TRANSFORMATIONS OF FERVENULIN-4-OXIDE IN REACTIONS WITH SEVERAL NUCLEOPHILES

Yu. A. Azev, I. I. Mudretsova,
E. L. Pidémskii, A. F. Goleneva,
G. A. Aleksandrova, and N. A. Klyuev

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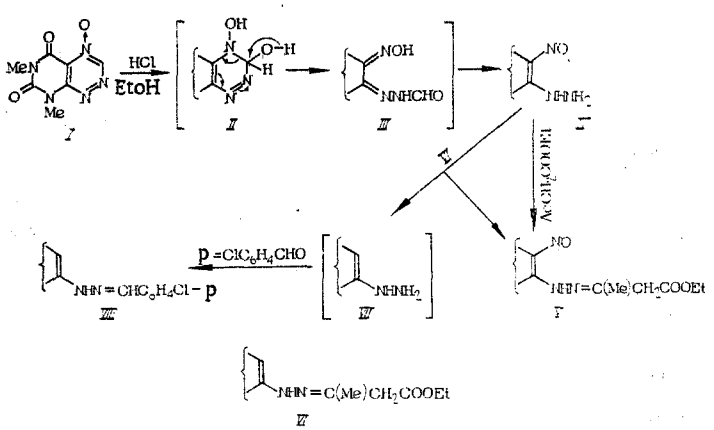
The study of chemical transformations of analogs of pyrimidotriazine antibiotics is of interest in a plan for a search for new biologically-active compounds as well as for a study of the possible routes for transformation of compounds of this type in the animal organism.

The transformation of derivatives of pyrimido[5,4-e][1,2,4]-triazin-5,7-dione under the influence of alcoholic base into 6-azapurine is known [7]. Fervenulin-4-oxide (I) is transformed by heating with 0.5% NaOH into 5,7-dimethylimidazo[4,5-e]as-triazin-6(7H)-one: [3]. Information on the reactivity of I with respect to C-nucleophiles is absent from the literature. It is known, however, that other heterocyclic N-oxides easily react with different C-nucleophiles in the presence of acylating agents [4].

In the present work, we studied the transformations of I under the influence of several nucleophiles, i.e., the reactions of I in ethanol in the presence of mineral acids, and in dry DMF by triethylamine-activated nucleophiles.

It was established that in 96% ethanol in the presence of HCl, I cleaves to form the nitrosohydrazine IV (30% yield), as described in [5]. However, if the reaction is carried out in the presence of acetoacetic ester, a 75-80% yield of 1,2,3,4-tetrahydro-1,3-dimethyl-5-nitrose-6(1'-methyl-2'-carboethoxymethylethylidinehydrazino)pyrimidin-2,4-dione (V) is formed, which indicates that the transformation of compound I into IV occurs with a yield of higher than 30%.



It should be noted that compound V is formed from I in the presence of HCl but also by addition of hydrazone VI. Thus, removal of hydrazone V and treatment of the mother liquor with 4-chlorobenzaldehyde gave 1,2,3,4-tetrahydro-1,3-dimethyl-6-(4-chlorophenylmethylidinehydrazino)pyrimidin-2,4-dione (VIII). Evidently the nitrosohydrazine IV formed by the acidic cleavage of I trans-hydrazinates the hydrazone VI. The trans-hydrazination of hydrazone VI by the hydrazine IV also takes place in the presence of triethylamine. The described cleavage of I evidently proceeds through a step opening the triazine ring through the covalent hydrate (II), followed by deformylation of the product III.

Compound I also reacts with acetoacetic ester in dry DMF in the presence of triethylamine. The reaction in this case gives 1,2,3,4-tetrahydro-1,3-dimethyl-5-nitroso-6-(2'-carboethoxy-2'acetylethylidinehydrazino)pyrimidin-2,4-dione (IX).

