PRECURSOR DIRECTED BIOSYNTHESIS OF PAULOMYCINS† A AND B. THE EFFECTS OF VALINE, ISOLEUCINE, ISOBUTYRIC ACID AND 2-METHYLBUTYRIC ACID

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Paulomycins A and B are structurally related antibiotics which are produced in approximately equal amounts by *Streptomyces paulus,* strains UC 5142[1,2,3], UC 5231[4,5,6] and UC 8560. *S. paulus* UC 5142 and 5231 are wild type isolates and UC 8560 was derived from UC 5142. Paulomycin A is an ester of 2-methylbutyric acid and paulomycin B is an ester of isobutyric acid[3]. They are related to proceomycin[7] and the senfolomycins A and B[5]. The paulomycins are effective against various pathogenic microorganisms including *Neisseria gonorrhoeae, Bacteroides fragilis, Streptococcus pyogenes, Streptococcus pneumoniae* and antibiotic resistant strains of *Staphylococcus aureus*[9].

Addition of isoleucine or 2-methylbutyric acid to fermentations of *S. paulus* results in the production of paulomycin containing *ca.* 90% paulomycin A relative to paulomycin B. The addition of valine or isobutyric acid results in production of paulomycin containing roughly 90% paulomycin B relative to paulomycin A. Both valine and isoleucine are known to be metabolized *via* deamination to their α-keto acids. These acids are then decarboxylated and converted to their coenzyme A thioesters. In this reaction sequence, isobutyryl-CoA and 2-methylbutyryl-CoA are formed respectively from valine and isoleucine[10]. In addition, isobutyric acid and 2-methylbutyric acid are converted to their coenzyme A thioesters by microorganisms[10]. As the paulomycins A and B are esters of 2-methylbutyric and isobutyric acids, it is reasonable that they are formed selectively in the presence of these acids, as well as in the presence of isoleucine and valine.

† Previously described as volonomycin[1] and U-43120[4,5,6].

Fermentation Conditions

S. paulus UC 8560 was maintained on Hickey and Tresner agar plugs stored over liquid nitrogen. The organism was introduced into a seed medium (GS-7) which contained Cerelose (C.P.C. International) and Pharmamedia (Procter and Gamble) each added at 25 g per liter of tap water. The medium was adjusted to pH 7.2 with NH_4OH and was autoclaved for 30 minutes. The inoculated 100 ml volumes of GS-7 were shaken in wide-mouth 500-ml fermentation flasks at 250 rpm for 48 hours at 28°C. The mature seed cultures were used as the source of inoculum (5% rate) for a fermentation medium known as 273F3. Medium 273F3 contained Cerelose 10 g, Pharmamedia 10 g, dextrin (C.P.C. International) 20 g, brewer's yeast (Fleishman Co.) 1 g and Ucon (Union Carbide) 10 g added per liter of tap water. After formulation, the medium was adjusted to pH 7.2 with NH_4OH and was sterilized in the manner described for GS-7. DL-Valine (Sigma Chemical Co.) and DL-isoleucine (Sigma Chemical Co.) were added in crystalline form in the indicated amounts at 48 and 72 hours after inoculation. Ammonium salts (pH 6.5) of isobutyric acid (Aldrich Chemical Co.) and 2-methylbutyric acid (Aldrich Chemical Co.) were added as sterile solutions in the indicated amounts at 24 hours after inoculation.

HPLC Assay

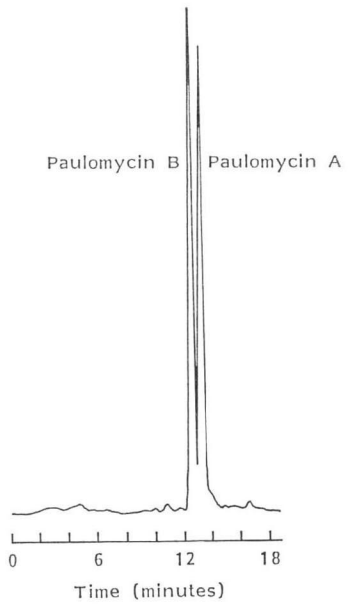
An experimental 100 ml fermentation yielded adequate material for HPLC analysis. Paulomycins A and B were extracted from the unfiltered beer at pH 5.3~5.5 with one-half volume methylene chloride.

