INHIBITION OF PROTEIN BIOSYNTHESIS: THE FIRST ACTIVE SPARSOMYCIN ANALOG

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SUMMARY

A (dl) S-deoxo-S-propyl sparsomycin analog has been prepared and examined as an inhibitor of the peptidyl transferase reaction with bacterial ribosomes. A double reciprocal plot and Dixon analysis indicate that the sparsomycin analog is a competitive inhibitor of phenylalanyl-puromycin formation. The inactivity of the L-isomer has established that the chiral carbon of sparsomycin analogs must be identical with the chirality of D-cysteinol for ribosomal binding.

Sparsomycin, a broad spectrum antibiotic from Streptomyces sparsogenes (1), is toxic to both pro- and eukaryotic cells (2). The biological activity of the antibiotic is a consequence of its ability to inhibit protein synthesis at the ribosome level (3). In the formation of polylysyl-puromycin (4,5) and acetyl-phenylalanyl-puromycin (6), sparsomycin acts as a competitive inhibitor with respect to puromycin. Thus, sparsomycin is generally regarded as a peptidyl transferase inhibitor.

Sparsomycin has proved to be a useful tool in the study of the biochemistry and physiology of protein synthesis in living cells (7), and its antitumor activity has been evaluated in phase I clinical studies (8). However, the availability of this agent is extremely limited: a total synthesis of sparsomycin has yet to appear in the literature. The proposed structure of this antibiotic was reported in 1970 (9), and a few related structures have recently been synthesized (10,11). However, none of these

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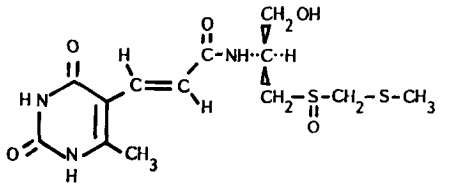
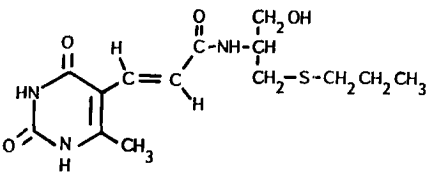


SPARSOMYCIN



S-DEOXO-S-PROPYL SPARSOMYCIN

Figure 1. Sparsomycin and its biologically active analog.

compounds exhibit biological activity. In this communication, we present the first biologically active sparsomycin analog (Fig. 1). Furthermore, a preliminary study on the structural requirements for the interaction of sparsomycin analogs in transpeptidation is reported.

MATERIALS AND METHODS

Puromycin dihydrochloride was obtained from Nurtitional Biochemicals, [^{14}C]-L-phenylalanine was purchased from New England Nuclear. Escherichia coli cell paste (B, mid log) was purchased from General Biochemicals. The polynucleotides were purchased from Miles Laboratories, and ATP, GTP, phosphoenolypyruvate, and pyruvate kinase were purchased from Sigma. Preparation of ribosomes, S-100, factors washable from ribosomes (FWR), and Ac[^{14}C]-L-Phe-tRNA were as previously described (12).

The S-deoxo-S-propyl sparsomycin analog, (dl) N-(1-hydroxy-3-n-propylthio-2-propyl)-3-(E)-(1,2,3,4-tetrahydro-6-methyl-2,4-diox-5-pyrimidine)propenamide (Fig. 1) was prepared by condensation of (E)-3-(1,2,3,4-tetrahydro-6-methyl-2,4-dioxo-5-pyrimidine) propenoic acid (13) with (dl)-S-n-propylcysteinol in dimethylformamide using dicyclohexylcarbodiimide and N-hydroxysuccinimide. The sparsomycin analog was isolated as a white solid, mp 241-244°; spectra (infrared, ultraviolet, proton magnetic, mass) were consistent with the structure present in Fig. 1. Elemental analysis for C, H, N agreed with calculated values. Details of the synthetic method are being presented elsewhere (C.K. Lee and R. Vince, J.Med.Chem., submitted).

RESULTS AND DISCUSSIONS

The lack of a facile synthetic route to the unique structural features of the aminoalcohol moiety of sparsomycin has severely limited the availability of this antibiotic for biological studies. Thus, a preliminary assessment of the binding affinities of spar-