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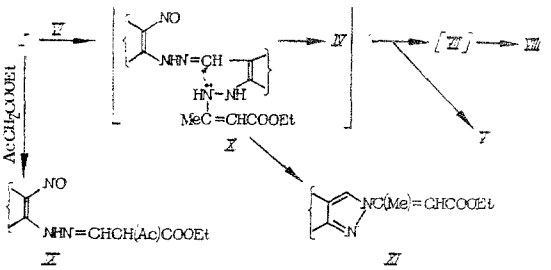

The molecular weight of compound IX, determined mass-spectrometrically, corresponded to the calculated value. The electronic spectrum of this compound showed the character of the curves and the positions of the maxima to be similar to the spectrum of hydrazone V.

We discovered interesting transformations by studying the interaction of the oxide I with the hydrazone VI in dry DMF in the presence of triethylamine. The reaction in this case gave pyrazole[3,4-d]pyrimidine (XI) in 40-50% yield. The molecular weight of compound XI, determined mass-spectrometrically, corresponded to the calculated value. The PMR spectrum of the product showed proton signals corresponding to the proposed structure. This reaction also formed hydrazone V. In addition, treatment of the mother liquor (after separating compounds V and XI) with 4-chlorobenzaldehyde gave hydrazone VIII, which supports the presence of the hydrazinouracil VII in the reaction mixture. The isolation of products V, XI, and VII from the reaction mixture shows that under the action of hydrazone VI in the presence of triethylamine, the cleavage of the triazine ring of I also takes place. The formation of products V, XI, and VII can be represented as the result of the intramolecular trans-hydrazination of intermediate X: Compounds IV and V are formed as a result of this reaction, while V trans-hydrazinates a second molecule of hydrazone VI analogously to that described above.

EXPERIMENTAL (CHEMICAL)

The PMR spectrum of compound XI was obtained in $CDCl_3$ with a Perkin-Elmer R-12B instrument with a working frequency of 60 MHz. Chemical shifts are given on the δ-scale in ppm relative to TMS.

The mass-spectra were obtained on a Varian MAT 311A with 3kV acceleration potential, cathode emission of mkA and ionization potential of 75 V; ionization temperature = 200°C.

The electronic spectra were determined on a Specord UV-VIS in ethanol.

Hydrazone VI was prepared according to [6].

Cleavage of Fervenulin-4-oxide (I) in Acidic Medium. A mixture of 0.084 g (0.4 mmole) of compound I, 4 ml of 96% ethanol, and 0.06 ml of concentrated HCl was boiled for 20 min. The reaction mixture was cooled and neutralized with saturated aqueous NaOAc. The precipitate (0.23 g, 29%) of nitrosohydrazine was filtered off; it was identified by melting point and IR spectrum, which were compared to an earlier-prepared sample [4].

1,2,3,4-Tetrahydro-1,3-dimethyl-5-nitroso-6(1'-methyl-2'-carboethoxyethylidinehydrazino)pyrimidin-2,4-dione (V). To a solution of 0.084 g (0.4 mmole) of I in 5 ml of 96% ethanol was added 0.06 ml of concentrated HCl and 0.06 ml of acetoacetic ester. The reaction mixture was kept for 2 h at 20-25°C and the precipitate was filtered to give 0.100 g (80%) of V, mp 159-160°C (ethanol). Found, %: C 46.7; H 5.7; N 22.3. $C_{12}H_{17}N_5O_7$. Calculated, %: C 46.3; H 5.5; N 22.5. UV spectrum, λ_{max}, nm (log ε) (ethanol): 247 (4.120); 296 (4.124); 444 (3.730).

Trans-Hydrazination of Hydrazone VI to Hydrazine IV. A. To a solution of 0.084 g (0.4 mmole) of I in 5 ml of 96% ethanol was added 0.06 ml of concentrated HCl and 0.12 g (0.4 mmole) of hydrazone VI. The reaction mixture was kept at 20-25°C for 10-15 h, and the precipitate was filtered off to give 0.099 g (80%) of V. The mother liquor was treated with 0.056 g (0.4 mmole) of 4-chlorobenzaldehyde in 3 ml of ethanol. The mixture was boiled for 3-5 min and cooled. The resulting precipitate was filtered off to give 0.082 g (70%) of VIII, mp 245-246°C (ethanol). Found, %: C 53.6; H 4.6. $C_{13}H_{13}ClN_4O_2$. Calculated, %: C 53.4; H 4.5.

