SYNTHESIS AND ANTITUMOR PROPERTIES OF SOME N-2,5-DIMETHYLOXAZOLO[5,4-d]PYRIMIDYL-7-AMINO ACIDS

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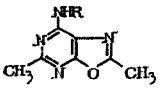
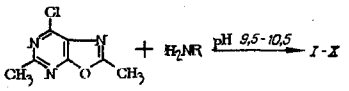
Because of the importance of N-(6-purinyl)-L-aspartic acid 5-phosphate in the biosynthesis of adenosine monophosphate (AMP) [6, 7, 9], we have undertaken the synthesis of a number of purinylamino acids with antitumor properties [8, 10].

As a part of this work, and also in connection with our work on the chemistry of oxazolo [5,4-d]pyrimidines [1-4], we have synthesized some oxo analogs of purinylamino acid, N-2,5-dimethyloxazolo[5,4-d]pyrimidyl-7-amino acids (I-X):



I-X

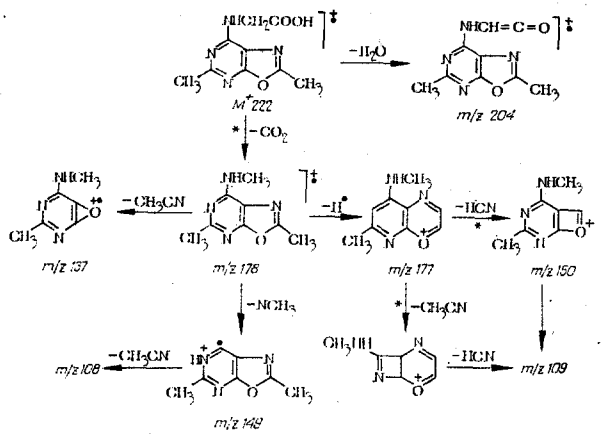
where R represents (I) glycine, (II) D-leucine, (III) L-leucine, (IV) D-α-alanine, (V) L-α-alanine, (VI) (D,L)-valine, (VII) sarcosine, (VIII) β-phenyl-β-alanine, (IX) γ-aminobutyric acid, and (X) L-proline.



Compounds I-X were prepared by the reaction of 2,5-dimethyl-7-chlorooxazolo[5,4-d]pyrimidine [1] with the corresponding amino acids in aqueous-alcoholic solution; the reaction was monitored by TLC and the pH value of the solution (9.5-10.5) was adjusted for each amino acid.

The compounds prepared were examined by chromatography and elemental analysis; structures were confirmed by mass spectrometry.

The mass spectra of compounds I-X contained moderately intense peaks due to molecular and fission ions, enabling the compounds to be identified unambiguously. This dissociative ionization of I-X is generally associated with decarboxylation, and, as shown in the example of compound I, proceeds in the same way as the decay of the molecular ions of 7-aminosubstituted 2,5-dimethyloxazolo[5,4-d]pyrimidines [4].



A possible route to the pyrimidylamino acid is shown for N-2,5-dimethyloxazolo[5,4-d]pyrimidyl-7-β-phenyl-β-alanine.

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TABLE 1. Properties of Compounds I-X

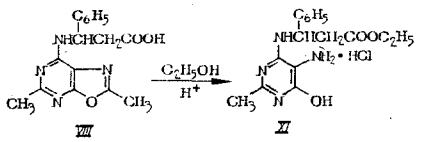
Compound	Yield, %	mp, °C	R_f	pH of reaction mixture	pH of acidified solution	$[\alpha]_{436}^{24*}$	Found, %			Empirical formula	Calc., %		
							C	H	N		C	H	N
I	45,1	250—251	0,49	9,5—9,7	3,5	—	48,46	5,07	25,06	$C_9H_{10}O_3N_4$	48,64	4,54	25,21
II	50,01	187—188	0,73	9,5	3,7	+5±0,6	56,6	6,20	20,27	$C_{13}H_{18}O_3N_4$	56,10	6,51	20,12
III	57,5	189—190	0,73	9,5	3,7	−16,0±0,6	56,30	7,20	19,88	$C_{13}H_{18}O_3N_4$	56,10	6,51	20,12
IV	30,02	165—166	0,65	9,5	3,5	−12,0±0,6	50,30	5,0	24,06	$C_{10}H_{12}O_3N_4$	50,84	5,11	23,71
V	42,1	164—165	0,62	9,5	3,5	+10,0±0,6	51,25	5,50	23,25	$C_{10}H_{12}O_3N_4$	50,84	5,11	23,71
VI	47,3	190—191	0,69	10,0	3,8	—	53,95	5,90	21,26	$C_{12}H_{16}O_3N_4$	54,53	6,09	21,20
VII	42,3	219—220	0,78	10,5	3,4	—	51,20	5,33	24,05	$C_{10}H_{12}O_3N_4$	50,84	5,11	23,71
VIII	32,1	170—171	0,79	9,5—10,0	4,5	—	61,45	5,72	18,50	$C_{16}H_{16}O_3N_4$	61,52	5,16	17,93
IX	16,3	174—175	0,72	9,7—10,0	4,0	—	52,55	6,01	21,75	$C_{11}H_{14}O_3N_4$	52,79	5,63	22,38
X†	68,3	184—185	0,57	9,8	4,0	+17,0±0,6	44,40	5,80	18,15	$C_{12}H_{15}O_3N_4Cl$	48,24	5,06	18,76

*c 1.0; ethanol.

