SYNTHESIS AND ANTITUMOR ACTIVITY OF AMINOPHOSPHONIC ACIDS

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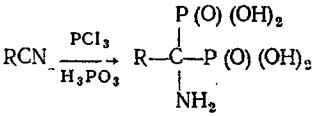
Aminophosphonic acids were first synthesized by M. I. Kabachnik and T. Ya. Medved' as structural analogs of natural amino acids [1, 2] while recently some aminophosphonic acids have been isolated from various mammalian and human tissues and identified [3, 4]. Moreover, aminophosphonic acids are known to have antimicrobial and antiviral activity [5, 6] and some have been prepared for use as pharmaceutical agents [7]. This has induced us to synthesize new representatives of aminophosphonic acids and examine their antitumor activity.

We prepared aminodiphosphonic acids from the nitriles by reaction with phosphorus trichloride in the presence of phosphoric acid followed by hydrolysis [7].

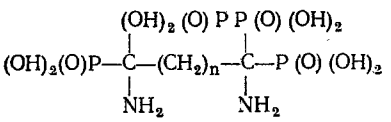


Changes in the published method affected temperature and time of standing. We prepared α-amino-β-(p-nitrophenyl)ethylidene-α,α-diphosphonic acid (I) by nitration of benzyl cyanide to p-nitrobenzyl cyanide [8] followed by phosphorylation in the same way. From cyanoacetamide we prepared α-amino-β-(carboxamide)ethylidene-α,α-diphosphonic acid (II).

Amino acids and peptides pass through cancer cells four or five times more easily than through normal cells [9]. Consequently we decided to synthesize aminophosphonic acids attached to certain peptides, namely glycylglycine, glycylalanine, glycylleucine, and alanylmethionine. We first prepared the cyanoethyl derivatives of these peptides following [10] (Table 1) and then converted the nitrile function to the aminodiphosphonic acid unit [compounds (III)-(IV)].

We synthesized an aminophosphonic acid containing three phosphonic acid groups by cyanoethylation of diethyl phosphite [11]. We prepared α-amino-γ-(diethylphosphono)propylidene-α, α-diphosphonic acid (VII) from γ-(diethylphosphono)propiononitrile by the general method.

We prepared diaminotetraphosphonic acids [12] of the general formula



from the dinitriles of malonic, succinic, glutaric, and adipic acids [compounds (VII)-(XI)].

Compounds (I)-(XI) are colorless, high-melting crystalline substances, soluble in water and insoluble in organic solvents. We verified their compositions and structures by elemental analysis (Table 2) and from their IR spectra, which show bands in the 1250 cm^{-1} region (P=O stretching) and in the 1030 cm^{-1} region [P(O)OH stretching] [13].

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TABLE 1. N-Cyanoethyl Derivatives of Peptides, RCH_2CH_2CN

R	Yield, %	Melting point, °C	Found, % N	Formula	Calculated, % N	R_f	Literature
Glycylglycine	78,5	144	22,71	$C_7H_{11}N_3O_3$	22,68	—	[10]
Glycylalanine	51,6	135—6	21,00	$C_8H_{13}N_3O_3$	21,1	0,12*	—
Glycylleucine	83,5	168	17,59	$C_{11}H_{19}N_3O_3$	17,46	0,29*	—
Alanylmethionine	89,8	205	15,61	$C_{11}H_{21}N_3O_4S$	15,40	0.23†	—

*System benzene—acetic acid—water 4:1:2.
†System benzene—acetic acid—water 4:1:5.

We tested all the synthetic compounds for antitumor activity, compound (V) *in vitro* and the others *in vivo*. Compound (V) showed some inhibition of nucleic acid synthesis in a culture of lympholeucosis NK/LY (100 μg/ml), Ehrlich ascites tumor (> 100 μg/ml), and Fischer tumor (> 100 μg/ml). We determined the toxicity of all the synthetic compounds. Compounds (II)-(XI) were relatively nontoxic (LD_{50} 300-500 mg/kg); only compound (I) had LD_{50} 70 mg/kg. The greatest antitumor potency was that of compound (VIII), which caused 52% inhibition of the growth of Lewis lung carcinoma and 80% inhibition of adenocarcinoma 755. Compounds (I)-(III) displayed weak antitumor activity, causing up to 30% inhibition of the growth of adenocarcinoma 755 and up to 47% inhibition of sarcoma 37. The other compounds were inactive.

EXPERIMENTAL BIOLOGICAL PART

We evaluated the antitumor activity in tests on inbred, noninbred, and hybrid mice with the following experimental tumors: adenocarcinoma 755, Lewis lung carcinoma, and sarcoma 37. Compounds were administered to animals intraperitoneally as solutions in water or $NaHCO_3$. We recorded the rate of growth of the tumor in the test mice (treated with the test compound) and in the control mice (intact), the general state of the animals, and the times of death, and made macroscopic examinations of the internal organs. Toxicity was determined on a single intraperitoneal administration.

