DERIVATIVES OF PYRROLO[3,2-d]PYRIMIDINES.

II. SYNTHESIS OF 4-SUBSTITUTED DERIVATIVES OF PYRROLO[3,2-d]PYRIMIDINE AND INVESTIGATION OF THEIR BIOLOGICAL ACTIVITY

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Among the 4-substituted derivatives of pyrrolo[3,2-d]pyrimidine with phenyl or methyl substitution in the 2 and 4 positions, one finds compounds which inhibit the growth of lactic acid bacteria and exhibit high tuberculostatic activity [1-3]. The present work concerns the synthesis of new derivatives of pyrrolo[3,2-d]pyrimidines and their biological activity.

Earlier we reported the preparation of the previously unknown 2-methyl-4-oxo-3,4-dihydropyrrolo[3,2-d]pyrimidine-7-aldehyde, and from it the nitrile (I) and the pyrrolo[3,2-d]-pyrimidine unsubstituted at position 7 (II). Nitrile I and pyrrolopyrimidine II were used in the present work for the preparation of pyrrolo[3,2-d]pyrimidines containing, as substituents in position 4, amino groups (Va-e and VIa-d) or sulfhydryl (Vf and VIe). To prepare these compounds, 4-oxopyrrolo[3,2-d]pyrimidines I and II were treated with phosphorus oxychloride, and the 4-chloro derivatives (III and IV) thus obtained, were treated with nucleophilic reagents, such as amines or sodium hydrosulfide. It was observed that the presence of the nitrile group in the 7 position increased the reactivity of the chlorine, compared to the pyrrolopyrimidine unsubstituted at the 7 position. The chloroderivative III reacts with amines in boiling ethanol to form the amino derivatives Va-c. III reacts with aniline hydrochloride or with glycine methyl ester in water to form Vd or Ve. Under analogous conditions the chlorine atom in compound IV is not replaced by an amine residue. The reaction proceeds only in boiling butanol.

It should be noted that, in the reaction of IV with amines, the corresponding amine product was isolated as the free base only in the case of piperidine. When the other three amines were employed, the products were recovered from the reaction in the corresponding amine hydrochloride form. The yields, properties, and elemental analyses are given in Table 1.

The synthesized compounds were investigated for antibacterial activity *in vitro* and for antitumor activity *in vivo*. Antibacterial activity was studied on synthetic and semi-synthetic media with regard to the lactic acid bacterium *Lactobacillus casei* 7469; the human bacillus, strain H 37 Rv, and *Escherichia coli*, strain 335. It was shown that substituted pyrrolo[3,2-d]- pyrimidines VIa-e inhibit the growth of lactic acid bacteria much less than the previously studied pyrrolo[3,2-d]pyrimidines containing phenyl residues at position 6 [1-3]. Derivatives of pyrrolo[3,2-d]pyrimidine, with a C≡N group in the 7 position retain their tuberculostatic effects (compounds Va, Vc, Vd) but the bacteriostatic properties against lactic acid bacteria are reduced or entirely lost, when compared with the previously studied pyrrolo[3,2-d]pyrimidines [1-3].

We conducted *in vivo* experiments on mice with sarcoma 180, using 400 animals, to study the antiblastic properties and toxicity of derivatives Va, Vb, Vd, Ve, and Vf and VId, on repeated dosage. The experimental substances were introduced *per os* as vegetable oil suspensions (for Va, Vb, Vd, Ve, and Vf), or intraperitoneally in distilled water (for VId), daily for 6-9 days, beginning with the day of the tumor inoculation. The experiments lasted between 11 and 13 days. It was shown that compounds Vb and Ve in tolerable doses inhibit the growth of sarcoma 180 in mice by 30-60%. The other derivatives (Va, Vd, Vf, and VId) have no effect on the growth of these tumors. The toxicity of derivatives of pyrrolo[3,2-d]pyrimidine depends on the structure of the substance and on the mode of administration. Thus, compounds Va, Vd, Ve, and Vf administered *per os* in vegetable oil were moderately toxic. Their maximum

