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SYNTHESIS AND RADIOPROTECTIVE PROPERTIES OF AMIDES OF PHOSPHORIC AND THIOPHOSPHORIC ACIDS

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It is known that many nitrogen-containing compounds, including amines, have radioprotective properties [3, 5]. High radioprotective activity (RPA) has been observed for thiophosphoric acid amide [6]. In the present research we have accomplished the synthesis and studied the RPA of a number of aliphatic amides of phosphoric and thiophosphoric acids.

We have previously obtained some amides of thiophosphoric acid by the alkaline hydrolysis of amidothiophosphoric acid dichlorides. This method of synthesis was limited by the low stability of the final compounds, which are readily hydrolyzed with cleavage of the P—N bond. In particular, the unstable phosphoric acid amides cannot be obtained. The silylation of phosphates and phosphonates with subsequent removal of the silyl groups under mild conditions is often used to obtain labile derivatives of phosphoric and phosphonic acids. Trialkylhalosilanes are used as silylating agents [8, 11]. We have successfully used intermediate bis(silyl) ethers for the synthesis of difficult-to-obtain amides of phosphoric and thiophosphoric acids.

$$\underset{\text{Ia-b}}{(MeO)_2P(=X)NR_2} \longrightarrow \underset{\text{IIa-c}}{(Me_3SiO)_2P(=X)NR_2} \longrightarrow \underset{\text{IIIa-d}}{(NaO)_2P(=X)NR_2}$$

NR_2 = piperidine (Ia, IIa, IIc, IIIa, IIIc), cyclohexylamino (Ib, IIb, IIIb, d);

X = O (Ia, b, IIa, b, IIIa, b), S (IIc, IIIc, d).

For the silylation we used trimethylbromosilane in the case of phosphoric acid derivatives and trimethylchlorosilane in the presence of an equivalent amount of potassium iodide for thiophosphoric acid derivatives. The silylation was accomplished in absolute solvents at 20°C in the course of 24 h. The bis(silyl) ethers of alkylamidophosphoric and -thiophosphoric acids were distilled liquids that were readily hydrolyzed by air moisture. They were converted to disodium salts by the action of sodium methoxide.

TABLE 1. Bis(silyl) Ethers II and Disodium Salts III of Alkylamidophosphoric and -thiophosphoric Acids

Compound	Yield, %	bp, °C (mm)	Found, %				Empirical formula	Calc., %			
			C	H	N	P		C	H	N	P
IIa	81	86 (2)	42,0	9,08	4,9	9,8	$C_{11}H_{28}NO_3PSi_2$	42,7	9,06	4,5	10,0
IIb	52	122 (2)	45,0	9,32	4,5	10,1	$C_{12}H_{30}NO_3PSi_2$	44,6	9,29	4,3	9,6
IIc	65	92 (2)	39,9	8,51	4,2	9,5	$C_{11}H_{28}NO_2PSi_2$	40,6	8,62	4,3	9,5
IIIa	95	Melts with decomposition below 100 °C	28,3	4,55	6,3	15,0	$C_5H_{10}NO_3PNa_2$	28,7	4,78	6,7	14,8
IIIb	87	The same	32,0	5,22	6,0	14,5	$C_6H_{12}NO_3PNa_2$	32,3	5,38	6,3	13,9
IIIc	91	» »	26,4	4,16	6,7	13,2	$C_5H_{10}NO_2PSNa_2$	26,7	4,45	6,2	13,8

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TABLE 2. Radioprotective Effectiveness and Toxicity of the Disodium Salts of Alkylamidophosphoric and -thiophosphoric Acids, Cyclohexylamine and Its Salts, and Butyric Acid Cyclohexylamide

Compound	LD_{50}, mg/kg	Radioprotective activity		
		dose, mg/kg	no. of animals	
			total	% surviving
IIIa	400	100	15	6
		200	15	0
IIIb	300	100	15	40
		200	15	13
IIIc	430	200	15	33
IIId*	335	96	15	73
IIIg*	510	200	15	0
IV	129	45	10	10
V	506	209	10	0
VI	946	384	10	20
VII**	203	84	15	60
Control	—	—	110	0

*See [2] for the synthesis.
**See [10] for the synthesis.

