ISOLATION AND CHARACTERIZATION OF THE METHIONINE ANTAGONIST L-2-AMINO-4-METHOXY-*TRANS*-3-BUTENOIC ACID FROM *PSEUDOMONAS AERUGINOSA* GROWN ON *n*-PARAFFIN

URSEL SAHM, GERRIT KNOBLOCH and FRITZ WAGNER

Gesellschaft für Molekularbiologische Forschung mbH, D-3301 Stöckheim, Mascheroder Weg 1, Germany

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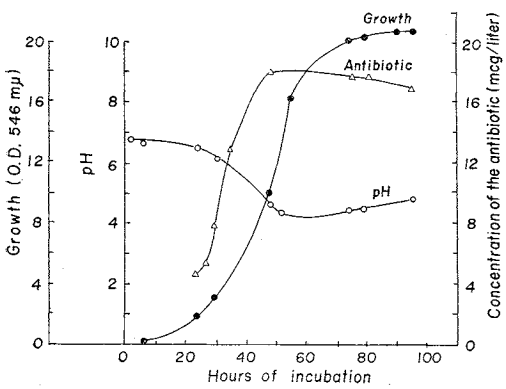
Only few antibiotics are known, which were isolated from the culture broth of bacteria utilizing *n*-paraffin[1~4]. In the course of our screening of antibiotics produced by microorganisms growing on *n*-paraffins as the sole source of carbon and energy we isolated a strain of *Pseudomonas aeruginosa*, designated as strain 15, which produces an amino acid antagonist active against gram-positive and gram-negative bacteria grown on minimal medium according to DAVIS and MINGIOLI[5]. This antibiotic is not formed when the strain utilizes methanol as the only carbon source.

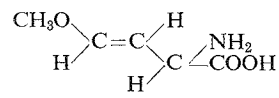
The cells were grown at 30°C in flasks on a rotary shaker or in a 80-liter fermentor in a medium containing *n*-hexadecane, 1 % (v/v), as described by SAHM and WAGNER[6], until the antibiotic activity reached a maximum. Fig. 1 shows the production of the antibiotic on *n*-hexadecane. Hexadecane supported the growth more effectively than glucose, but we did not observe an increase in the formation of the antibiotic in *n*-hexadecane medium.

Fig. 1. Growth response and antibiotic production together with pH-changes during incubation of *P. aeruginosa* on a 1% (v/v) *n*-hexadecane-basal salt medium at 30°C.



For the isolation of the antibiotic the culture broth containing 18 mg/liter antibiotic was treated with activated charcoal (1 %, w/v) and the compound was isolated from the charcoal filtrate by adsorption onto a cation-exchange column (Dowex 50 WX2, H^+-form) followed by elution with 0.25 N NH_4OH. The material recovered from active fractions was further purified by means of preparative thin-layer chromatography on silica gel with methanol-acetic acid-pyridine-water (80 : 1 : 4 : 20) and rechromatography on cellulose powder with *n*-propanol-water (8 : 2) as solvent system. The overall recovery was 19 %.

Recently, SCANNELL *et al.*[7] reported the isolation of an amino acid antagonist from *Pseudomonas aeruginosa* ATCC-7700 grown on cerelose medium and established its structure as L-2-amino-4-methoxy-*trans*-3-butenoic acid by spectroscopic evidence and chemical degradation. The antibacterial activity against *Bacillus* sp. 1283 B as well as the characteristic reversal pattern of growth inhibition on this organism by a variety of amino acids led to the suggestion that our product was identical with L-2-amino-4-methoxy-*trans*-3-butenoic acid (**I**)

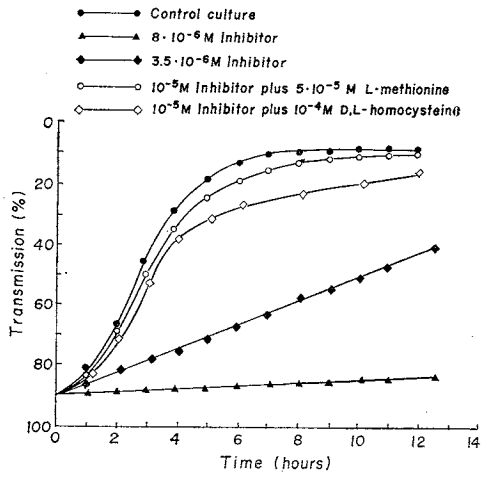


(I)

This was proven by comparing TLC behaviour, colour reaction with ninhydrin, ORD and ^{1}H-NMR spectra of both our compound and an authentic sample of **I** kindly sent to us by Dr. A. SCHOCHER (Hoffmann-La Roche, Basle). The spectra were in agreement with the recorded data.

In addition we found a strong inhibitory activity of the amino acid antagonist against *Escherichia coli* K12 and a quite different reversal pattern of the growth inhibition as reported for *Bacillus* sp. 1283 B by SCANNELL *et al.*[7]. Fig. 2 shows that addition of $8 \cdot 10^{-6}$ M antibiotic completely inhibited the growth of *E. coli* K12 in minimal medium, when the strain was precultivated in the same medium. When *E. coli* K12 was pregrown in complete medium, the minimal inhibitory concentration decreased significantly to $3 \cdot 10^{-6}$ M. The inhibi-

Fig. 2. Inhibition of growth of *E. coli* K12 on minimal medium by L-2-amino-4-methoxy-*trans*-3-butenoic acid and reversal of growth inhibition by L-methionine and D, L-homocysteine.



tion of growth does not depend on the growth phase.

The growth inhibition of *E. coli* K12 by L-2-amino-4-methoxy-*trans*-3-butenoic acid was reversed by methionine as well as by the two intermediates in methionine biosynthesis, homocysteine (Fig. 2) and cystathionine. No reversal is brought about by homoserine, the common precursor of methionine, threonine and lysine. This reversal pattern leads to the conclusion that L-2-amino-4-methoxy-*trans*-3-butenoic acid inhibits the biosynthetic pathway of methionine at a step prior to formation of cystathionine by mimicking methionine in regulating the formation and the activity of homoserine O-transsuccinylase[8]. The examination of the influence of this methionine antagonist on the enzymes regulated by methionine is under investigation.

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