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SYNTHESIS OF CERTAIN DERIVATIVES OF A NEW HETEROCYCLIC SYSTEM — PYRIMIDO[3,4-b]-1,2,4-TRIAZINE — AND THEIR ANTITUMORIGENIC ACTIVITY

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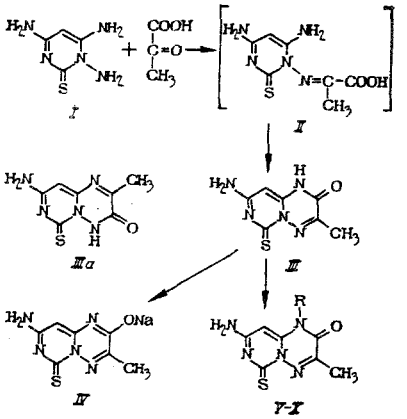
In the course of a search for biologically active compounds, we studied the reaction of 1,4,6-triamino-2(1H)pyrimidine-thione (I) [4] with pyruvic acid, and synthesized the derivatives of a new pyrimido[3,4-b]-1,2,4-triazine heterocyclic system (III–X), isomeric with the biologically important pteridine.

The reaction was carried out in 30% alcohol and 5% acetic acid, and the ratio of the pyrimidine-thione/pyruvic acid reagents was 1:3. In aqueous alcohol, the reaction took 12 h, while in acetic acid, it was complete in 2 h. The TLC, IR, PMR and mass spectroscopy data showed that the product of this reaction (III) was isolated in individual state and had the same structure, irrespective of the solvent used. We did not observe the formation of a mixture of compounds with an isomeric structure (III and IIIa)



R = Me (V), Et (VI), Pr (VII), Bu (VIII), CH_2Ph (IX), $CH_2C_6H_4NO_2$-*n* (X).

According to the literature data, the reaction of diaminopyrimidines with pyruvic acid can proceed in two directions, depending on the character of the medium. In a strongly acid medium, the reaction is of an acylation type, while in a weakly acid medium, the condensation of the more basic amino group of pyrimidine with the keto group of the acid predominates, followed by closure into the isomeric pteridine [1].

Taking into account the literature data, the results of our quantum-chemical calculations for compound I, carried out by the CNDO method [3], according to which the N-amino group is more basic than the amino at the 6-position, and also the character of the medium

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TABLE 1. Derivatives of Pyrimido[3,4-b]-1,2,4-triazine

Compound	Yield, %	Found, %			Empirical formula	Calculated, %		
		N	C	H		N	C	H
III	99,0	33,8	40,6	3,4	$C_7H_7N_5SO$	33,5	40,2	3,4
IV	88,5	30,1	36,5	2,7	$C_7H_6N_5SONa$	30,3	36,4	2,6
V	78,0	31,1	43,2	3,9	$C_8H_9N_5SO$	31,4	43,0	4,0
VI	73,1	29,8	45,4	4,5	$C_9H_{11}N_5SO$	29,5	45,6	4,6
VII	81,2	27,8	47,7	5,2	$C_{10}H_{13}N_5SO$	27,9	47,8	5,2
VIII	79,6	26,2	49,9	5,8	$C_{11}H_{15}N_5SO$	26,4	49,8	5,7
IX	97,1	23,6	56,4	4,4	$C_{14}H_{13}N_5SO$	23,4	56,2	4,4
X	95,0	24,5	48,9	3,7	$C_{14}H_{13}N_6SO_3$	24,4	48,8	3,9

Note. Melting point of compounds III-X ~ 300°C.

(weakly acid or neutral), it can be assumed that of the two possible reaction products with an isomeric structure, only one of them is formed. Thus, the reaction probably proceeds through an intermediate compound (II) followed by closure in [I]. The presence of a carbonyl absorption band at 1735 cm^{-1} in the IR spectrum of compound [I] indicates its oxo structure. In the PMR spectrum singlet signals are observed of the 2-CH_3 (2.6 ppm) and 6-NH_2 groups (7.55 ppm), as well as the 5-H proton signal (6.15 ppm). The mass spectrum contains a molecular ion peak with m/z 209; the main direction of the fragmentation is characterized by the elimination of the $NCCH_3$ particle (168).

Compound [III] is a light yellow crystalline high-melting material, which is soluble in alkaline and DMFA and is insoluble in water and most organic solvents. To increase its solubility for biological investigations, a water-soluble salt (IV) was prepared. To carry out a primary biological screening, we studied the characteristic features of the chemical behavior of compound III in the reaction with alkyl and aryl(alkyl) halides and synthesized 4-alkyl- and aralkyl-substituted derivatives V-X. Pyrimidotriazine III was reacted with methyl iodide, benzyl chloride, and p-nitrobenzyl bromide in an alkaline medium at room temperature to form products V, IX, X, respectively, whose structure was confirmed by elemental analysis and spectroscopic data. There is a carbonyl absorption band at 1680 cm^{-1} in the IR spectra of the compounds. In the PMR spectrum of the methylation product V, besides the pyrimidine proton signal (δ 6.25 ppm), there are also proton signals of two methyl groups (δ 2.57 and 2.65 ppm), one of which belongs to the 2-CH, group, and the other indicates the presence of an N-CH_3 group in the molecule [2]. In the PMR spectra of compounds IX, X, signals are observed of 5-H protons (δ 6.32 and 6.45 ppm), aromatic protons in the 7.45 (IX) and 8.15 (X) ppm regions, methyl protons (δ 2.55 and 2.7 ppm) and methylene group protons (δ 4.5 and 4.75 ppm).

