DIRECTED BIOSYNTHESIS OF PAULOMYCIN A† THE EFFECT OF L-METHIONINE, L-THREONINE AND α-KETOBUTYRIC ACID

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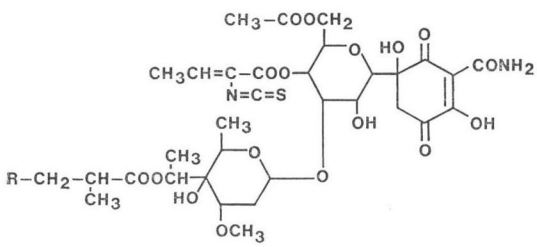
Paulomycins A and B belong to a class of novel antibiotics, produced by *Streptomyces paulus* UC 8560, that are equally active against a variety of pathogenic bacteria[1~3]. Earlier studies[4] indicated that a greater concentration of paulomycin A, relative to paulomycin B, could be achieved *via* the addition of isoleucine or 2-methylbutyric acid to fermentations. As paulomycin A is an ester of 2-methylbutyric acid[2] (Fig. 1), this result was not unexpected. The present paper describes the effect on paulomycin A production when L-methionine, L-threonine or α-ketobutyric acid were added to fermentations of *S. paulus*. Moreover, a partial biosynthetic scheme for paulomycin A is proposed.

Organism

S. paulus UC 8560 was used throughout this study and was maintained on Hickey and Tresner agar.

Fermentation Procedures

Fig. 1. Chemical structure of paulomycins A and B.



Paulomycin A R = CH_3

Paulomycin B R = H

† Previously described as volonomycin A[1].

A chemically defined medium was derived from various components of the media of NOSSAL and HEPPEL[5], GARDNER and LASCALLES[6], and MAH *et al.*[7] and was used for seed and fermentation media. The composition of this medium was as follows: NaCl 4.67 g, NH_4Cl 1.07 g, Na_2SO_4 0.426 g, $MgCl_2 \cdot 6H_2O$ 0.203 g, $CaCl_2 \cdot 2H_2O$ 0.029 g, $ZnCl_2$ 0.27 mg, K_2HPO_4 7.0 g, KH_2PO_4 3.0 g per liter of distilled H_2O. The pH of the medium was not adjusted. Sterile maltose was added aseptically to a final concentration of 5 g/liter. L-Threonine, L-methionine or α-ketobutyric acid were added as sterile solutions. All additions were made at the onset of fermentation. Fermentations were conducted on a rotary shaker (250 rpm) at 28°C. Cultures were analyzed for antibiotic production on the fourth day of fermentation.

Biological Assay

Quantitation of the amount of paulomycin complex produced was performed by biological assay using *Micrococcus luteus* ATCC 9341. Standard curves were made with authentic paulomycin (0.025 μg/disc ~ 80 μg/disc). Data for the standard curve were analyzed by a linear regression program on a microcomputer. Correlation coefficients for standard curves were routinely 0.99. Zones of inhibition *versus M. luteus* for fermentation samples were then entered and μg paulomycin complex per ml calculated.

Bioautography

Fermentations were analyzed by thin-layer chromatography on cellulose plates (Brinkman Polygram Cel 400) using 0.1 M sodium phosphate buffer (pH 7.0) as the mobile phase. Bioautography was performed on *M. luteus*.

Analytical Procedures

Antibiotic isolation was accomplished by adjusting the filtered broth to pH 5.3 ~ 5.5 and extracting 300 ml of this with methylene chloride (150 ml). The organic phase was dried over anhydrous $MgSO_4$ and filtered. The solvent was removed under a stream of nitrogen. The residue was dissolved in acetonitrile and contained *ca.* 3 mg paulomycin.

HPLC Chromatography

The chromatographic conditions were as described previously[4]. The percent paulomycin A or B was calculated on the basis of the integrated areas representing the absorptions (320 nm) of

Fig. 2. Bioautographic analysis of fermentations conducted with methionine, threonine and α-ketobutyric acid.

