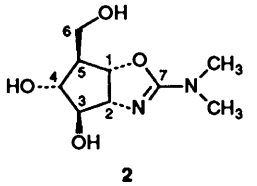
**Ze-Yang Zhou, Shohei Sakuda* and Yasuhiro Yamada***
*Department of Biotechnology, Faculty of Engineering, Osaka University, Yamada-oka 2-1, Suita-shi, Osaka 565, Japan*

Allosamidin **1** is a potent insect chitinase inhibitor produced by *Streptomyces sp.* The biosynthesis of compound **1** was studied by feeding experiments with labelled precursors. Incorporation experiments using [1-$^{13}$C]- and D-[6-$^{13}$C]glucose as well as doubly labelled D-[1-$^{13}$C,2-$^{15}$N]-glucosamine revealed that each skeleton of *N*-acetyl-D-allosamine and allosamizoline **2** was derived from D-glucosamine. Further experiments with L-[*guanidino*-$^{13}$C,$^{15}N_2$]arginine and [*methyl*-$^{13}$C]-methionine clarified the origin of the dimethylaminooxazoline moiety of compound **2**.

In 1986, allosamidin 1 was isolated from mycelia of *Streptomyces sp.* no. 1713 as the first chitinase inhibitor.[1] It possesses a novel pseudotrisaccharide structure[2,3] and interesting biological activities against chitin-containing organisms, such as insects and fungi.[4–10] Allosamidin consists of two characteristic components. One is *N*-acetyl-D-allosamine, which is a C-3 epimer of *N*-acetyl-D-glucosamine hitherto unknown in nature. Two units of *N*-acetyl-D-allosamine are connected with β-1,4 linkage and the reducing end is linked *via* the β form to the other unique component, named allosamizoline **2**. This has a



**1**



**2**

novel cyclopentanoid structure which is highly oxygenated and fused with an aminooxazoline ring. The absolute configuration of allosamizoline has been assigned as compound **2** in which the configurations of C-2 and -3 are identical with those of D-glucosamine.[11] Recently, the synthesis of racemic[12] and chiral **2**,[13,14] and the total synthesis of compound **1**[15,16] have been reported in succession. In this paper, we describe the first elucidation of the biosynthesis of **1**, which clarifies the origin of its carbon skeleton and nitrogen atoms, by means of feeding experiments with labelled precursors.

## Results and Discussion

*Streptomyces sp.* AJ 9463, which was found to be a high producer of compound **1**, was used throughout this work. Cultivation was performed in a 500 cm$^3$ Erlenmeyer flask containing Bennet medium (100 cm$^3$), on a rotary shaker. Labelled precursor was added in one portion to the culture at the 36th hour of cultivation, around which time production of allosamidin started. After 120 h cultivation, mycelia obtained by filtration were extracted with aqueous methanol and the extract was chromatographed on charcoal. Further purification by HPLC (high-performance liquid chromatography) afforded *ca.* 7 mg of compound **1** from 1 dm$^3$ of culture.

**Table 1** Incorporation of $^{14}$C-labelled precursors into allosamidin **1**

| Expt. | Precursor | Incorporation[a] (%) |
|---|---|---|
| 1 | D-[1-$^{14}$C]glucose | 0.008 |
| 2 | D-[1-$^{14}$C]glucose[b] | 0.03 |
| 3 | D-[1-$^{14}$C]glucosamine | 0.75 (0.46,[c] 0.22[d]) |
| 4 | L-[*guanidino*-$^{14}$C]arginine | 3.0 (0,[c] 2.7[d]) |

[a] Total radioactivity in **1**/total radioactivity in precursor. [b] Replacement culture. [c,d] Incorporation to allosamine and **2**, respectively, measured after hydrolysis.

Since the carbohydrate pool may be a biosynthetic origin for each moiety of D-allosamine and the cyclopentane ring of **2**, incorporation experiments with labelled glucose were carried out first. Very low incorporation was observed in the experiment with $^{14}$C-glucose (Table 1, expt. 1) due to dilution of the labelled glucose with the non-labelled form contained in the medium. Replacement culture was therefore carried out to avoid a high level of dilution. After 36 h of cultivation, the usual medium was exchanged with a replacement medium containing little glucose. The yield of **1** slightly decreased under the replacement conditions, but a higher incorporation was observed in the experiment with $^{14}$C-glucose (Table 1, expt. 2). Next, [1-$^{13}$C]- or [6-$^{13}$C]-D-glucose was administered to the culture under the same conditions. The $^{13}$C NMR spectrum of $^{13}$C-labelled **1** showed enrichment at C-1, C-1′ and C-1″, and C-6, C-6′ and C-6″, respectively (Table 2). These results indicated that each carbon skeleton of D-allosamine and the cyclopentane ring of **2** were derived from D-glucose.