The mass spectra of the compounds are characterized by molecular ion peaks with m/z 223 (V), 299 (IX), 344 (X), of the fragments $[M - CH_3]^+$ (208) (V), $[M - CH_2C_6H_5]^+$ (208) (IX), $[M - CH_2C_6H_4NO_2]^+$ (208) (X). The fragmentation of the molecular ions, as in III, involves the elimination of the $NCCH_3$ particle (182) (V), (258) (IX), (303) (X).

The synthesis of alkyl derivatives VI-VIII was carried out by reacting compound III with ethyl or propyl iodides, and butyl bromide in DMFA in the presence of potassium carbonate at 90°C.

EXPERIMENTAL

The IR spectra were recorded on a UR-20 spectrophotometer (KBr tablets), PMR spectra in CF_3COOH on a "Perkin-Elmer" spectrometer (60 MHz), using TMS as internal standards. The mass spectra were run on a "Varian MAT-112" mass spectrometer at a 70-eV ionizing voltage with direct introduction of the sample into the source; the temperature of the ionization chamber was 180°C. The individual state of the compounds synthesized was controlled by TLC on Silufol UV-254 plates in the systems n-propanol—0.2 N ammonia (3:1). R_{f_1} and ethanol—water—AcOH—AcONa (8:6:2:1), R_{f_2}.

The characterisitcs of the compounds synthesized are given in Table 1.

2-Methyl-3-oxo(4H)-6-amino-8-thioxopyrimido[3,4-b]- 1,2,4-triazine (III). A 0.5-g portion (3.2 mmole) of pyrimidine I is dissolved with heating in 100 ml of 30% alcohol, 0.7 ml (8 mmoles) of pyruvic acid are added, and the mixture is heated on a water bath for 12 h. The precipitate that separates on cooling, is filtered, purified by reprecipitation with acetic acid from 2 N NaOH, and dried in vacuo. The filtrate is concentrated and an additional

amount of material is isolated, which according to the TLC data, physicochemical and spectral characteristics is identical with the previously precipitated compound. R_{f1} 0.64, R_{f2} 0.89.

B. A 2-g portion (14.2 mmoles) of pyrimidine I is dissolved with heating in 200 ml of 5% AcOH, 3.3 ml (37.5 mmoles) of pyruvic acid are added, and the mixture is boiled for 2 h. The reaction product is isolated and purified as in the preceding method.

Sodium Salt of 2-Methyl-3-hydroxy-6-amino-8-thioxopyrimido-[3,4-b]-1,2,4-triazine (IV). A 0.54-g portion (2.6 mmoles) of compound III is dissolved with heating in 20 ml of 2 N NaOH. The precipitate that separates from the solution is filtered and crystallized from alcohol, R_{f1} 0.59.

2,4-Dimethyl-3-oxo-6-amino-8-thioxopyrimido[3,4-b]-1,2,4-triazine (V). A 0.3-g portion (1.4 mmole) of compound III is dissolved with heating in 50 ml of 2 N NaOH. The solution is cooled to room temperature, 3 ml (21.1 mmoles) of MeI are added, and the mixture is stirred for 2 h. The light-yellow precipitate is filtered and crystallized from DMFA. R_{f2} 0.79.

2-Methyl-3-oxo-4-ethyl-6-amino-8-thioxopyrimido[3,4-b]-1,2,4-triazine (VI). A suspension of compound III, EtI, and potassium carbonate (molar ratio 1:1, 1:1:1.5) in DMFA is heated on a water bath for 5 h. The mixture is filtered hot, and the precipitate that separates from the solution on cooling is filtered and crystallized from DMFA, R_{f1} 0.67.

2-Methyl-3-oxo-4-propyl-6-amino-8-thioxopyrimido[3,4-b]-1,2,4-triazine (VII) is obtained in a similar way as compound VI 0.83.

2-Methyl-3-oxo-4-butyl-6-amino-8-thioxopyrimido[3,4-b]-1,2,4-triazine(VIII) is obtained in a similar way as compound VI. R_{f1} 0.8.

2-Methyl-3-oxo-4-benzyl-6-amino-8-thioxopyrimido[3,4-b]-1,2,4-triazine (IX) is obtained in a similar way as compound V from 0.2 g of III and 0.24 ml of $PhCH_2Cl$. The product is purified by crystallization from DMFA, R_{f1} 0.64.

2-Methyl-3-oxo-4-p-nitrobenzyl-6-amino-8-thioxopyrimido[3,4-b]-1,2,4-triazine (X). A 0.52-g portion (2.39 mmoles) of p-nitrobenzyl bromide in a mixture of 50 ml of diethyl ether and 25 ml of ethanol is added to a solution of 0.5 g (2.39 mmoles) of compound III in 30 ml of 2 N NaOH. The mixture is stirred at room temperature for 4 h, the precipitate is filtered and crystallized from DMFA R_{f1} 0.78.

EXPERIMENTAL BIOLOGICAL

The antitumorignic activity of compounds III-X was studied by a method proposed at the All Union Oncologic Scientific Center of the Academy of Medical Sciences of the USSR, on line $C57Bl_6$ mice and nonpedigree mice. A mammary gland adenocarcinoma Ca 755, an epidermoidal Lewis lung cancer, a leucosis La, sarcoma 37 and 180 were used as experimental models of tumors. The compounds were administered intraperitoneally in a starch paste daily for 5 days.

The experimental data showed that the compounds tested are slightly toxic (LD_{50} 750-1000 mg/kg) and have a moderate anticardinogenic activity with respect to sarcoma 37 (up to 64% inhibition of the tumor growth). The compounds studied did not inhibit the growth of other tumors. Compound [sic] stimulated the growth of Ca 755 and the Lewis lung cancer.

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