B. To 0.020 g (0.1 mmole) of hydrazine IV in 2 ml of 96% ethanol was added 0.06 ml of concentrated HCl and 0.028 g (0.1 mmole) of hydrazone VI. The reaction mixture was kept at

20-25°C for 30 min and filtered to give 0.028 g (90%) of V. The hydrazone VIII was separated as in A, 75-80% yield.

C. To 0.020 g of hydrazine IV in 1 ml of dry DMF was added 0.060 ml of triethylamine and 0.028 g of hydrazone VI. The reaction mixture was kept at 20-25°C for 30 min and then diluted with water (1:1). The precipitate was filtered off to give 0.017 g (55%) of IV. Hydrazone VIII was isolated as in A, 65-70% yield.

1,2,3,4-Tetrahydro-1,3-dimethyl-5-nitroso-6-(2'-carboethoxy-2'-acetylethylidinehydrazino)pyrimidin-2,4-dione (IX). To a solution of 0.084 g of I in 1 ml of dry DMF was added 0.06 ml of Et_3N and 0.06 ml of acetoacetic ester. The reaction mixture was kept at 20-25°C for 20-25 h, diluted with water (1:1), and acidified with concentrated HCl to pH 2.0-3.0. The resulting precipitate was filtered off to give 0.095 g (70%) of IX, mp 162-163°C (ethanol). Found, %: C 45.9; H 5.1; N 20.6. $C_{13}H_{17}N_5O_6$. Calculated, %: C 46.0; H 5.0; N 20.6. UV spectrum, λ_{max}, nm (log ε) (ethanol): 247 (4.058); 306 (3.876); 417 (3.808).

Reaction of I with Hydrazone VI in the Presence of Et_3N. To 0.84 g (0.4 mmole) of I in 1 ml of dry DMF was added 0.224 g (0.8 mmole) of hydrazone VI and 0.06 ml of Et_3N. The reaction mixture was kept for 5-6 h at 20-25°C, and the precipitate of 4,5,6,7-tetrahydro-2-(1'-methyl-2'-carboethoxyvinyl)-5,7-pyrazolo[3,4-d]pyrimidine-4,6-dione (XI) was filtered off, 0.049 g (42%), mp 203-204°C (ethanol). Found, %: C 53.5; H 5.5; N 19.5. $C_{13}H_{16}N_4O_4$. Calculated, %: C 53.4; H 5.5; N 19.2. PMR spectrum ($CDCl_3$): 1,26 t (3H, CH_2CH_3); 2.7 s (3H, C—CH_3); 3.35 s (3H, NCH_3); 3.50 s (3H, NCH_3); 4.20 q (2H, CH_2CH_3); 6.64 s (1H, 2'H), 8.26 s (1H, 3H). The mother liquor from the reaction mixture was diluted with water (1:1), and the resulting precipitate was filtered off to give 0.082 g (74%) of V. The mother liquor after removal of V and XI was treated with a solution of 0.056 g (0.4 mmole) of 4-chlorobenzaldehyde in 43 ml of ethanol to give 0.089 g (77%) of compound VIII.

EXPERIMENTAL (BIOLOGICAL)

As was shown earlier [1], the hydrazinouracils IV and VII possess antiinflammatory and antimicrobial properties. In this connection, interest was aroused to test for the presence of antiinflammatory, analgesic, and antimicrobial activity in the new derivatives of hydrazinouracils prepared in the present work.

The acute toxicity (LD_{50}) and the analgetic activity of the preparations was studied on tetrahybrid mice weighing 18-20 g. The toxicity was determined by a single peroral dose and observation of the death of the animals over five days.

The study materials were introduced in the form of a suspension in 2% starch gel in a dose of 1/5 to 1/10 of the LD_{50}.

The analgesic activity was determined by the hot plate method.

The anti-inflammatory activity was studied in white Wistar rats weighing 180-220 g by means of the carrageenin inflammation. The magnitude of the inflammatory reaction was determined oncometrically by methods analogous to [1].

The antimicrobial action of the compounds was studied by the double serial dilution method [2] with the bacteria *Staph. aureus* (strain 209) and *E. coli* (strain M17).