In the next experiment, labelled glucosamine was evaluated as a precursor as the nitrogen atom on C-2 of allosamine or **2** strongly suggested that glucose may be incorporated into it *via* glucosamine. D-[1-$^{14}$C]Glucosamine was administered to the culture first. In this case, a high degree of incorporation into each moiety of allosamine and **2** was observed without the use of replacement conditions (Table 1, expt. 3). Next, in order to verify the incorporation of the nitrogen of D-glucosamine into each nitrogen atom on C-2, C-2′ and C-2″ of **1**, a feeding experiment with doubly labelled D-[1-$^{13}$C,2-$^{15}$N]glucosamine was undertaken. The $^{13}$C NMR spectrum of the resulting compound **1** showed enriched peaks at C-1, C-1′ and C-1″ (Table 2). These enriched peaks were slightly broadened, but under the experimental conditions used for $^{13}$C NMR spectroscopy, an expected clear two-bond coupling with $^{15}$N on C-2,

**Table 2** $^{13}C$ Abundances in **1** obtained from feeding experiments with $^{13}C$-labelled precursors

| Carbon no. | $\delta_C$[b] | Relative $^{13}C$ abundance[a] | | | |
|---|---|---|---|---|---|
| | | D-[1-$^{13}C$]Glucose | D-[6-$^{13}C$]Glucose | D-[1-$^{13}C$,2-$^{15}N$]-Glucosamine | L-[*Guanidino*-$^{13}C$,$^{15}N_2$]-arginine |
| 1 | 87.4 | 2.3 | 1.2 | 6.1[c] | 1.0 |
| 2 | 64.9 | 1.1 | 1.2 | 1.0 | 0.8 |
| 3 | 81.1 | 1.0 | 1.0 | 1.0 | 1.0 |
| 4 | 85.6 | 0.9 | 1.2 | 1.0 | 1.0 |
| 5 | 52.1 | 0.9 | 1.2 | 1.0 | 0.9 |
| 6 | 59.8 | 1.3 | 2.3 | 1.0 | 1.1 |
| 7 | 161.2 | 1.0 | 1.1 | 1.0 | 63.2 ($^1J_{NC}$ 29 Hz) |
| 1′ | 100.5 | 2.3 | 1.1 | 10.4[c] | 1.1 |
| 2′ | 53.2 | 0.9 | 1.0 | 1.1 | 1.1 |
| 3′ | 69.6 | 1.1 | 1.0 | 1.1 | 1.2 |
| 4′ | 77.5 | 0.9 | 1.2 | 1.0 | 1.2 |
| 5′ | 73.1 | 0.9 | 1.0 | 1.1 | 1.1 |
| 6′ | 61.5 | 1.0 | 1.8 | 1.0 | 1.0 |
| 1″ | 101.2 | 2.0 | 0.9 | 9.5[c] | 0.8 |
| 2″ | 53.4 | 0.7 | 1.0 | 1.0 | 0.8 |
| 3″ | 70.6 | 1.0 | 1.1 | 0.9 | 1.1 |
| 4″ | 66.9 | 0.8 | 0.9 | 0.9 | 0.9 |
| 5″ | 74.1 | 0.8 | 1.0 | 1.0 | 1.0 |
| 6″ | 61.5 | 1.0 | 1.7 | 0.9 | 0.9 |

[a] Peak height ratio of $^{13}C$ enriched to natural abundance. [b] Assignments cited from ref. 3. [c] Deduced from peak area because of peak broadening.