EXPERIMENTAL CHEMICAL PART

The IR spectra were recorded on a UR-20 instrument in potassium bromide tablets. Chromatography was carried out on Silufol UV-254 plates.

α-Amino-β-(p-nitrophenyl)ethylidene-α,α-diphosphonic Acid Dihydrate (I). A mixture of p-nitrobenzyl cyanide (5 g, 0.03 mole), phosphorus trichloride (14.105 g, 0.1 mole), and H_3PO_3 (12.5 g, 0.15 mole) was stirred at room temperature for 3 h, at 60°C for 6 h, and at 100°C for 6 h. The reaction mixture was cooled with ice, and cold water (50 ml) was added. The oily precipitate was filtered off. The filtrate was treated with activated charcoal and, after filtration, poured into acetone (200 ml). The resulting precipitate was filtered off and dried over phosphorus pentoxide to give colorless crystals, mp 224°C (decomposition).

α-Amino-β-(carboxamido)ethylidene-α,α-diphosphonic acid (II) was prepared in the same way, except that the reaction mixture was kept at 60°C for 16 h.

N-(α-Amino-α,α-diphosphonopropyl)glycylglycine (III). A mixture of N-(cyanoethyl)glycylglycine (3.7 g, 0.02 mole), phosphorus trichloride (6.95 g, 0.05 mole), and H_3PO_3 (13.5 g, 0.15 mole) was stirred at room temperature for 3 h and at 60°C for 12-14 h. The reaction mixture was cooled with ice and water (50 ml) was added. The oily precipitate was filtered off. Acetone (200 ml) was added to the filtrate and the resulting precipitate was filtered off and dried over phosphorus pentoxide. The yield was 6.7 g (88.3%).

N-(α-Amino-α,α-diphosphonopropyl)glycylalanine dihydrate (IV), N-(α-amino-α,α-diphosphonopropyl)glycylleucine dihydrate (V), and N-(α-amino-α,α-diphosphonopropyl)alanylmethionine dihydrate (VI) were prepared in the same way.

α-Amino-γ-(diethylphosphono)propylidene-α,α-diphosphonic Acid Dihydrate (VII). A mixture of β-(ethylphosphono)propiononitrile (5 g, 0.03 mole), H_3PO_3 (13.5 g, 0.165 mole) and phosphorus trichloride (13.9 g, 0.09 mole) was kept at room temperature for 3 h and heated at 55-60°C for 8 h. After cooling with ice, absolute ethyl alcohol (100 ml) was added. The mixture was filtered and the filtrate was poured into acetone (200 ml). The precipitate was filtered off and dried over phosphorus pentoxide.

TABLE 2. Aminophosphonic Acids (I)-(VII), $R-C(NH_2)[P(O)(OH)_2]_2$

Compound	R	Yield, %	Melting point, °C	Found, %		Formula	Calculated, %	
				N	P		N	P
I	$4\text{-}NO_2—C_6H_4—CH_2—$	34,5	224 (decomp.)	8,0	17,81	$C_8H_{12}N_2O_8P_2\cdot 2H_2O$	7,73	17,12
II	$CONH_2—CH_2—$	36,5	>300	10,00	21,44	$C_3H_{10}N_2O_7P_2\cdot 2H_2O$	9,86	21,82
III	$HOOC—CH_2—NHCO—CH_2—NH—CH_2—CH_2—CH_3$	88,3	263—4	9,87	16,3	$C_7H_{17}N_3O_9P_2\cdot 2H_2O$	10,01	16,09
IV	$COOH—CH(COOH)—NHCO—CH_2—NH—CH_2—CH_2—$	47,0	247—9	10,42	15,46	$C_8H_{19}N_3O_9P_2\cdot 2H_2O$	10,52	15,52
V	$(CH_3)_8CH—CH_2—CH(CONH—CH_2—NH—CH_2—CH_2—)$	54,5	258—9	8,92	14,5	$C_{11}H_{25}N_3O_9P_2\cdot 2H_2O$	9,02	14,04
VI	$HOOC—CH(CH_2—CH_2—S—CH_3)—NHCO—CH(CH)_3—NH—CH_2—CH_2—$	39,0	268—9	8,54	13,67	$C_{11}H_{25}N_3O_9SP_2\cdot 2H_2O$	8,87	13,09
VII	$(C_2H_5O)_2—P(O)CH_2—CH_2—$	19,4	210	4,03	24,76	$C_7H_{20}NO_9P_3\cdot H_2O$	3,75	24,76

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