The salts of amidothiophosphoric acids were crystalline compounds that were quite soluble in water and could be stored without decomposition at 20°C for a long time. At the same time, the salts of amidophosphoric acids decompose readily in air with splitting out of the corresponding amine.

In order to study the relationship between the structure of the substances and the RPA we obtained and tested cyclohexylamine (IV) and its hydrochloride (V) and phosphate (VI), as well as the cyclohexylamides of butyric (VII), phosphoric, and thiophosphoric (IIId) acids.

EXPERIMENTAL CHEMICAL

The yields, constants, and results of elementary analysis of the compounds obtained are presented in Table 1. All of the reactions were carried out in absolute solvents.

Bis(trimethylsilyl) Piperididophosphate (IIa). A 6.12-g (0.04 mole) sample of trimethylbromosilane [9] was added dropwise with stirring to a solution of 3.86 g (0.02 mole) of dimethyl piperididophosphate (Ia) [7] in 5 ml of methylene chloride. After 24 h at 20°C, the reaction mass was evaporated, and the residue was distilled in vacuo. The yield of IIa was 5 g.

Bis(trimethylsilyl) Cyclohexylamidophosphate (IIb). This compound was similarly obtained from 0.02 mole of dimethyl cyclohexylamidophosphate (Ib) [7]. The yield was 3.36 g.

Bis(trimethylsilyl) Piperididothiophosphate (IIc). A solution of 4.32 g (0.04 mole) of trimethylchlorosilane in 15 ml of acetonitrile was added dropwise at 5-10°C to a mixture of 4.18 g (0.02 mole) of dimethyl piperididothiophosphate (Ic) [1] and 6.64 g (0.04 mole) of calcined potassium iodide in 15 ml of acetonitrile, the temperature of the reaction mass was raised to 20°C, and the mixture was stirred at this temperature for 24 h. The precipitated potassium chloride [2.7 g (90%)] was removed by filtration, the solution was evaporated in vacuo, and the residue was distilled. The yield of IIc was 4.23 g.

General Method for Obtaining Disodium Salts of Amidophosphoric and Amidothiophosphoric Acids (IIIa-c). A 0.015-mole sample of the corresponding bis(silyl) ether II was added with stirring at 5-10°C to a solution of 0.69 g (0.03 mole) of sodium in 20 ml of methanol. The precipitated salt was removed by filtration, washed with ether, and dried in a vacuum desiccator.

EXPERIMENTAL (BIOLOGICAL)

The acute toxicity of the substances was investigated in white mongrel male mice with masses of 19-26 g and was determined by the method of Suslikov and co-workers [4]. The radioprotective effectiveness was evaluated on F_1 (CBA × C57Bl) hybrid mice with masses of 19-23 g. The preparations were administered to the animals in the form of aqueous solutions in a volume of 0.2 ml intraperitoneally for 15-20 min prior to irradiation. Irradiation was carried out with an IGUR gamma apparatus in a dose of 900 rad at a rate of 210 rad/min. The effectiveness of the compounds was judged from the survival rate of the animals on the 30th day after irradiation. The results of the experiments are presented in Table 2. All of the compounds have moderate and low acute toxicities.

The radioprotective activity of the preparations is due to the nature of both the amide and acid parts of the molecule. Cyclohexylamides IIIb, d and VII have marked RPA as compared with the piperidides (IIIa, c) and piperazide $(NaO)_2PSNC_4H_8NPS(ONa)_2$ (IIIe) of these acids. The blocking acidic group evidently controls the rate of liberation of the amine in vivo and its passage through the cell membrane. In our case the thiophosphoryl (IIId) and butyryl (VII) derivatives of cyclohexylamine were found to be most effective. Phosphoric acid cyclohexylamide (IIIb) was considerably less active, while the amine itself and its salts IV-VI did not display appreciable RPA.

Thus it was established that in some cases the acylation of amines increases their bioaccessibility and leads to compounds with pronounced RPA. In particular, butyric and thiophosphoric acid residues are promising blocking groupings; this evidently makes it possible to obtain substances with a spectrum of therapeutic activity that is broader than in the case of the substances presented in this paper. Because of the narrow spectrum of therapeutic activity of the latter, there is some doubt about the possibility of the practical utilization of III-IV.

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