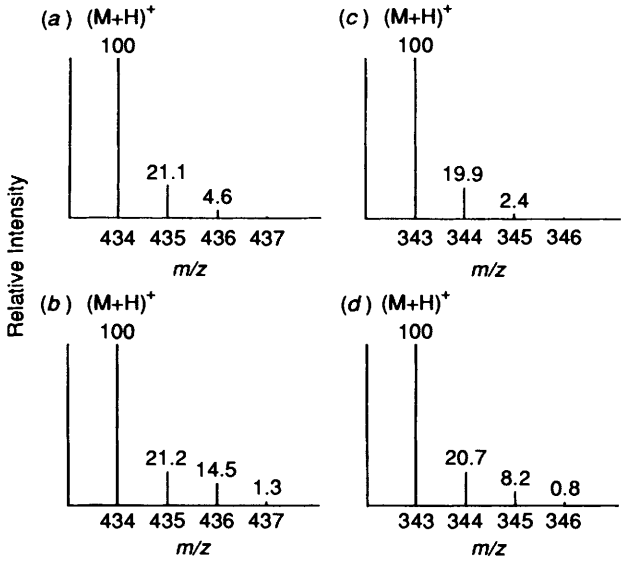



**Fig. 1** CI Mass spectra of allosaminitol peracetate, (*a*) and (*b*), and allosamizoline triacetate, (*c*) and (*d*): (*a*) and (*c*) natural abundance; (*b*) and (*d*) derived from D-[1-$^{13}C$,2-$^{15}N$]glucosamine

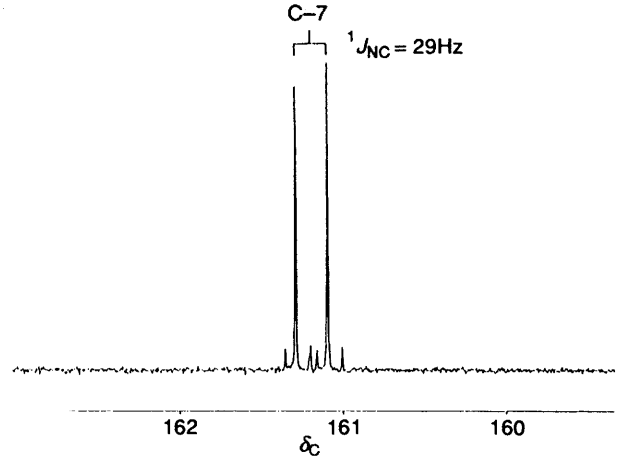



**Fig. 2** $^{13}C$ NMR spectrum of **1** derived from L-[*guanidino*-$^{13}C$,$^{15}N_2$]-arginine (150 MHz; 2.8 mg in 0.6 $cm^3$ of $D_2O$ + 0.3% $CD_3CO_2D$, 7944 transients)

C-2′ or C-2″ could not be observed at any of the enriched carbon signals, due to its small value.[17] As the intact incorporation of $^{15}N$ could not be verified by the $^{13}C$ NMR spectrum, the labelled compound **1** was hydrolysed to afford labelled D-allosamine and **2**, which were then converted to allosaminitol peracetate and the triacetate of **2** for mass spectrometric (MS) analysis. The CI-MS spectra of labelled allosaminitol peracetate and the triacetate of **2** indicated that the mono- and di-labelled molecules increased by 0.1 and 9.0%, and 0.8 and 5.2%, respectively; these values were deduced from the relative intensities of ion peaks[18] around the pseudo-molecular mass range on the spectra of labelled and natural analogues (Fig. 1). In both cases, the increased ratios of monolabelled molecules were negligible and those of dilabelled molecules were approximately consistent with the increased $^{13}C$ percentage estimated by NMR spectroscopy of C-1″ (9.1%) or C-1′ (10.1%) and C-1 (5.5%). These facts indicated that the doubly labelled glucosamine was incorporated into each moiety of D-allosamine and **2** without cleavage of the $^{13}C$–C–$^{15}N$ bond. Considering the results obtained with the labelled glucoses mentioned earlier, it was concluded that the carbon skeleton and nitrogen atom of D-glucosamine were incorporated into each moiety of D-allosamine and **2** in an intact form.