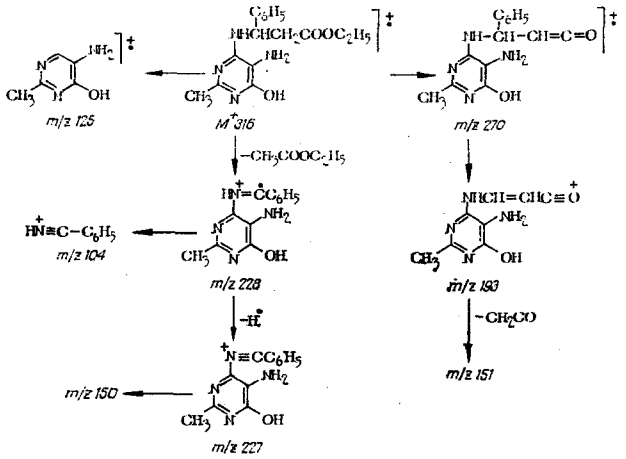
†Chlorine, calculated 11.86%, found 11.64%.

TABLE 2. Toxicity and Antitumor Activity of Compounds I-X

Compound	Toxicity for mice		Inhibition of tumor growth, T %				
			rats			mice	
	LD_{100}, mg/kg	MTD, mg/kg	dose, mg/kg	sarcoma 45	Pliss lympho-sarcoma	dose, mg/kg	sarcoma 180
I	2500	2000	150	29	0	250	0
II	1250	1000	80	49	35	125	27
III	750	500	60	32	0	75	0
IV	1500	1250	100	41	54,7	150	0
V	1500	1250	120	0	32	150	50
VI	1250	1000	80	42,6	0	100	23
VII	2500	2000	150	40	0	250	23
VIII	1000	750	60	0	0	75	30
IX	500	400	30	34,5	20	50	0
X	400	300	25	43,6	40,7	25	20



The structure of XI is determined by the method by which it is synthesized [3], and was confirmed by the mass-spectral decay of the molecular ion:



CHEMICAL EXPERIMENTAL

Mass spectra were obtained on an MX-1303 with direct introduction of the sample into the ionization chamber at a temperature of 40-50°C (below the melting point); energy of the ionizing electrons, 30 eV. Optical rotation was determined on a "Polamat A" polarimeter (Carl Zeiss, GDR). Chromatography was carried out on Silufol UV-254 plates in ether-butanol (10:1 for I and X; 12.5:1 for II, III, and VI), ether-butanol 8:1 for IV, V, VII-IX. Spots were developed with a UI-1 ultrachemoscope.

Preparation of Compounds I-III and VI-IX. To a mixture of 0.02 moles of the appropriate amino acid in 50 ml of water were added 10 ml of 2 N sodium hydroxide and a solution of 1.83 g (0.01 moles) of 2,5-dimethyl-7-chlorooxazolo[5,4-d]pyrimidine in 20 ml of ethyl alcohol. The mixture was heated for 2 hours at 70°C and pH 9.5-10.5 and 2 N sodium hydroxide then added dropwise. After cooling, the solution was extracted twice with chloroform (total 20 ml), and the aqueous layer separated and acidified to pH 2.5-3.8 with formic acid. The crystals which separated were filtered off, washed with water, and dried at 100°C for 48 hours (Table 1).

Compounds IV, V, and X were prepared in the same way from 0.02 moles of amino acid, 0.01 moles of sodium carbonate, and 0.01 moles of 2,5-dimethyl-7-chlorooxazolo[5,4-d]pyrimidine. The acidified aqueous solution was extracted with chloroform, the chloroform evaporated, ether added and the crystals which formed filtered off. In the case of compound X, the product was an oil and was therefore characterized as the hydrochloride (see Table 1).

Ethyl Ester of N-5-Amino-2-methyl-4-hydroxypyrimidyl-6-phenyl-β-alanine Hydrochloride. A mixture of 0.5 g (0.001 moles) of VIII, 1 ml of a concentrated solution of hydrochloric acid, and 20 ml of ethyl alcohol was refluxed for 3 hours. The alcohol was evaporated off, and the residue recrystallized. The crystals were washed with ether and dried. Yield, 0.3 g (56.2%), mp above 280°C, R_f 0.67 (in alcohol–water 2:1); M^+ (mass spectometer) 316.