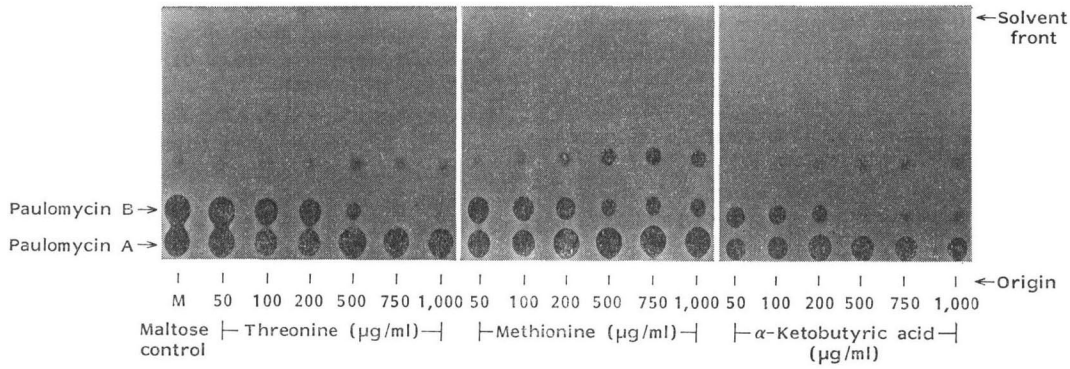


Fig. 3. The effects of L-methionine, L-threonine and α-ketobutyric acid on paulomycin A production.

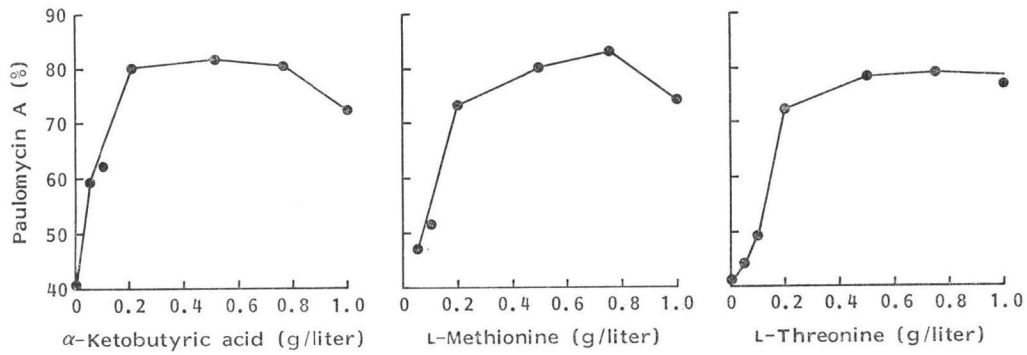
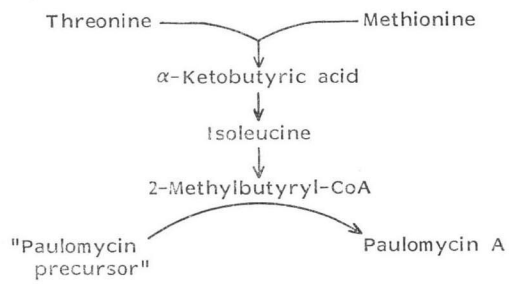


Fig. 4. Proposed scheme for paulomycin A biosynthesis.



the paulomycins A and B.

Paulomycin Biosynthesis

Fig. 2 shows the bioautographic analyses of fermentations conducted with methionine, threonine or α-ketobutyric acid. Levels of precursor addition were from 0.05 g/liter to 1.0 g/liter. A fermentation containing no added precursor was included as a control. The levels of paulomycin produced in fermentations were *ca.* 5~10 μg/ml irregardless of concentration of precursors. In all instances, higher precursor concentrations resulted in greater paulomycin A production as compared to paulomycin B.

Fig. 3 shows the percentage of paulomycin A produced, relative to paulomycin B, when various concentrations of methionine, threonine or α-ketobutyric acid were added to the fermentations. In each instance, paulomycin A production increased with increasing precursor levels. Fermentations containing threonine or α-ketobutyric acid showed a slight decrease in paulomycin A when their concentration was at 1.0 g/liter.

Our results support the hypothesis that α-ketobutyric acid plays a central role in paulomycin A biosynthesis. The initial metabolism of both methionine and threonine is known to yield α-ketobutyric acid, which is a precursor of isoleucine[8]. As shown in Fig. 4, the metabolic production of α-ketobutyric acid would favor isoleucine biosynthesis and thus the selective production of paulomycin A *via* 2-methylbutyryl-CoA.

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