Next, the biosynthetic origin of the dimethylaminooxazoline moiety of **2** was elucidated. A high level of incorporation into **2** was observed in the feeding experiment of L-[*guanidino*-$^{14}C$]arginine (Table 1, expt. 4). Then, multiple labelled L-[*guanidino*-$^{13}C$,$^{15}N_2$]arginine was administered to the culture in order to confirm the carbon position incorporated and investigate the origin of the nitrogen atom of the dimethylamino group. The relevant region of the $^{13}C$ NMR spectrum of the labelled **1** obtained is shown in Fig. 2. In the spectrum, the enriched C-7 showed mostly doublet signals with a coupling constant of 29 Hz, indicating that the $^{13}C$ and one of the $^{15}N$ of the guanidino group of labelled arginine were incorporated together into compound **2** without cleavage of the bond. The position of the labelled nitrogen should be the dimethylamino group because it had already become clear that the nitrogen

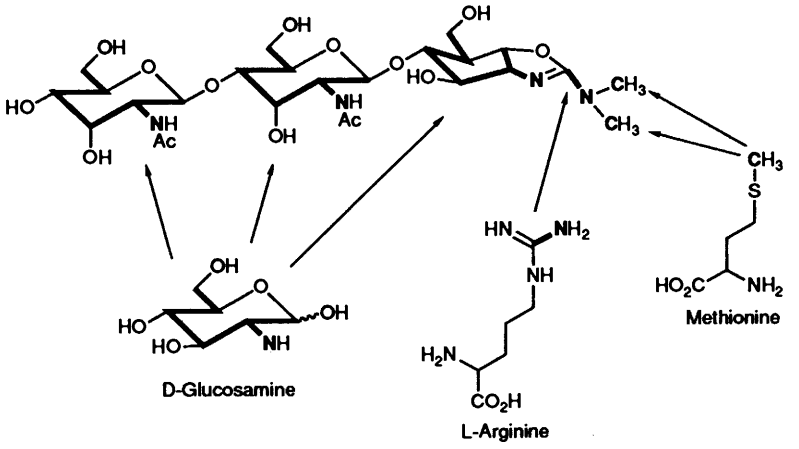



**Fig. 3** Biosynthetic origin of allosamidin

atom on C-2 originated from glucosamine, as mentioned above. This $^{13}C$-$^{15}N$-$(CH_3)_2$ connection was also supported by the fact that each double-doublet pattern having $J$ values of 2.9 and 1.5 Hz, and 3.7 and 1.4 Hz, which correspond to $^3J_{CH}$ or $^2J_{NH}$, was observed in the region of two *N*-methyl signals in the $^1H$ NMR spectrum of labelled **1**. Small double-doublet signals of labelled C-7 were also observed in the $^{13}C$ NMR spectrum (Fig. 2), probably due to the metabolism of $^{15}N$ of labelled arginine and its incorporation into the nitrogen on C-2 of **2**. Finally, feeding of [*methyl*-$^{13}C$]methionine was performed. The $^1H$ and $^{13}C$ NMR spectra of the compound **1** obtained showed very high $^{13}C$ enrichment (*ca.* 70%) of two *N*-methyl carbons, indicating that both *N*-methyl groups come from methionine.

The basic building blocks of allosamidin are summarized in Fig. 3. D-Allosamine is structurally a simple and fundamental amino sugar, but this is the first study of its biosynthesis because its occurrence in **1** is the first example found in Nature. It is easily speculated that a derivative of D-glucosamine might be converted to that of D-allosamine by the action of an epimerase. For example, uridine-5′-diphosphate(UDP)-*N*-acetyl-D-glucosamine would be epimerized to UDP-*N*-acetyl-D-allosamine as in the case of UDP-*N*-acetyl-D-galactosamine biosynthesis by UDP-*N*-acetyl-D-glucosamine-4-epimerase.

A cyclopentanoid structure of carbohydrate origin is relatively rare in natural products. It is known that the cyclopentane ring of pactamycin,[19] bacteriohopane[20] and aristeromycin[21] is biosynthesized from glucose. This time, it was revealed that the cyclopentanoid portion of allosamizoline is also derived from glucose and, moreover, glucosamine is its close precursor. In order to investigate the mechanism of the formation of the cyclopentane ring, feeding experiments using specifically $^3H$-labelled glucose or glucosamine mixed with $^{14}C$-labelled glucose or glucosamine were carried out.[21] However in all the cases tested, unexpectedly large tritium loss, for example in an experiment with mixtures of [6-$^3H$]- and [1-$^{14}C$]-glucosamine, was observed at the portion of not only allosamizoline, but also allosamine (data not shown), perhaps due to a rapid turnover in the carbohydrate pool. As already assumed for the biosynthesis of the cyclopentanoid portion of pactamycin, glucosamine might cyclize to form the cyclopentane ring *via* an intermediate 4-keto or 6-aldehyde glucosamine, which would undergo an aldol condensation on C-5 with C-1. It is thought that a cell-free system is necessary to study the cyclization mechanism.