BIOLOGICAL EXPERIMENTAL

The antitumor activity and toxicity of compounds I-X were studied by standard methods [5] using white non-pedigree mice and rats of both sexes weighing 18-20 g and 90-110 g respectively; a total of 250 mice and 200 rats were used. The acute toxicity in mice was determined from a single intraperitoneal injection. Compounds which were insoluble in water were injected into the animals as suspensions in 0.5% carboxymethylcellulose solution. For each substance the absolute lethal dose (LD_{100}) and the maximum tolerable dose (MTD) were determined.

Chemotherapeutic experiments were conducted on rats and mice with the following transplanted tumors — sarcoma 45, Pliss lymphosarcoma, and 180 sarcoma. Compounds were injected into the animals intraperitoneally in doses 1/10-1/15 of the LD_{100}.

The results of the study of toxicity and antitumor activity are given in Table 2. Of the tested substances, the most toxic were compounds containing γ-aminobutyric acid (IX), proline (X), and L-leucine (III) in their structure; the LD_{100} of these compounds was 400-750 mg/kg. Compounds III and I, containing sarcosine and glycine, were considerably less toxic (LD_{100} 2500 mg/kg); the rest of the compounds were intermediate in toxicity.

In chemotherapeutic experiments, it was established that the majority of compounds possessed moderate antitumor activity against sarcoma 45, retarding growth by 32-49%. Compounds containing β-phenyl-β-alanine (VIII), L-α-alanine (V), and glycine (I), were ineffective. Only compounds II, IV, V, and X exhibited significant antitumor activity towards Pliss lymphosarcoma (retardation of growth, 32-55%). Sarcoma 180 was found to be relatively resistant to the therapeutic action of the test compounds; only compounds containing L-α-alanine (V) and β-phenyl-β-alanine (VIII) groups showed some growth-inhibiting activity (30-50%).

Thus, some compounds in the oxazolo[5,4-d]pyrimidyl-7-amino acid series, in addition to their low toxicity, possessed significant activity against transplanted tumors; further research in this area is therefore recommended.

LITERATURE CITED

1. R. G. Melik-Ogandzhanyan and T. A. Khachatryan, A. p. 810699 (USSR). Otkrytiya, No. 9 (1981).
2. R. G. Melik-Ogandzhanyan, T. A. Khachatryan, Zh. S. Manukyan, et al., Arm. Khim. Zh., 34, No. 4, 324-327 (1981).
3. R. G. Malik-Ogandzhanyan, T. A. Khachatryan, V. S. Mirzoyan, et al., Khimiya Geterotsikl. Soedin., No. 1, 112-114 (1983).
4. R. G. Mirzoyan, K. A. Basentsyan, P. B. Terent'ev, et al., ibid., No. 9, 1255-1258 (1982).
5. V. A. Chernov, Methods of Experimental Chemotherapy, Moscow (1971), pp. 357-403.
6. R. Abrams and M. Bentley, J. Amer. Chem. Soc., 77, 4179-4186 (1955).
7. E. Carter and L. H. Cohen, J. Biol. Chem., 222, 17-30 (1956).
8. C. E. Carter, ibid., 223, pp. 139-254.
9. I. Lieberman, J. Amer. Chem. Soc., 78, 251-254 (1956).
10. D. N. Ward, J. Wade, E. F. Walborg, et al., J. Org. Chem., 26, 5000-5005 (1961).

SYNTHESIS AND BIOLOGICAL ACTIVITY OF DERIVATIVES OF 4,6-DIAZATRYPTOPHAN AND 4,6-DIAZAHETEROAUXIN

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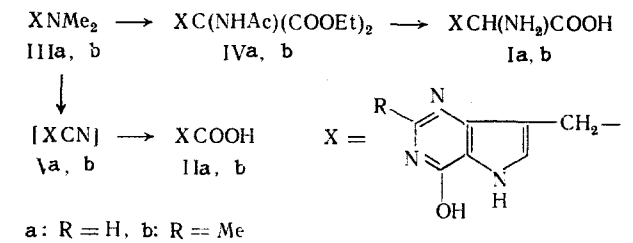
Pyrrolo(3,2-4)pyrimidines (9-desazapurines) have attracted attention for study because of the discovery of anti-tumor activity in some compounds of this series [4]. In continuing studies in a search for a substance with anti-tumor activity, the present work describes the synthesis of diaza-analogs of tryptophan (Ia and b) and heteroauxin (IIa and b) starting from our previously-described 2-R-4-hydroxy-7-dimethylaminomethylpyrrolo(3,2-d)pyrimidine hydrochlorides (IIIa and b) [3] according to the scheme:



a: R = H, b: R = Me

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