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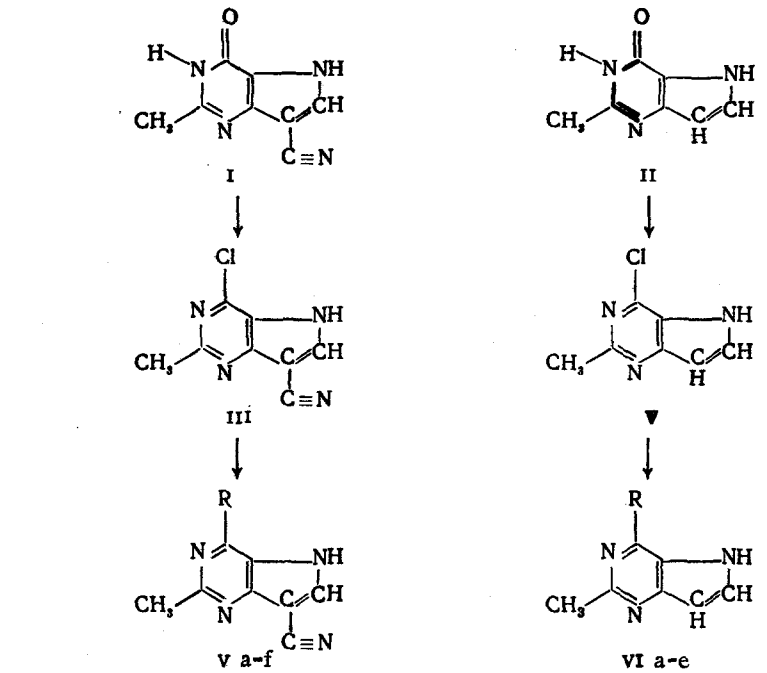

a R = $NHCH_2C_6H_5$
b R = $NHCH_2CH{=}CH_2$
c R = 2-$\Delta^{1,2}$- cyclohexenylethylamino
d R = NHC_6H_5
e R = $NHCH_2COOH$
f R = SH

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d R = $NHCH_2CH{=}CH_2$
e R = SH

tolerable doses (MTD), on daily administration for 7 days, varied between 300 mg/kg (for Vb) and 750 mg/kg (for Va). Derivative VId, similar in structure to Vb, but soluble in distilled water and administered intraperitoneally, was highly toxic, having an MTD of 5 mg/kg. On single dose administration, the MTD of VId was 50 mg/kg.

EXPERIMENTAL

IR spectra were obtained on a Perkin Elmer 457 apparatus, using the crystalline samples dispersed as a paste in a vaseline oil.

2-Methyl-4-chloro-7-cyanopyrrolo[3,2-d]pyrimidine (III). A mixture of 6.5 g of nitrile I and 180 ml of phosphorus oxychloride was heated and kept at a slow boil for 3 h after which the phosphorus oxychloride was distilled off under vacuum. The residue was treated with ice. Filtration yielded 5.2 g of III. IR spectrum: 2230 cm^{-1} (C≡N).

2-Methyl-4-chloropyrrolo[3,2-d]pyrimidine (IV). A mixture of 1 g of 2-methyl-4-hydroxypyrrolopyrimidine and 30 ml of phosphorus oxychloride was boiled for 5 h. The phosphorus oxychloride was then distilled off under vacuum, and the residue treated with ice. A 30% sodium hydroxide solution was added until the solution was neutral. Extraction with ethyl acetate and distillation of the ethyl acetate yielded 0.6 g of IV.

2-Methyl-4-benzylamino-7-cyanopyrrolo[3,2-d]pyrimidine (Va). A mixture of 1.5 g (7.7 mmoles) of III and 2.1 g (18.7 mmoles) of benzylamine in 40 ml of ethanol was boiled for 3 h, the solution cooled, and the precipitated product filtered off, yielding 0.7 g of Va.

2-Methyl-4-allylamino-7-cyanopyrrolo[3,2-d]pyrimidine (Vb). A mixture of 2 g (10.4 mmoles) of III and 2.6 g (45.5 mmoles) of allylamine in 45 ml of ethanol was heated for 3 h in a closed vessel at 80°C. Evaporation of the solution afforded 1.2 g of Vb.

2-Methyl-4-cyclohexenylethylamino-7-cyanopyrrolo[3,2-d]pyrimidine (Vc). A mixture of 1 g (5.2 mmoles) of III and 1.5 g (12 mmoles) of 2-$\Delta^{1,2}$-cyclohexenylethylamine in 30 ml of ethanol was heated for 3 h. After evaporation of the solution the residue was treated with a 5% solution of acetic acid. The crystalline precipitate was filtered off, yielding 0.5 g of Vc.

