

BIOSYNTHETIC PATHWAY OF 2-DEOXYSTREPTAMINE

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Four 2-deoxystreptamine (DOS) related compounds including S-11-P, isolated as an intermediate of DOS biosynthesis, were supplemented to the culture of a DOS⁻ mutant of *Bacillus circulans*. Among the tested aminocyclitol compounds, S-11-P alone was converted to DOS. Addition of other aminocyclitols neither produced antibiotic nor inhibited the incorporation of S-11-P and DOS into butirosins. By these facts, S-11-P was confirmed clearly to be an intermediate of DOS biosynthesis.

2-Deoxystreptamine (DOS, **1**) is a widely distributed and important component of many aminoglycoside antibiotics. Recently we isolated an aminocyclitol, S-11-P, from the culture broth of a DOS⁻ mutant of *Bacillus circulans*, and its structure was elucidated as (1L)-1,3,5/2,4-5-amino-cyclohexanetetrol (**2**)^{1,2}. This compound was converted to DOS by an another kind of DOS⁻ mutant¹, and we suggested that the biosynthesis of DOS proceeds from glucose through S-11-P. However, the conversion of the enantiomer (**4**) of S-11-P, the C-1 epimer (**3**) of **2** and the C-1 epimer of **4** (**5**) to DOS have not been checked until now. In this paper we examined this point using the chemically synthesized optically active compounds. We also examined the possibility of mutational biosynthesis of new antibiotics and whether the synthesized aminocyclitols could interfere with the conversion of S-11-P and DOS to butirosin (BTN).

Materials and Methods

Strain

The DOS⁻ mutant *B. circulans* 236, in the converter class of such mutants used in this experiment was described in the previous paper¹.

Aminocyclitols

Syntheses of S-11-P, (1L)-1,3,5/2,4-5-aminocyclohexanetetrol (**2**) and its epimer, (1D)-1,2,4/3,5-5-aminocyclohexanetetrol (**3**) were reported in the previous paper². The enantiomer of S-11-P, (1D)-1,3,5/2,4-5-aminocyclohexanetetrol (**4**) and its epimer, (1L)-1,2,4/3,5-5-aminocyclohexanetetrol (**5**) were also synthesized in our research laboratory, and the details will be published elsewhere.

Bioconversion of aminocyclitols

Seed medium S-4 and fermentation medium F-5 were described in the previous paper¹. A loopful of *B. circulans* 236 grown on a soybean agar slant was inoculated into 10 ml of seed medium S-4, which was incubated at 28°C for 24 hours on a reciprocal shaker. Next, 0.4 ml of seed culture was transferred into 10 ml of fermentation medium F-5 and incubated for 24 hours at 28°C on a reciprocal shaker. Aminocyclitol was supplemented to this culture and after 6-day incubation antibiotic activity of the cultured broth was assayed by a paper disc diffusion method using 6-mm diameter paper discs and peptone agar (peptone 0.5%, agar 1.5%) seeded with *Bacillus subtilis* PCI 219 as a test organism.

Harvested culture filtrate was adsorbed on Amberlite IRC-50 (NH₄⁺). The material eluted with 1 N aqueous ammonia was concentrated under vacuum. The concentrated sample was examined by TLC-bioautography.

Results

Among the four tested aminocyclitols, **2**, **3**, **4**, and **5**, only the S-11-P (**2**) supplemented culture produced antibiotic activity, which consists of BTNs A and B. The other three aminocyclitols were obviously not converted to DOS (Table 1). Another possibility, of **3**, **4**, and **5** being incorporated intact to form new aminoglycoside antibiotics by mutational biosynthesis, was examined, because S-11-P (**2**) was incorporated intact into a new antibiotic, S-11-A, by *B. circulans* S-11⁵⁾. But TLC-bioautography of *ca.* one hundredfold concentrated samples prepared from the **3**, **4**, and **5**-supplemented culture broths exhibited no clear antibiotic spots. Finally, we examined whether these aminocyclitols (**3**, **4**, and **5**) inhibit the conversion of the biosynthetic intermediate S-11-P (**2**) to DOS (**1**) and incorporation of DOS to BTN. As shown in Table 2, tenfold amounts of **3**, **4**, and **5** added with S-11-P (**2**) or DOS (**1**) showed essentially no inhibition against the conversion of S-11-P to DOS and DOS to BTN.

