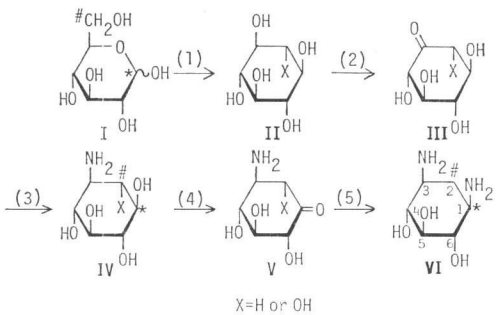
ACCUMULATION OF 2-DEOXY-*SCYLLO*-INOSAMINE BY A 2-DEOXYSTREPTAMINE-REQUIRING IDIOTROPH OF *MICROMONOSPORA SAGAMIENSIS*

Sir:

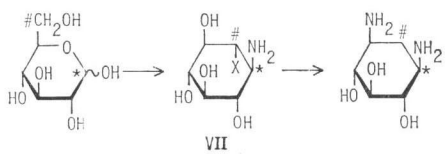
2-Deoxystreptamine (DOS) is a component of many clinically important aminoglycoside antibiotics. A biosynthetic pathway for DOS has been proposed by REINHART and coworkers[1] on the basis of the incorporation of [6-^{13}C] glucose into the aminocyclitol (Fig. 1). The route involves initial oxidation and amination at C-3 of the aminocyclitol ring (which is derived from C-5 of glucose), followed by similar reactions at C-1 of the aminocyclitol ring (from C-1 of glucose). An alternative route (also shown in Fig. 1), involving the reverse sequence—oxidation-amination at the ring's C-1 first, followed by oxidation-amination at its C-3—cannot be ruled out. Feeding of putative biosynthetic intermediates to DOS idiotrophs[2,3] and cell free enzymic studies[4] have suggested 2-deoxy-*scyllo*-inosose (2,3,4,5-tetrahydroxycyclohexanone 3,5/2,4, compound **III**, X=H, or its enantiomer) and 2-deoxy-*scyllo*-inosamine (2,3,4,5-tetrahydroxycyclohexylamine 3,5/2,4, compound **IV**, X=H, or its enantiomer, compound **VII**, X=H)

Fig. 1. Possible biosynthetic pathway from glucose to 2-deoxystreptamine. (RINEHART *et al.*[1])

(a) Postulated pathway:



(b) Alternative pathway:



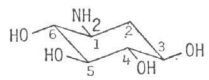
*, #: Carbon atoms of 2-deoxystreptamine labeled by [1-^{13}C]glucosamine(*) and [6-^{13}C]glucose(#).

(DOI) as intermediates in the biosynthetic pathway for DOS. However, it should be noted that no intermediates in DOS biosynthesis were isolated in any of above studies.

In this communication, we described the isolation of DOI in the culture broth of a DOS-requiring idiotrophic mutant of *Micromonospora sagamiensis* which produces the DOS-containing antibiotic, sagamicin. We will also present data which will support the route (a) in Fig. 1.

The DOS idiotrophic mutant, KY11509, was derived from a sagamicin producer, *M. sagamiensis* KY11505. Mutant KY11509 was grown in 150 liters of a medium consisting of 4% Stabilose K (soluble starch), 1% soy bean meal, 2% Pharmamedia (cottonseed flour), 0.1% corn oil, 0.5% casein, 0.2% phytate, 0.015% $FeSO_4 \cdot 7H_2O$, 0.05% $MgSO_4 \cdot 7H_2O$ and 0.025% KH_2PO_4. The fermentation was carried out in a 300-liter tank by stirring at 180 rpm at 34°C with aeration of 150 liters/min. Mutant KY11509 produced a number of aminocyclitols. One of them, K14-1 had a similar chromatographic behavior to DOI. K14-1 was isolated from the fermentation broth by an ion exchange procedure. The fermentation broth was harvested at 73 hours and the pH was adjusted to 2.0. After filtration, the filtrate was neutralized, applied to an Amberlite IRC-50 resin (NH_4^+) column and eluted with aqueous ammonia. The eluate was concentrated to dryness and then applied to an Amberlite CG-50 resin (NH_4^+) column. The resin was eluted with a linear gradient of 0 to 0.08 N ammonium hydroxide and fractions containing K14-1 were collected. Lyophilization of the pooled fractions gave a crude powder of K14-1. The crude K14-1 was further chromatographed on Bio-Rex 70 (NH_4^+) column.

Fig. 2. ^{13}C chemical shifts of K14-1 (2-deoxy-*scyllo*-inosamine) (ppm from DSS).



	Free base (pD 10.0)	DCl-D_2O (pD 2.5)
1	50.3	50.8
2	36.8	33.2
3	70.3	69.5
4	75.7	75.2
5	77.8*	77.0*
6	78.0*	73.7*

* tentative assignment

Table 1. Effect of DOS, DOI and deoxyinosose on sagamicin production by DOS idiotrophs of *M. sagamiensis*, KY 11508 and KY 11509.