The origin of the dimethylaminooxazoline moiety partly resembles that of the methylaminooxazolinone ring of indolmycin, in which the C-2 of the ring is derived from guanidino carbon of arginine.[22] As for the formation of the oxazoline ring, it is assumed that an amidino group of arginine or an amido group of citrulline might be transferred to a hydroxy group on C-1 or a nitrogen atom on C-2 of the cyclopentane ring; cyclization would then subsequently occur.

No information is available regarding the assembly steps of the two allosamine units and allosamizoline unit at present. In a trial, we examined whether **2** could be a possible precursor by means of a feeding experiment with [7-$^{14}C$]-**2**, which was prepared by the feeding with [*guanidino*-$^{14}C$]arginine. As a result, labelled **2** was incorporated into the cell, but no incorporation was observed into **1**. A search for a biosynthetic intermediate of **1** in metabolites of the wild type strain, as well as in allosamidin minus mutants, is now in progress.

## Experimental

*General Methods.*—$^{13}C$ NMR spectra were recorded on a Bruker AM 600 spectrometer at 150 MHz with power-gated broad-band proton decoupling (sweep width = 38 462 Hz, 128 K data points, acquisition time = 1.704 s), using dioxane $\delta_C$ 67.4 as an external reference for $D_2O$ solutions. $^1H$ NMR spectra were recorded at 600 MHz on a Bruker 600 spectrometer. All $J$-values are given in Hz. Mass spectra were obtained on a JEOL JMS-DX303 spectrometer. Radiolabelled compounds were purchased from Amersham International plc and NEN Research Products. Radioactivity was assayed by scintillation counting with a Beckman LS 6000IC instrument.

*Culture and Isolation of Allosamidin.*—One strain of *Streptomyces sp.* AJ 9463, which was selected as a high producer of allosamidin and demethylallosamidin[5] by single-cell isolation, was used in this study. Spores of the strain maintained on Bennet agar slants were innoculated into Bennet medium (100 $cm^3$), which consisted of glucose (1%), peptone (0.2%), meat extract (0.1%) and yeast extract (0.1%) (pH 7.2) for preculture in a 500 $cm^3$ Erlenmeyer flask. The flask was incubated at 28 °C and 150 rpm on a rotary shaker for 48 h. This culture (2 $cm^3$) was transferred into the same medium (100 $cm^3$) for the main culture. Incubation was carried out for 120 h under the same conditions as that of preculture. The culture broth (5 × 100 $cm^3$) was filtered and the mycelial cake obtained was extracted with 80% aqueous methanol (500 $cm^3$). The extract was concentrated under reduced pressure to an aqueous solution (50 $cm^3$), which was then adsorbed onto a charcoal (50 $cm^3$, activated charcoal, Wako Pure Chemical Ind.) column. After being washed with water (100 $cm^3$), the column was eluted successively with portions (100 $cm^3$ each) of 10% ethanol and 25% ethanol and 50% ethanol (500 $cm^3$). Allosamidin was eluted with 50% ethanol, and the fraction was finally purified by HPLC (column: Capcell-Pak $C_{18}$, 4.6 × 250 mm, Shiseido;

mobile phase: gradient elution of 0–50% $CH_3CN$ in 10 mmol $dm^{-3}$ $AcONH_4$–$NH_4OH$ pH 8.9 in 30 min; flow rate: $cm^3$ $min^{-1}$). The peak having a retention time of 15.7 min afforded *ca.* 3.5 mg of allosamidin.

Acid hydrolysis of allosamidin was performed with the procedure previously described.[3] The hydrolysate obtained was purified by HPLC using the same conditions as used in the isolation of allosamidin, to give D-allosamine and allosamizoline (retention times of 3.0 and 13.2 min, respectively). Allosaminitol peracetate and allosamizoline triacetate were prepared by the method as previously described.[3]