Table 1. Production of BTN by *B. circulans* 236 culture supplemented with aminocyclitols.

Aminocyclitol	Concentration ($\mu\text{g/ml}$)	Inhibition diameter (mm)
None		9.0
2	97	21.0
3	150	8.9
	960	6.5
4	274	10.8
	1,280	9.0
5	113	8.9

Aminocyclitol was supplemented to a culture of *B. circulans* 236 after 24-hour incubation. Antibiotic activity of 6-day culture was expressed as inhibition zone diameter against *B. subtilis* using 6-mm paper discs.

Fig. 1. Compounds tested for incorporation by a DOS⁻ mutant of *B. circulans*.

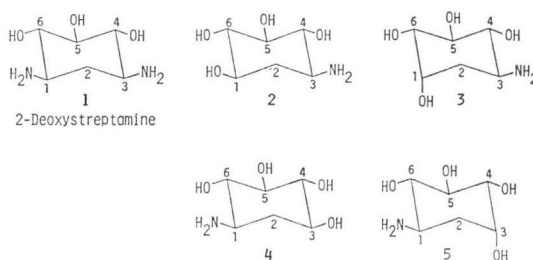


Table 2. Effect of non-intermediate aminocyclitols on the incorporation of S-11-P and DOS into BTN.

Aminocyclitols	Inhibition diameter (mm)
None	7.9
2 (S-11-P), 10 $\mu\text{g/ml}$	15.5
2 (S-11-P), 10 $\mu\text{g/ml}$, + 3 , 100 $\mu\text{g/ml}$	14.9
2 (S-11-P), 10 $\mu\text{g/ml}$, + 4 , 100 $\mu\text{g/ml}$	15.4
2 (S-11-P), 10 $\mu\text{g/ml}$, + 5 , 100 $\mu\text{g/ml}$	15.2
1 (DOS), 10 $\mu\text{g/ml}$	16.2
1 (DOS), 10 $\mu\text{g/ml}$, + 3 , 100 $\mu\text{g/ml}$	15.9
1 (DOS), 10 $\mu\text{g/ml}$, + 4 , 100 $\mu\text{g/ml}$	16.1
1 (DOS), 10 $\mu\text{g/ml}$, + 5 , 100 $\mu\text{g/ml}$	16.0

Both aminocyclitols were supplemented to a 24-hour culture. Other experimental conditions were the same as those of Table 1.

Discussion

From the results shown in Table 1, S-11-P was re-confirmed to be an intermediate of DOS biosynthesis. Inability of the enantiomer (**4**) of S-11-P to be converted to DOS indicates that DOS was formed from only one of the enantiomers when the chemically synthesized racemic aminocyclitol or cyclose was supplemented to a DOS⁻ mutant^{4,5)}.

As SHIER, *et al.*⁶⁾, already discussed, there are three possible explanations for the failure of **3**, **4**, and **5** to be incorporated into an antibiotic-enzyme specificity, cell wall impermeability, and bioactivity of the product of incorporation. More than five examples have been reported concerning the bioconversion of cycloses and aminocyclitols to aminocyclitol antibiotics by a DOS⁻ mutant of *B. circulans*.

2-Deoxy-*scyllo*-inosose⁴⁾, and S-11-P¹⁾ were incorporated into BTN. *Scyllo*-inosose⁴⁾, *scyllo*-inosamine⁴⁾, and streptomine⁷⁾ were incorporated into 2-hydroxy-BTN. 2,5-Dideoxystreptomine⁷⁾ was also incorporated into 5-deoxybutirosamine. These facts mean that a reasonable amount of the above-mentioned substances entered into the cells of a DOS⁻ mutant, and there is little possibility that **3**, **4**, and **5** were specifically excluded from permeating into the cells of *B. circulans* 236. The results of the inhibition experiment (Table 2) also excluded the possibility of bioinactive antibiotic analogs being produced by competing with DOS incorporation into BTN. Thus, we assume the first explanation is the most plausible. The enzyme(s) concerning the conversion of S-11-P to DOS may require strict stereospecificity. On the other hand, the enzyme concerning glycosidation of DOS seems to have low specificity, but **3**, **4**, and **5** could not be used as substrates.

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