	DOI production μg/ml	Sagamicin production (μg/ml) from		
		DOS	DOI	2-Deoxy-*scyllo*-inosose
M. sagamiensis KY 11509	105	20	0	0
KY 11508	0	20	18	18

DOS idiotroph was cultivated in 5 ml of the production medium with or without 100 μg/ml of each cyclitol in a large test tube. Production of DOI (in 7 days cultured broth) and sagamicin (in 5 days cultured broth) was determined by fluorometric assay with NBD chloride and bioassay.

Elution was performed with a linear gradient of 0 to 0.05 N ammonium hydroxide. Fractions containing K14-1 were collected and the pooled fractions were lyophilized to yield pure K14-1 base.

The compound thus obtained showed a protonated molecular ion at *m/e* 164 (Calcd. for $C_6H_{14}NO_4$: 164.0923, Found: 164.0923) in the mass spectrum. Its pmr spectrum* (DCl-D_2O, pD 1.1) showed signals at δ 1.60 (1H, q, J=12.3, H-2ax), 2.30 (1H, dt, J=12.0, 4.0, H-2eq) and 2.8~3.8 (5H, m). Chemical shifts of its cmr* are shown in Fig. 2. K14-1 was positive to ninhydrin and RYDON-SMITH reactions, and had similar Rf values on silica gel thin-layer chromatography to those of authentic *dl*-DOI in the following solvent systems: *n*-butanol - methanol - chloroform - 28% ammonium hydroxide (4: 5: 2: 5, v/v) (Rf 0.22); 10% ammonium acetate - methanol - 28% ammonium hydroxide (5: 1: 1, v/v) (Rf 0.43); and chloroform - methanol - 28% ammonium hydroxide (1: 2: 2, v/v) (Rf 0.40). These data show that K14-1 is DOI. Absolute configuration of K14-1 has not been determined so far.

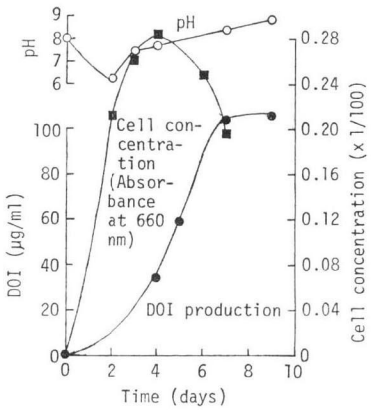
A time course of DOI production by KY11509 is shown in Fig. 3. Synthesis of DOI takes place at the late logarithmic phase of growth, reaching its peak (105 μg/ml) at 7th day.

Another DOS idiotrophic mutant, KY11508, which produces no DOI has been derived from *M. sagamiensis*.

When mutant KY11508 was incubated in the filtrate obtained from a 48-hours culture broth of mutant KY11509, sagamicin was produced in the culture broth. However, no antibiotics were produced when mutant KY11509 was incubated with the filtrate of mutant KY11508. These results indicate that the blockage in the biosynthesis of DOS in mutant KY11508 precedes that in mutant KY11509.

Fig. 3. Time course of DOI production by *M. sagamiensis* KY11509.

Mutant KY11509 was cultivated in 40 ml of the production medium in a 300 ml flask. DOI production was determined by fluorometric assay with NBD chloride and by bioassay using a DOS idiotroph KY11508.



Mutant KY11508 yielded sagamicin when DOI (either *dl*-DOI or K14-1) or 2-deoxy-*scyllo*-inosose was added to the growing culture, whereas these compounds did not produce the antibiotic when supplemented to a growing culture of mutant KY11509 (Table 1). Mutant KY11509 is apparently blocked in the biosynthetic ability between DOI and DOS (probably the oxidation step (4) in Fig. 1). The location of the block in mutant KY11508 may be at some point prior to the formation of the deoxyinosose

* Pmr and cmr spectra were taken on a JEOL PFT 100A spectrometer in the FT mode at 100 MHz for pmr and 25 MHz for cmr at ambient temperature. The chemical shifts are given in ppm downfield from TMS.

from glucose.

These results indicate that DOI is an intermediate of DOS biosynthesis in *M. sagamiensis*. 2-Deoxy-*scyllo*-inosose has been reported to be utilized by a DOS idiotroph of *Bacillus circulans* to produce butirosins.[3] The compound can also be utilized by KY11508 (but not by KY11509) to produce sagamicin and is probably an intermediate prior to DOI in the DOS biosynthesis in *M. sagamiensis*.

CHEN and WALKER[4] reported that DOS was a very active amino donor with keto-*scyllo*-inositol as acceptor in cell-free extracts from *M. purpurea* and *Streptomyces fradiae*. From the preliminary data, they suggested that the 1-amino group of DOS participated in the transamination, which may possibly indicate that the amino group in the 3-position of DOS is added first in the DOS biosynthesis. We have isolated many shunt products from the culture filtrates of mutant KY11509, all of which do not contain the 1-amino group of DOS[5]. The results suggest that the absolute configuration of K14-1 is **IV** but not **VII** in Fig. 1. Thus it may be concluded that, in the biosynthesis of DOS, the amination of position 3 occurs first, followed by oxidation-amination at position 1 (pathway (a) in Fig. 1).

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