D-[1-$^{13}$C,2-$^{15}$N]*Glucosamine Hydrochloride*.—This was synthesized by the procedure of Taniguchi *et al.*,[23] with modifications. A mixture of D-arabinose (818 mg) and [$^{15}$N]-benzylamine (815 mg), which was prepared from [$^{15}$N]-ammonium nitrate (98 atom% $^{15}$N; Sigma) *via* [$^{15}$N]benzamide, was refluxed for 20 min in absolute ethanol (5 $cm^3$) to give an ethanolic solution of *N*-benzyl-D-[$^{15}$N]arabinosylamine. Separately, an ethanolic solution (15 $cm^3$) of anhydrous toluene-*p*-sulfonic acid (2.0 g) was added to K$^{13}$CN (1.0 g, 99 atom% $^{13}$C; Sigma) with cooling in ice-cold water. The reaction mixture was stirred and the H$^{13}$CN generated was obtained as an ethanolic solution by distillation together with ethanol under atmospheric pressure, carefully protecting this system from moisture with a $CaCl_2$ tube. The ethanolic solution of H$^{13}$CN was mixed with that of *N*-benzyl-D-[$^{15}$N]arabinose, and the mixture was stirred for 4 h at room temp. The resulting precipitate of 2-[$^{15}$N]benzylamino-2-deoxy-D-[1-$^{13}$C]glucononitrile was collected by filtration and hydrogenated in 0.5 mol $dm^{-3}$ HCl (48 $cm^3$) in the presence of 5% palladium-on-barium sulfate (895 mg). After 7 h, the catalyst was filtered off, and the filtrate was evaporated under reduced pressure and lyophilized to dryness. A small volume of methanol was added to the residue, and the resulting precipitate was collected to obtain a white powder of D-[1-$^{13}$C,2-$^{15}$N]glucosamine (207 mg), m.p. 190–200 °C (decomp.), with an overall yield of 18% based on D-arabinose; FAB-MS *m/z* 182 $(M + H)^+$; $\delta_H$(600 MHz; $D_2O$) 5.47 (0.85 H, dd, *J* 3.6, $^1J_{CH}$ 171, 1-H of β-anomer), 4.97 (0.15 H, dd, *J* 8.6, $^1J_{CH}$ 162, 1-H of α-anomer), 3.32 (0.85 H, ddd, *J* 3.6 and 10.7, $^2J_{NH}$ 2.6, 2-H of β-anomer) and 3.07 (0.15 H, ddd, *J* 8.6 and 10.7, $^2J_{NH}$ 10.5, 2-H of α-anomer); $\delta_C$(150 MHz; $D_2O$) 56.7 (dd, $^1J_{CC}$ 43.7, $^1J_{NC}$ 6.4, C-2 of β-anomer).

L-[Guanidino-$^{13}$C,$^{15}N_2$]*arginine*.—This was synthesized from ornithine and [$^{13}$C,$^{15}N_2$]urea (99 atom% $^{13}$C; 99 atom% $^{15}$N; MSD Isotopes) *via* *O*-methyl isoureatosylate as described by Martinkus *et al.*,[24] in 26% yield; $\delta_C$(150 MHz; $D_2O$) 157.5 (t, $^1J_{NC}$ 21.3, *guanidino*-C).

*Administration of Labelled Compounds to* Streptomyces sp. AJ 9463.—The labelled precursor was dissolved in distilled water or the production medium, and the solution was autoclaved or passed through a sterile Millipore filter before administration. The solution (1 $cm^3$) was added in one portion to each 500 $cm^3$ flask containing the medium (100 $cm^3$) at the 36th hour of cultivation. In this manner, 2.7, 2.8 and 8.0 mg of **1** was obtained from broths (4 × 100, 4 × 100 and 10 × 100 $cm^3$) in feeding experiments in which each flask received D-[1-$^{13}$C,2-$^{15}$N] glucosamine (50 mg), L-[*guanidino*-$^{13}$C,$^{15}N_2$]arginine (80 mg) and [*methyl*-$^{13}$C]methionine (50 mg; 99.6 atom% $^{13}$C; Sigma).

In the feeding experiments with labelled glucose, the following replacement culture was used. Fermentation was initiated under the same conditions as described above. After 36 h, the fermentation broth (100 $cm^3$) was transferred to a sterile centrifuge bottle and the mixture was centrifuged at 6000 g for 20 min. The supernatant was decanted and the remaining mycelial pellet was resuspended in modified Bennet medium (100 $cm^3$) in which the glucose content was reduced to 0.2%. After replacement, fermentation was carried out in the usual manner. Under the replacement conditions, a mixture of D-[1-$^{13}$C]glucose (50 mg; 98.7 atom% $^{13}$C; Sigma) or D-[6-$^{13}$C] glucose (50 mg), which was synthesized with K$^{13}$CN,[25] and unlabelled D-glucose (150 mg) was added to each flask at the replacement time as a component of the medium. After cultivation and work-up, 0.8 and 2.6 mg of **1** was obtained from broths (4 × 100 and 7 × 100 $cm^3$) in the experiments with [1-$^{13}$]- and [6-$^{13}$C]-D-glucose, respectively.

