Effect of Thio- and Hydrazino-derivatives of Uracil, 6-Azauracil and 6-Azathymine on the Growth of Some Microorganisms in Vitro

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Hydrazino-Pyrimidine Derivatives, Hydrazino-Azapyrimidine Derivatives, Antibacterial Activity

Numerous purine and pyrimidine derivatives have been synthesized in the last years and they have been studied as inhibitors of nucleic acids biosynthesis. The inhibitors of the biosynthesis of nucleic acids possess antitumour, antivirus, antibacterial and immunodepressive activity.

The synthesis and the examination of the antibacterial activity of thiao- and hydrazino-derivatives of uracil, 6-azaracil and 6-azathymine are described in the present article. No reference data about the biological activity of hydrazino-derivatives have been found. However there are in literature data concerning the antibacterial activity of thio-derivatives which are in good agreement with our results1-3. In order to establish the similarity or the difference in behaviour of thiao- and hydrazino-derivatives several parallel comparative tests with different microorganisms were carried out.

Methods and Materials

A. Synthesis of thio- and hydrazino-derivatives

The formulas of the derivatives of pyrimidine and azapyrimidine synthesized and examined for antibacterial activity, are shown in Table 1.

1) 2-Thiouracil (1) a commercial preparation of Fluka is used.

2) 2-Methylthiouracil (2) was synthesized by methylation of 2-thiouracil with dimethyl sulphate according to the general method for the preparation of (alkylthio) pyrimidines proposed by KOPPEL et al.4.

3) 2-Hydrazinouracil (3). Synthesized from 2-methylthiouracil and hydrazine hydrate in alcohol solution. The substance is described by YUOH-FONG CHI et al.5.

4) 2,4-Dithiouracil (4) was prepared according to BROWNS6, using 2-thiouracil and phosphorus pentasulphide.

5) 2-Thio-4-hydrazinouracil (5), first described by LIBERMAN and ROUAI57, was synthesized according to a modified method. 2,4-Dithiouracil is treated with 100% hydrazine hydrate in alcoholic solution in the presence of pure acetic acid as catalyst. The reaction is carried out for three hours at 0 °C. The yield is 94%.

6) 4-Thio-6-azaracil (6) was synthesized according to GUT et al.9, using 6-azaracil and phosphorus pentasulphide.

7) 4-Hydrazino-6-azaracil (7), not described in the literature, was prepared by suspending 4-thio-6-azaracil in ethanol in the presence of pure acetic acid as catalyst and by adding 100% hydrazine hydrate. The reaction was carried out for 2 — 3 hours under continuous stirring at 20°. After cooling for one night 80% yield.

C₃H₄N₂O (127.11)

Calculated: C 28.34 H 3.96,

Found: C 28.38 H 4.21.

8) 2-Thio-6-azathymine (8) was prepared by cyclization of pyruvic acid thiosemicarbazone10.

9) 2-Methylthio-6-azathymine (9) was synthesized by methylation of 2-thio-6-azathymine with methyl iodide11.
10) 2-Hydrazino-6-azathymine (10) was synthesized by the interaction of 2-methylthio-6-azathymine with 50% hydrazine hydrate.

11) 2,4-Dithio-6-azathymine (11) was prepared from 2-thio-6-azathymine and phosphorus pentasulphide.

12) 2-Thio-4-hydrazino-6-azathymine (12). It is described by TADASHI SASAKI et al., but we synthesized it with a greater yield (65%) by a much easier method and similar to the synthesis of 4-hydrazino-6-azauracil (7).

13) 2,4-Dimethylthio-6-azathymine (13) was synthesized by methylation of 2,4-dithio-6-azathymine with methyl iodide.

14) 2-Methylthio-4-hydrazino-6-azathymine (14) was prepared from 2,4-dimethylthio-6-azathymine with 50% hydrazine hydrate.

B. Study of the antibacterial activity

For determination of the antibacterial activity all compounds were previously purified to a constant melting point (see Table 1).

I. Test microorganisms: Staphylococcus aureus 209, Streptococcus faecalis 775, Escherichia coli 387, Pseudomonas aeruginosa, Bacillus subtilis, Candida tropicalis and Neurospora crassa 9863.

II. The used nutritive mediums are different in dependence with the character of the microorganisms: M9, medium of Saburo, medium of Fries.

Methods

1) All microorganisms are grown up under optimal temperature. The growth rate was determined turbidometrically.

2) The investigation with Neurospora crassa is carried out as described earlier.

