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CONVERSION OF 2-MERCAPTOPYRIMIDINE INTO *S*-(PYRIMIDIN-2-YL)-CYSTEINE IN GROWING *ESCHERICHIA COLI* CELLS

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1. Introduction

In the course of our investigations on the biological activity of simple pyrimidine derivatives we have been interested in the effect of 2-mercaptopyrimidine derivatives upon bacterial cells *in vivo*. In this connection, we have also studied the behaviour of the parent compound, 2-mercaptopyrimidine (fig. 1, I) in the system mentioned.

2. Materials and methods

2-Mercaptopyrimidine (I) was prepared by the reported procedure [1] and purified by a 3-fold crystallisation from 50% aqueous ethanol. Bacterial cell cultures were grown in a synthetic medium [2]. The cell growth inhibition was followed by absorbancy measurement of the culture suspension at 575 nm and by counting the bacterial colonies. Paper chromatography was performed on Whatman No. 3 MM paper in systems: S1, 2-propanol–conc. aqueous ammonia–water (7:1:2); S2, 1-butanol–acetic acid–water (10:1:3); paper electrophoresis was performed on the same paper, in (S3) 0.1 M triethylammonium hydrogen carbonate pH 7.5 at 20 V/cm, in an apparatus of Markham and Smith. Amino acid analysis was performed on an automatic analyzer under standard conditions.

The isolation of *S*-(pyrimidin-2-yl) cysteine (fig. 1 II) from the culture medium: to 3 × 100 ml cultures of *E. coli* B growing exponentially in a synthetic medium, there was repeatedly added 50 μg/ml of 2-mercaptopyrimidine. The first portion was added at 3.5×10^8 cells/ml and the other portions after additional 40 and 80 min. After 120 min over-all incubation time, the cell suspension was centrifuged under cooling, the supernatant filtered through nitrocellulose membrane filters (Synpor 6, VChZ Synthesia, Czechoslovakia) and the filtrate brought to pH 3 by addition of Dowex 50 × 8 (H^+-form) ion exchange resin previously washed with water. The suspension was applied to a 200 ml column of the same resin, washed previously with water and the column was eluted with water (rate 3 ml/min) till the UV-absorption and conductivity dropped to the original values. Afterwards, the elution was performed with 5% aqueous ammonia, the UV-absorbing band was collected, evaporated to dryness at 30°/15 Torr and the residue chromatographed on 4 sheets of Whatman No. 3 MM paper in the system S1. The UV-absorbing bands of the product II were eluted with 50 ml total of 1% aqueous ammonia, evaporated as above on 2 sheets of the same paper in the system S2. On eluting with water and freeze-drying there was obtained 43 mg (54%, referred to 45 mg of I of white amorphous compound II, m.p. 158–160°). For $C_7H_9N_3O_2S$ (M.W. 199.2), calculated: 21.10% N, 16.09% S; found: 20.81% N, 16.52% S. S1, R_F 0.44, S2, R_F 0.21, S3, E_{Up} 0.2 (referred to Up); UV-spectrum (pH 7): λ_{max} 243 nm, λ_{min} 221 nm.

3. Results and discussion

2-Mercaptopyrimidine (I) inhibits strongly bacterial growth in a stationary culture of *E. coli* B: 88% at 10

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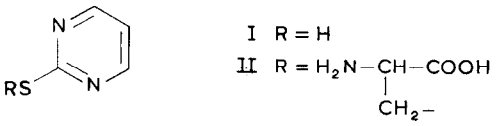

Fig. 1.

μg/ml, full inhibition at 30 μg/ml. On the other hand, with the compound I added to the exponentially growing culture *E. coli* B at 50 μg/ml, there was no growth inhibition. After 100 min of incubation, the viable counts of the 2-mercaptopyrimidine-treated culture were 1.1×10^9 (compared with the control culture of 1.75×10^9). The compound was added at 3.4×10^8 cells/ml.

Upon analysis of the culture medium it was found that there is no 2-mercaptopyrimidine present after the first 60 min of incubation time. The culture medium contains another UV-absorbing material which was isolated as above. Its electrophoretic mobility corresponds to a weak acid, whereas the negative results of phosphate and sugar tests eliminate the possibility of nucleotidic material. On the other hand, the product exhibits a strong positive ninhydrin reaction (violet) suggesting thus an amino acid structure. The UV-absorption maxima correspond to the *S*-substituted derivatives of I [3]. On acid hydrolysis (6 N HCl, 16 hr, 120°) the only amino acid material present was shown to be cysteine (affording cysteic acid by performic acid oxidation). After 2 min treatment of the 5% aqueous solution of the unknown material with Raney-Ni (W8) at 100°, the compound was quantitatively converted to alanine. The above results suggest the structure of *S*-(pyrimidin-2-yl) cysteine for the material isolated. This was confirmed by an independent chemical synthesis of the compound II [4] (m.p. 160°).

The conversion of 2-mercaptopyrimidine (I) into the compound II by growing *E. coli* cells represents possibly a simple way of detoxication of the mercapto derivative to a non-toxic amino acid derivative. The most embarassing is the rate of the conversion and its quantitative course. There is no transformation of compound I in the culture medium itself, or, with bacteria precultivated, washed and resuspended in a synthetic medium without glucose or other organic source. This suggests a biochemical reaction utilizing a simple metabolite of glucose, probably L-serine or its *O*-phosphoryl derivative. On treatment of the bacterial growing culture with compound I in the presence of ^{14}C-L-serine, the radioactivity was found in the transformation product II, *without any significant isotope dilution.* Thus, L-serine seems to be the reaction partner in the reaction which is evidently catalyzed enzymatically (there is no reaction of I with L-serine in the medium with or without ATP).

Owing to the fast conversion of L-serine into compound II, an effect of 2-mercaptopyrimidine upon L-serine synthesis de novo, and, thereby, the synthesis of L-serine metabolites could be expected. Therefore, the protein synthesis should be affected throughout the period of detoxication reaction. This was found to be the case: the protein synthesis inhibition reaches 23% (as measured by ^{14}C-leucine incorporation).

The transformation reaction described above is not limited to the parent compound; the preliminary experiments with some of its substituted derivatives demonstrated the occurrence of the same reaction type taking place with 4- or 5-alkyl derivatives of 2-mercaptopyrimidine. On the other hand, the presence of 4-hydroxy or 4-amino group (2-thiouracil, 2-thiocytosine) results in a lack of the enzymatic activity towards the mercapto group. Therefore, the necessary condition for the reaction seems to be the presence of an acidic SH-function in the molecule.

We have not yet succeeded in the localisation of the enzyme responsible for the transformation reaction, or to ascribe its activity to any other enzyme catalyzing the sulfuration reactions or their reversals in bacterial cells. This problem, as well as the specificity of the enzyme towards both reaction partners, are under present study.

References

[1] Org. Syntheses 43 (1963) 68.
[2] J. Škoda, V. Hess and F. Šorm, Collection Czech. Chem. Commun. 22 (1957) 1330.
[3] A. Albert and G.B. Barlin, J. Chem. Soc. (1962) 3129.
[4] A. Holý and K. Jošt, Collection Czech. Chem. Commun., in press.