TABLE 1. 4-Substituted Pyrrolo[3,2-d]pyrimidines

Compound	Yield, %	Mp*, deg C	Found, %					Empirical formula	Calculated, %				
			C	H	Cl	N	S		C	H	Cl	N	S
III	70	275—7	50.1	2,5	18,0	29,0	—	$C_8H_5ClN_4$	49,9	2,6	18,4	29,1	—
IV	54	140—2	50,1	3,5	21,4	25,5	—	$C_7H_6ClN_3$	50,1	3,6	21,2	25,1	—
Va	34	>320	68,0	4,9	—	26,7	—	$C_{15}H_{13}N_3$	68,4	4,9	—	26,6	—
Vb	54	306—8	61,8	5,3	—	32,9	—	$C_{11}H_{11}N_5$	62,0	5,2	—	32,9	—
Vc	34	297—9	68,1	7,1	—	24,6	—	$C_{16}H_{19}N_5$	68,3	6,8	—	24,9	—
Vd	46	315—7	67,5	4,4	—	28,0	—	$C_{14}H_{11}N_5$	67,5	4,4	—	28,1	—
Ve	55	>300	51,5	3,9	—	30,5	—	$C_{10}H_9N_5O$	51,9	3,9	—	30,3	—
Vf	50	>300	50,3	3,2	—	29,2	16,8	$C_8H_6N_4S$	50,5	3,2	—	29,5	16,8
VIa	49	251—3	66,4	7,4	—	25,8	—	$C_{12}H_{16}N_4$	66,7	7,4	—	25,9	—
VIb	48	261—3	61,8	7,4	12,1	19,0	—	$C_{15}H_{20}N_4\times$ $\times$ HCl	61,5	7,2	12,1	19,1	—
VIc	45	247—9	61,1	5,6	12,7	20,6	—	$C_{14}H_{14}N_4\times$ $\times$ HCl	61,2	5,4	12,9	20,4	—
VId	45	214—6	63.8	6,4	—	29,5	—	$C_{10}H_{12}N_4$	63,8	6,4	—	29,8	—
VIe	56	297—9	50,7	4,5	—	25,4	18,9	$C_7H_7N_3S$	50,9	4,2	—	25,4	19,3

*Compounds III, VIb, VId, and VIe were crystallized from water; IV from ethyl acetate—petroleum ether (1:1); Va from dimethylformamide; Vb and Vc from aqueous alcohol; VIa from dichloroethane; and VIc from 1% alcoholic HCl.

2-Methyl-4-phenylamino-7-cyanopyrrolo[3,2-d]pyrimidine (Vd). A mixture of 1.5 g (7.7 mmoles) of III and 2.3 g (17.7 mmoles) of aniline hydrochloride in 50 ml of water was boiled for 4 h. The residue was filtered off, affording 0.9 g of Vd. Ve was obtained by an analogous procedure. The compounds were purified by reprecipitation from an aqueous alkaline solution by the addition of acetic acid.

2-Methyl-4-mercapto-7-cyanopyrrolo[3,2-d]pyrimidine (Vf). A mixture of 2 g (10.4 mmoles) of III and a 10% aqueous solution of sodium sulfide (18 mmoles) in 40 ml of ethanol was boiled for 3 h. The sodium salt of Vf was filtered off, dissolved in water, and acidified with acetic acid to precipitate 1 g of the mercaptan Vf.

2-Methyl-4-piperidinopyrrolo[3,2-d]pyrimidine (VIa). A mixture of 0.3 g (1.78 mmoles) of IV and 0.35 g (4.1 mmoles) of piperidine in 80 ml of butanol was boiled for 1 h. The solution was evaporated and the residue treated with water to yield 0.19 g of the amine VIa.

2-Methyl-4-cyclohexenylethylaminopyrrolo[3,2-d]pyrimidine Hydrochloride (VIb). A mixture of 0.3 g (1.78 mmoles) of IV and 0.56 g (4.5 mmoles) of 2-$\Delta^{1,2}$-cyclohexenylethylamine in 10 ml of butanol was boiled for 2 h. After evaporation the residue was extracted with ethyl acetate, yielding 0.25 g of the hydrochloride of VIb.