The studies showed that compounds I (LD_{50} = 50 mg/kg), V (LD_{50} = 400 mg/kg), and VI (LD_{50} = 500 mg/kg), exhibited only mild analgetic action; less than that of amidopirin (aminopyrine).

Compounds V, IX (LD_{50} = 200 mg/kg), and XI (LD_{50} = 400 mg/kg) showed antiinflammatory properties. The most active was hydrazone V, which inhibited the carrageenin edema by 36 and 34%, after 3 and 4 h, respectively. Compound XI inhibited the edema by 17 and 24%, and derivative IX, by 14 and 13%.

The highest antimicrobial activity was shown by the hydrazone VI. The minimum bacteriostatic concentration (MBSC) of this compound against *E. coli* was 7.8 μg/ml, and against *Staph. aureus*, it was 62.5 μg/ml. The bactericidal concentrations (MBC) for this compound were 31.2 and 125 μg/ml against *E. coli* and *Staph. aureus*, respectively. Hydrazone V suppressed the growth of the coli bacillus at a concentration of 500 μg/ml, and *Staph. aureus* at 1000 μg/ml. This same concentration was bactericidal to *E. coli*. The MBSC and MBC of compound IX against cultures of *E. coli* were 62.5 and 125 μg/ml, respectively. Against *Staph. aureus*, compound IX showed only bactericidal activity (MBC = 1000 μg/ml).

These studies confirm the expediency of a search for biological active compounds in the hydrazine derivatives of uracil. In addition, this work establishes the usefulness of a study of the biological properties of pyrimido-as-triazine, which, as shown in the earlier work, can transform into hydrazinouracils.

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SYNTHESIS AND PHARMACOLOGICAL ACTIVITY OF DERIVATIVES OF 2,4-DICHLORO-5-SULFAMOYLBENZOIC ACID

A. A. Lebedev, L. I. Mironova, M. G. Pleshakov, A. K. Matveeva, and I. A. Timokhina

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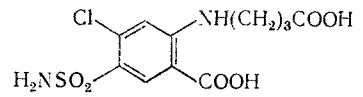
Furosemide [4-chloro-N-(2-furylmethyl-5-sulfamoylanthranilic acid] is a highly active diuretic which is used extensively in medicine in various types of edema, and for the treatment of cardiovascular conditions [3].

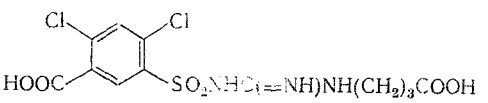
Furosemide is synthesized by condensing 2,4-dichloro-5-sulfamoylbenzoic acid (I) with furfurylamine [1].

We have synthesized 1-, 2-, and 5-substituted 2,4-dichloro-5-sulfamoylbenzoic acids in order to examine the dependence of diuretic activity on the type of substituent.

Compounds (II) and (III) have been obtained previously; they were obtained from γ-aminobutyric acid (GABA) and γ-guanidinobutyric acid, the second component being 2,4-dichloro-5-sulfamoylbenzoic and 2,4-dichloro-5-chlorosulfonylbenzoic acids [2].



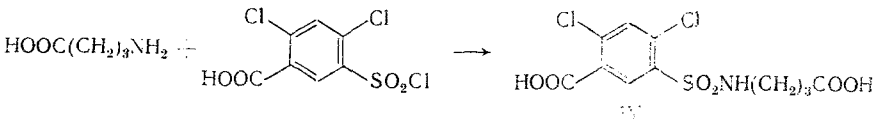
II



III

In (III), the amino-group was modified by replacement by the guanidino-group, which itself possesses many unique chemical and biological properties.

Compound (IV) was obtained by reacting 2,4-dichloro-5-chlorosulfonylbenzoic acid with GABA.



Compound (V) is the furfurylamine salt of (I), and (VI) was obtained by reacting (I) with glycine.

D. I. Ul'yanov Kuibishev Institute of Medicine, and Affiliated Branch of the S. Ordzhonikidze All-Union Research Institute for Pharmaceutical Chemistry, Moscow Region.

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