Results and Discussion

Those substances, which showed highest inhibitory effect against N. crassa were tested on the remaining test organisms mentioned above. A high inhibitory effect – 100% at a concentration 10⁻³ M show 2-thio-4-hydrazinouracil, 4-thio-6-azauracil, 2-thio-4-hydrazino-6-azathymine, 2,4-dimethylthio-6-azathymine and 2-methylthio-4-hydrazino-6-azathymine. The growth of N. crassa is depressed to a smaller degree by 2-hydrazino-derivatives of uracil (67%) and 6-azathymine (33%). The depression of growth by thio-derivatives is smaller. The difference in activity of hydrazino- and thio-derivatives can be seen on Fig. 1 in which the inhibition in percentage
at two concentrations ($1 \cdot 10^{-5} \text{M}$ and $10 \cdot 10^{-3} \text{M}$) is expressed. At a concentration $1 \cdot 10^{-3} \text{M}$ the inhibitory effect of 2-thiouracil is 7% while the inhibitory effect of 2-hydrazinouracil is 42%. At a concentration $10 \cdot 10^{-3} \text{M}$ the percentage of inhibition is 34% and 67% respectively. The same dependence is clearly expressed for 2,4-dithiouracil and 2-thio-4-hydrazinouracil, where the substitution of thio-group at position 4 by a hydrazino-group increases the inhibitory effect from 48% to 100% at a concentration $10 \cdot 10^{-3} \text{M}$. The opposite effect is valid for 6-azauracil. The inhibition caused by 4-hydrazino-derivatives is with 34% lower than the inhibition caused by thio-derivatives. Thio- and 4-hydrazino-derivatives of 6-azathymine do not show any considerable difference in their inhibitory effect on the growth of N. crassa. Furthermore, the position of the hydrazino-radical in the pyrimidine ring appears to have an important role for the activity of the compound. In all cases 4-hydrazino-derivatives are more active than the respective 2-hydrazino-derivatives. For example the inhibition caused by 2-thio-4-hydrazino-6-azathymine is 100%, while that caused by 2-hydrazino-6-azathymine is only 33%. Our results help us to conclude that thio-derivatives are less active against N. crassa than the corresponding methylthio-derivatives. For example the inhibitory effect of 2-thiouracil is 34% while the inhibitory effect of 2-methylthiouracil is 72%.

Some of the more active compounds which depress the growth of E. coli are shown on Fig. 2. The inhibitory effect given in percentage 5 hours after the treatment at a concentration $1 \cdot 10^{-3} \text{M}$ (towards which are expressed all the results of the microbiological investigations) of the respective compounds is: 100% for 2-thio-4-hydrazino-6-azathymine, 93% - 4-thio-6-azauracil, 91% - 2-methylthio-4-hydrazino-6-azathymine, 87% - 4-hydrazino-6-azauracil and 78% for 2-methylthiouracil. A lower inhibitory effect is found for 2-hydrazino-derivatives of uracil and 6-azathymine. In this case also as in the case with N. crassa the highest inhibition effect is exhibited by compounds having hydrazino-group at the fourth place.

The effect of some compounds on the growth of Pseudomonas aeruginosa is given on Fig. 3. The growth of the microorganisms is depressed with 80% 21 hours after the treatment, by 2-hydrazinouracil and 4-hydrazino-6-azauracil. 4-Thio-6-aza-
pressed to 42% by 2-thio-4-hydrazinouracil and 2-hydradino-6-azathymine and to 37% by 2-methylthiouracil. The percentage of inhibition for all other tested compounds is between 15 and 30%.

The greatest inhibitory effect on *Staphylococcus aureus* is caused by 2-methylthiouracil which depresses the growth of bacteria with 75%, 12 hours after the treatment. The inhibition due to the treatment with 2,4-dithio-6-azathymine is lowered to 46% and 42% respectively for 2-thio-6-azathymine. The inhibitory effect of all other tested substances is insignificant.

Most of the compounds have no effect on *Streptococcus faecalis*.

A comparison between the results of the microbiological study in vitro and the structure of the tested compounds shows that hydrazino-derivatives have a higher biological activity compared to the thio-derivatives. Most active are the compounds with hydrazino-group at position 4. It is possible that this is due to the fact that 4-hydrazino-derivatives are (compared to the derivatives with hydrazino-group at position 2) analogues antagonists of cytosine. It turned out that most active of the tested compounds are: 4-hydrazino-derivatives of 2-thiouracil, 6-azauracil, 2-thio-6-azathymine and 2-methylthio-6-azathymine. The mechanism of their action will be the subject of further experiments.

5. Yuoh-Fong Chi and Yuan-Lin Wu, Hua Hsiuch Hsiich Pao 23, 145 [1957]; (C. A. 52, 14627a [1958]).