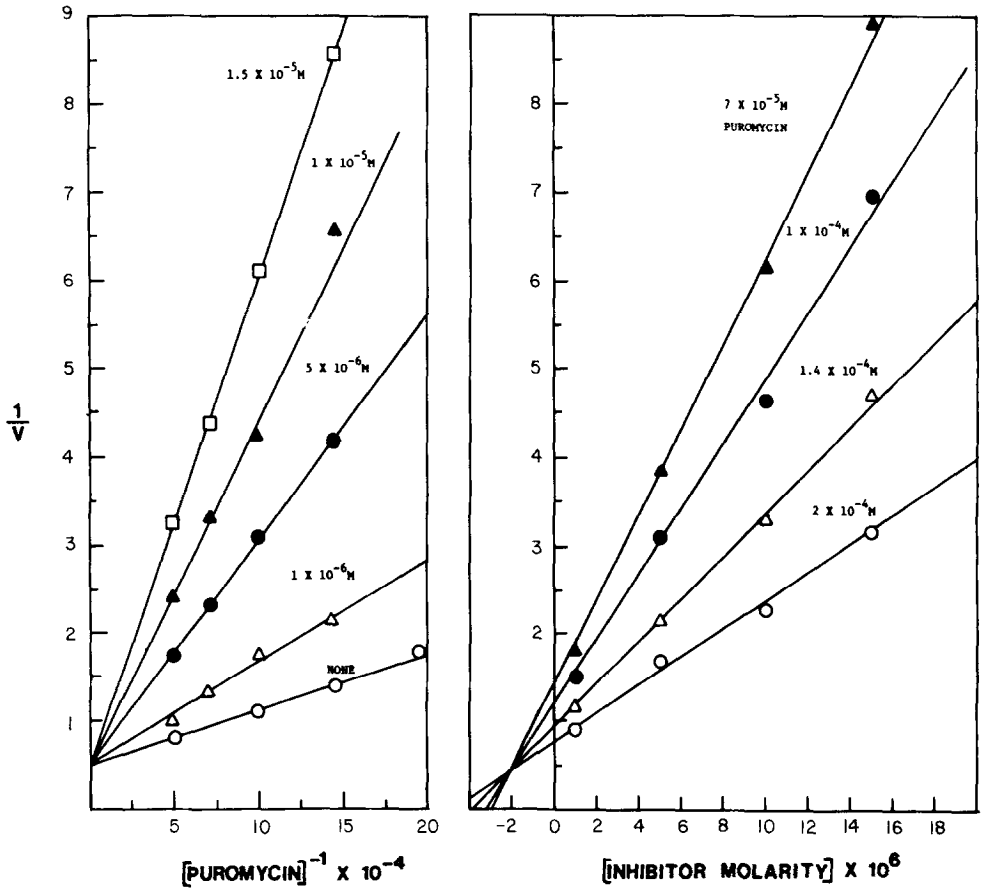


Figure 2. Competitive inhibition of N-acetyl-[^{14}C]phenylalanyl-puromycin synthesis by E. coli ribosomes with S-deoxo-S-propyl sparsomycin. The Ac-[^{14}C]Phe-tRNA was bound to the ribosomes in a reaction mixture containing 100mM Tris-Cl (pH 7.5), 100mM NH_4Cl (pH 7.6), 15 mM $Mg(OAc)_2$, 0.65 mM dithiothreitol, 2.78 A_{260} units of E. coli washed ribosomes, 1.2 mM GTP, 63 µg of FWR, 0.35 units of poly (U), and 20.8 pmoles of Ac-[^{14}C]Phe-tRNA (464 pCi/pmole). The binding mixture was incubated at 28°C for 8 minutes, and the peptidyl transferase reaction was initiated by 80 µl of the icubation cocktail to 20 µl of puromycin or a mixture of puromycin plus inhibitor. Reactions were incubated at 28°C for a specified time and product formation was measured as described in reference 12. Initial velocities were recorded as CPM of product formed per minute.

somycin analogs, possessing more easily accessible aminoalchols, may provide a basis for the design of more readily available inhibitors of transpeptidation. Specifically, a sparsomycin analog in which the unique $-\overset{\overset{O}{\|}}{S}-CH_2S-CH_3$ moiety is replaced by the

$-S-CH_2CH_2CH_3$ side chain was evaluated as an inhibitor of the peptidyl transferase reaction.

The effect of the S-deoxo-S-propyl analog of sparsomycin on the rate of acetyl [^{14}C]phenylalanyl-puromycin synthesis is shown in Fig. 2. A double reciprocal plot (left panel) indicates that the sparsomycin analog inhibits the puromycin reaction competitively with a K_i equal to 2×10^{-6}M. A Dixon analysis (14) of the effect of the sparsomycin analog on acetyl [^{14}C]phenylalanyl-puromycin formation also indicates competitive inhibition (right panel). The K_i of 2×10^{-6}M is in agreement with that obtained by the double reciprocal plot. These studies were performed with ribosomes form Escherichia coli. A double reciprocal plot also indicated that sparsomycin inhibits the peptidyl transferase reaction with a K_i equal to 5×10^{-7}M.

Since sparsomycin is highly toxic to mammalian cells including tumor cells, the sparsomycin analog was evaluated for toxicity against P-388 mouse lymphoid leukemia cells in a tissue culture assay previously described (15). The sparsomycin analog exhibited an LD_{50} concentration of 5×10^{-6}M in this assay.

The chiral carbon of sparsomycin has been shown to be identical with the chirality of D-cysteinol (9,13). Thus, it was anticipated that only the D-isomer of the racemic analog is capable of ribosomal binding. Sythesis of the L-isomer has confirmed that this is indeed the case; the L-isomer did not inhibit the peptidyl transferase reaction at a concentration of 10^{-4}M. Therefore, the S-deoxo-S-propyl sparsomycin analog exhibits a binding affinity approximately one-half that of sparsomycin. The ability of a simplified sparsomycin molecule to bind to the ribosomal site with a relatively high affinity has prompted a more extensive structure-activity relationship study of this antibiotic. In

addition, the replacement of the propyl side chain with a radioactive alkylating moiety for affinity labeling of the ribosomal binding site is being pursued.

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