Water was removed from the organic phase by the addition of anhydrous $MgSO_4$. The solution was then filtered and evaporated to dryness under N_2. The residue was triturated with 5 ml of heptane to remove Ucon, and the paulomycins were extracted into acetonitrile (5 ml). The acetonitrile phase was filtered and concentrated to 1/2 volume under N_2. The purity of this preparation was sufficient for HPLC analysis.

Analysis of samples was done with a Spectra-Physics System — SP8100 liquid chromatograph, SP8400 UV detector and SP4100 recorder/integrator. The column used was a Brownlee 250 RP8, 10 μm, 4.6 mm diameter × 250 mm length. A gradient chromatographic solvent system was composed of the following com-

Fig. 1. HPLC separation of authentic paulomycins A and B.

Quantitation of the paulomycins is based on the area percent of absorption at 320 nm. The area percentages for A and B, respectively, were 44.782 and 46.157.



ponents: Solvent A, acetonitrile (Burdick and Jackson); solvent B, 500 ml acetonitrile, 500 ml H_2O (Burdick and Jackson) and 1 ml glacial acetic acid (Mallinkrodt, AR); solvent C, H_2O. Fig. 1 provides an example of this separation.

Biological Assay

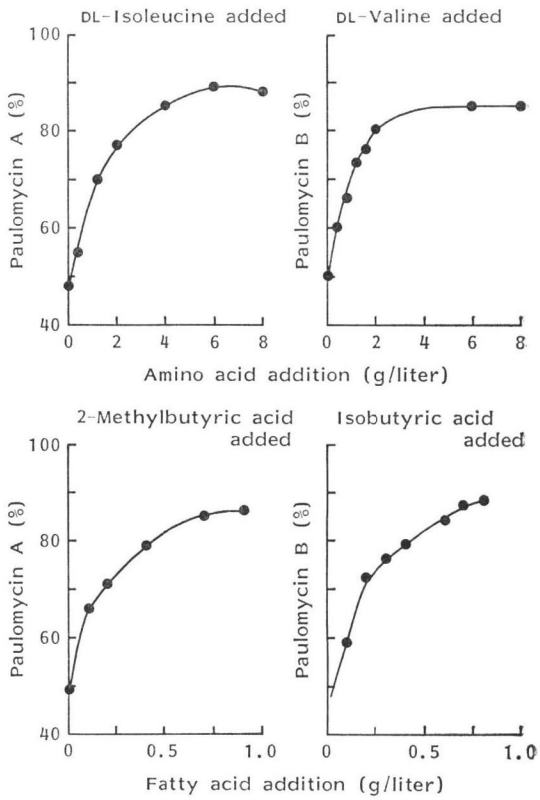
The amount of the paulomycin complex produced was determined by a biological assay. One biounit of anti-*Micrococcus luteus* UC 130 activity is the amount of paulomycin required to produce a zone of growth inhibition of 20 mm when applied to a 12.7 mm paper disc (Schleicher and Schuell No. 740-E). Using this assay, one biounit is equivalent to *ca.* 0.2 μg of paulomycin. The levels of paulomycin produced in all fermentations was about 100 mg per liter.

Precursor Directed Biosynthesis of Paulomycin

Fig. 2 shows the effects of added DL-isoleucine and DL-valine on the composition of the mixture of paulomycin A and paulomycin B produced by *S. paulus*. These amino acids were added to fermentations of *S. paulus* on the second and third days of fermentation. The amount of valine or isoleucine added to each flask was

Fig. 2. The effects of precursors on the biosynthesis of paulomycin.

Analysis was performed 2 days after the onset of antibiotic biosynthesis. The percent paulomycin A or B is calculated on the basis of the integrated areas representing A_{320}.



divided equally and added on the successive days. Fig. 2 additionally demonstrates the effects of added 2-methylbutyric acid and isobutyric acid on the composition of the mixture of paulomycin A and paulomycin B. These fatty acids were added to each fermentation 24 hours after inoculation.

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