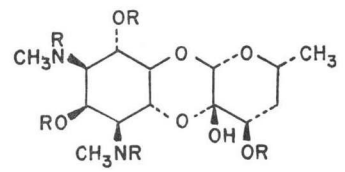
Communications to the editor

THE PRODUCTION OF DIHYDROSPECTINOMYCIN BY *STREPTOMYCES SPECTABILIS*

Sir:

In the course of a study of compounds produced by *Streptomyces spectabilis*,[1,2,3] contents of spectinomycin crystallization mother liquors were examined by gas chromatography/mass spectrometry.[4] Silylated samples were analyzed on a 3 % OV-101 column at 220°C. A peak emerging with a retention time of 28 minutes gave a molecular ion at *m/e* 622, and a mass spectrum that could not be distinguished from that of the tetra-trimethylsilyl derivative of dihydrospectinomycin. Because of the similarities of the mass spectra of the two dihydrospectinomycin[5] epimers, it could not be determined with certainty by this means which of the two was actually present in the mother liquor solids. The epimer produced by *S. spectabilis* was found to be that represented by structure **1** (Fig. 1) by the method described in the following discussion.

Fig. 1. Dihydrospectinomycin and penta-acetyl dihydrospectinomycin.

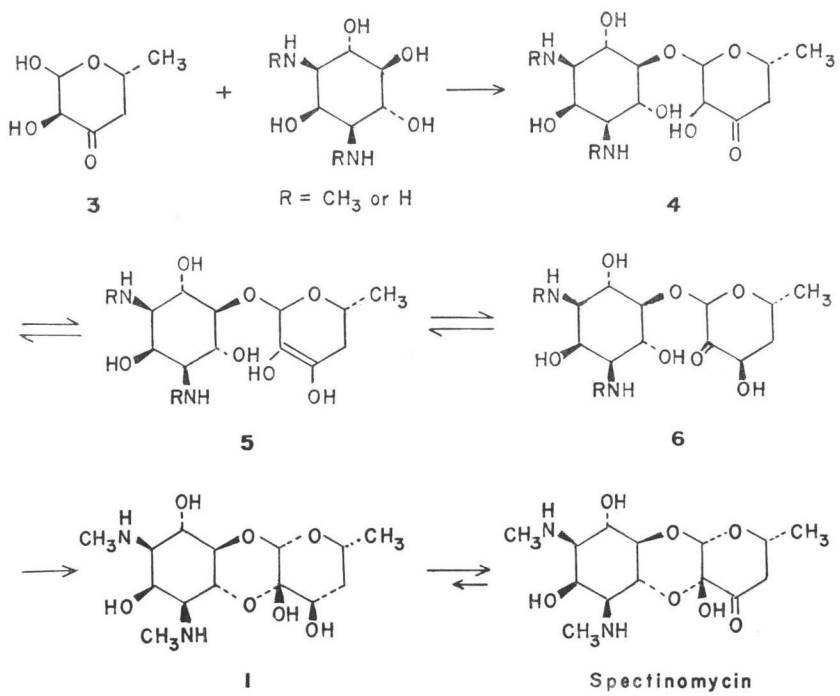


1 R = H
2 R = Ac

Acetylation of crude crystalline spectinomycin hydrochloride, followed by chromatography on silica gel using chloroform-methanol (95:5, v/v) afforded tetra-acetyl* spectinomycin as the major component and penta-acetyl dihydrospectinomycin (**2**, Fig. 1) as a minor component (about 1 % of the major component).

The NMR spectrum of the penta-acetyl di-

Scheme 1



* NMR studies have now shown that the substances which we earlier[2] had reported to be the tri- and tetra-acetyl derivatives of spectinomycin are actually the tetra- and penta-acetyl derivatives.

hydrospectinomycin was then compared with those of the two penta-acetyl dihydrospectinomycins prepared from the individual epimers. As in the case of the epimeric dihydrospectinomycins,[5] the location of the signal for the C-10a (anomeric) hydrogen distinguishes the epimeric penta-acetates. This signal for the compound represented by structure **2** occurs at 4.79 ppm while that of its epimer appears at 4.64 ppm. The corresponding signal for penta-acetyl dihydrospectinomycin isolated from fermentation sources was found at 4.79 ppm. Thus structure **1** (Fig. 1) represents the dihydrospectinomycin epimer produced by *S. spectabilis*.

This finding suggests that dihydrospectinomycin is involved in spectinomycin biosynthesis in the role of either intermediate or final product. If the reduced compound is the product, spectinomycin would likely be the immediate precursor with the equilibrium favoring the oxidized state. It may be more attractive to consider dihydrospectinomycin as the precursor in view of the role of glucose in actinospectose biosynthesis.[6] In such a case, an actinospectose precursor (not actinospectose itself as suggested by MITSCHER, *et al.*[6]) such as **3** would combine with actinamine[7] or N,N-desmethylactinamine,[6] forming a single glycoside. (Scheme 1)

Compound **3** has also been proposed as an intermediate in the synthesis from D-glucose of the macrolide sugars, D-lankavose and D-desosamine.[8]

In a preliminary experiment, however, in which the appropriate dihydrospectinomycin epimer was supplied to an actinamine-requiring *S. spectabilis* mutant, no conversion to spectinomycin could be observed by a spectinomycin biological assay.[9] Additional investigations with labeled substances in cell-free systems are warranted.

HERMAN HOEKSEMA
JOHN C. KNIGHT

Research Laboratories
The Upjohn Company
Kalamazoo, Michigan 49001 U.S.A.

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