## References


1 S. Sakuda, A. Isogai, S. Matsumoto and A. Suzuki, *J. Antibiot.*, 1987, **40**, 296.
2 S. Sakuda, A. Isogai, S. Matsumoto, A. Suzuki and K. Koseki, *Tetrahedron Lett.*, 1986, **27**, 2475.
3 S. Sakuda, A. Isogai, T. Makita, S. Matsumoto, K. Koseki, H. Kodama and A. Suzuki, *Agric. Biol. Chem.*, 1987, **51**, 3251.
4 D. Koga, A. Isogai, S. Sakuda, S. Matsumoto, A. Suzuki, S. Kimura and A. Ide, *Agric. Biol. Chem.*, 1987, **51**, 471.
5 A. Isogai, M. Sato, S. Sakuda, J. Nakayama and A. Suzuki, *Agric. Biol. Chem.*, 1989, **53**, 2825.
6 S. Sakuda, Y. Nishimoto, M. Ohi, M. Watanabe, S. Takayama, A. Isogai and Y. Yamada, *Agric. Biol. Chem.*, 1990, **54**, 1333.
7 Y. Nishimoto, S. Sakuda, S. Takayama and Y. Yamada, *J. Antibiot.*, 1991, **44**, 716.
8 G. W. Gooday, L. J. Brydon and L. H. Chappell, *Mol. Biochem. Parasitol.*, 1988, **29**, 223.
9 K. Dickinson, V. Keer, C. A. Hitchcock and J. Adams, *J. Gen. Microbiol.*, 1989, **135**, 1417.
10 A. R. Butler, R. W. O'Donnell, V. J. Martin, G. W. Gooday and M. J. R. Stark, *Eur. J. Biochem.*, 1991, **199**, 483.
11 S. Sakuda, A. Isogai, S. Matsumoto, A. Suzuki, K. Koseki, H. Kodama and Y. Yamada, *Agric. Biol. Chem.*, 1988, **52**, 1615.
12 B. M. Trost and D. L. Van Vranken, *J. Am. Chem. Soc.*, 1990, **112**, 1261.
13 M. Nakata, S. Akazawa, S. Kitamura and K. Tatsuta, *Tetrahedron Lett.*, 1991, **32**, 5356.
14 S. Takahashi, H. Terayama and H. Kuzuhara, *Tetrahedron Lett.*, 1991, **32**, 5123.
15 D. A. Griffi and S. J. Danishefsky, *J. Am. Chem. Soc.*, 1991, **113**, 5863.
16 J.-L. Maloisel, A. Vasella, B. M. Trost and D. L. Van Vranken, *J. Chem. Soc., Chem. Commun.*, 1991, 1099.
17 G. C. Levy and R. L. Lichter, *Nitrogen-15 Nuclear Magnetic Resonance Spectroscopy*, Wiley, New York, 1979.
18 R. M. Caprioli, in *Biochemical Applications of Mass Spectrometry*, ed. G. R. Waller, Wiley, New York, 1972, p. 735.
19 D. D. Weller and K. L. Rinehart, Jr., *J. Am. Chem. Soc.*, 1978, **100**, 6757.
20 G. Flesch and M. Rohmer, *Eur. J. Biochem.*, 1988, **175**, 405.
21 R. J. Parry, V. Bornemann and R. Subramanian, *J. Am. Chem. Soc.*, 1989, **111**, 5819.
22 U. Hornemann, L. H. Hurley, M. K. Speedie and H. G. Floss, *J. Am. Chem. Soc.*, 1971, **93**, 3028.
23 M. Taniguchi, R. F. Nystrom and K. L. Rinehart, Jr., *Carbohydr. Res.*, 1982, **109**, 161.
24 K. J. Martinkus, C.-H. Tann and S. J. Gould, *Tetrahedron*, 1983, **39**, 3439.
25 D. L. Williams and T. W. Whaley, *J. Labelled Compds. Radiopharm.*, 1981, **19**, 669.


*Paper* 2/00735E
*Received* 12*th February* 1992
*Accepted* 23*rd March* 1992