2-Methyl-4-benzylaminopyrrolo[3,2-d]pyrimidine Hydrochloride (VIc). A mixture of 0.6 g (3.56 mmoles) of IV and 0.96 g (9.0 mmoles) of benzylamine in 15 ml of butanol was boiled for 1 h. After evaporation the residue was treated with water, yielding 0.45 g of the hydrochloride of VIc.

2-Methyl-4-allylaminopyrrolo[3,2-d]pyrimidine (VId). A mixture of 1.8 g (10.7 mmoles) of IV and 3.4 g (59.5 mmoles) of allylamine in 50 ml of butanol was boiled for 5 h in a closed vessel at 130°C. The solution was evaporated, and the residue treated with ethyl acetate, yielding 0.9 g of the hydrochloride of VId, which was crystallized from ethyl acetate—alcohol (1:1). For conversion to the base, it was dissolved in 10 ml of water, and the solution neutralized with dry sodium bicarbonate. The precipitate of VId was filtered off.

2-Methyl-4-mercaptopyrrolo[3,2-d]pyrimidine (VIe). A mixture of 0.3 g (1.78 mmoles) of IV and 2 ml of a 10% aqueous solution of sodium sulfide (3.57 mmoles) in 7 ml of chloroform was boiled for 4 h. The precipitated sodium salt was filtered off, yielding 0.17 g of the mercaptan VIe.

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SYNTHESIS AND MUTAGENIC PROPERTIES OF 6-CHLOROMETHYL, CYANOMETHYL, AND β-AMINOETHYL DERIVATIVES OF 1,3,5-TRIAZINES

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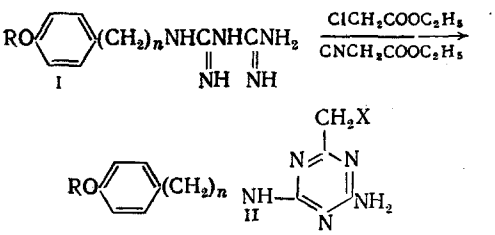
The available literature data show some interesting biological properties of symm-triazine derivatives. Some diamino-symm-triazines exhibit antipyretic, vasodilatory, antiviral, diuretic, and hypoglycemic activity [1-3].

We prepared some substituted diamino-symm-triazines based on biguanidines reported earlier [4, 5] in order to study their biological properties.

It is known that acidic reagents can cyclize biguanidines to 1,3,5-triazines. It was, therefore, of some interest to study the chemical behavior of the starting biguanidines (I) in the condensation reactions with chloroacetic and cyanoacetic esters.

For the cyclization of biguanidines with chloroacetic ester we took into account the fact that a similar reaction takes place in low-boiling alcohols with the use of alcoholates of alkaline metals [6]. This synthesis was accomplished in methanol in the presence of sodium methylate as catalyst according to the following scheme:

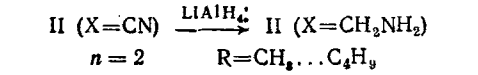


R=CH_3,..., iso-C_4H_9; $n = 1, 2$; X=Cl, CH.

The structure II (X = Cl) was confirmed by mass spectroscopy (see Fig. 1a). The spectrum of II where R = CH_3, n = 1, X = Cl shows an intensive peak of the molecular ion (M^+ 279) and ion peaks at 243, 161, 147, 136, 121, and 77.

Triazines II where X = CN were prepared by cyclizing biguanidine I with cyanoacetic ester in methanol on prolong keeping. The compounds (X = CN) are obtained as colorless stable crystals. The mass spectra of II where X = CN and R = CH_3, n = 1 and R = C_2H_5, and n = 2 showed peaks corresponding to the molecular ions together with the characteristic fragmentation pattern (see Fig. 1b, c).

The reduction of the cyano group in triazines II (X = CN) with lithium aluminum hydride in ether did not take place apparently due to a low solubility of the starting nitriles in absolute ether. Better results were obtained when tetrahydrofuran was used as a solvent.



Amines II were treated with an ether solution of hydrogen chloride to give the corresponding hydrochlorides. The hydrochlorides were hygroscopic. The mutagenic activity of triazines II was studied using biochemical mutants: *Escherichia coli* P678 for threonine, leucine, and

*Deceased.

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