PRECURSOR DIRECTED BIOSYNTHESIS OF PAULOMYCIN C BY METHIONINE

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Paulomycin C (Fig. 1) is a member of a novel class of antibiotics, the paulomycins[1,2], which are produced by *Streptomyces paulus* UC 8560 and are active against a variety of pathogenic bacteria, including methicillin-resistant *Staphylococcus aureus*[3]. Paulomycins A and B are esters of methylbutyric acid and isobutyric acid[1], respectively, and can be produced selectively through procedures involving precursor directed biosynthesis[4,5]. Paulomycin C is an ester of propionic acid and has been detected in fermentations of *S. paulus* in synthetic medium to which methionine has been added. We now wish to report the effect of various concentrations of methionine on paulomycin C production.

Organism

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

Fermentation Procedures

All fermentations were conducted in the chemically defined medium described in ref 5. The pH of the medium was not adjusted. Sterile maltose was added aseptically to a final concentration of 5 g/liter. L-Methionine was also added as a sterile solution. All additions were made at the onset of fermentation. Fermentations were conducted on a rotary shaker (250 rpm) at 28°C.

Fig. 1. Paulomycin C.

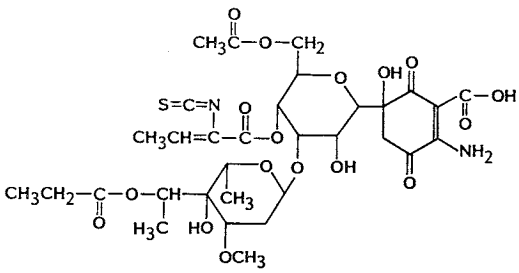


Table 1. Effect of methionine on production of paulomycin C by *Streptomyces paulus*.

Methionine concentration (mg/ml)	Paulomycin C relative to the control[a] (%)	Paulomycin C relative to the total amount of the paulomycin complex produced (%)
0.0	100.0	4.5
0.1	91.0	8.6
0.5	112.0	11.1
1.0	226.0	22.7
2.0	188.0	22.0

[a] Control fermentation is without added methionine.

Biological Assay

Quantitation of the amount of paulomycin complex produced was performed by biological assay using *Micrococcus luteus* UC 130[4,5].

Analytical-HPLC

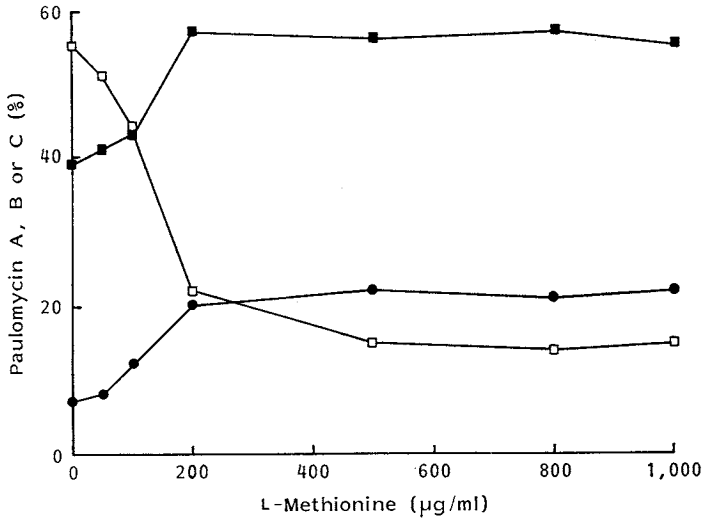
All HPLC chromatography was carried out with a Varian Model 5560 (Varian Instruments, Sugarland, Texas) instrument equipped with a LKB Rapid Spectral Detector (LKB, Broma, Sweden). Data was stored in an IBM-AT microcomputer and integration of peak areas was done using Nelson Analytical Software version 3.6. The percent paulomycin A, B or C was calculated on the basis of the integrated areas representing the absorptions (320 nm) of the paulomycins A, B and C.

Paulomycin C Biosynthesis

Table 1 shows the effect of various concentrations of methionine on production of paulomycin C in fermentations of *S. paulus*. A fermentation containing no added precursor was included as a control. The level of paulomycin complex produced in fermentations was 5~15 μg/ml. As can be seen in Table 1, the concentration of paulomycin C increased relative to the control lacking added methionine, achieving maximum concentration at 1.0 mg/ml methionine. In addition, the proportion of paulomycin C relative to total paulomycin production also increased with increasing concentrations of methionine.

Fig. 2. The effect of methionine on production of paulomycins A, B and C in fermentations of *Streptomyces paulus*.

■ Paulomycin A, □ paulomycin B, ● paulomycin C.



As shown in Fig. 2, methionine also affected changes in the ratio of paulomycins A, B and C produced. Increased levels of the amino acid resulted in an increase in production of paulomycin A with a concomitant decrease in paulomycin B. This finding has been previously reported[5].

Our results indicate that methionine plays a central role in the directed biosynthesis of paulomycin C. As paulomycin C is an ester of propionic acid, it is presumably synthesized *via* catabolism of α-ketobutyric acid to propionyl-CoA. The initial metabolism of methionine is known to yield α-ketobutyric acid[6]. The increased production of paulomycin A also is the result of higher levels of α-ketobutyric acid being generated in the presence of methionine.

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