THE SYNTHESIS AND RADIOPROTECTIVE ACTIVITY OF ACETAMIDINE THIOPHOSPHORIC ACID DERIVATIVES

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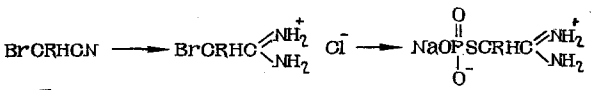
The radioprotective effect of certain N- and or S-substituted mercaptoacetamidines has recently been indicated [1]. In order to search for new radioprotective agents and to determine the connection between structure and effect, we achieved the synthesis of certain C-alkyl-(Ib-f) and N-adamantyl-(IIa-c) substituted 2-acetamidine thiophosphoric acids, and studied their radioprotective activity and toxicity.

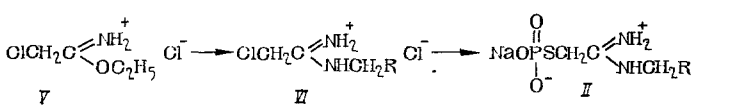
The synthesis of compounds I started from the 2-bromoalkylcyanides (III), which were treated with methanol in the presence of a catalytic amount of sodium methylate followed by ammonium chloride to give the corresponding 2-alkyl substituted 2-bromoacetamidine hydrochlorides (IVb-f). The thiophosphoric derivatives (I) were obtained by interaction of IV with an aqueous solution of sodium thiophosphate with subsequent precipitation of the monosodium salt from ethyl alcohol. Compounds obtained by this procedure frequently gave only one spot on thin-layer chromatography. If necessary, the products were repeatedly reprecipitated from aqueous alcohol; the sodium salt of 2-S-(1-amidinocapryl)thiophosphoric acid (Id) was purified by conversion into the free acid and then isolated in the form of the ammonium salt. The unsubstituted acetamidine thiophosphate (If) was prepared for comparison according to [1] from the hydrochloride of α-chloroacetamidine. The results are presented in Table 1.



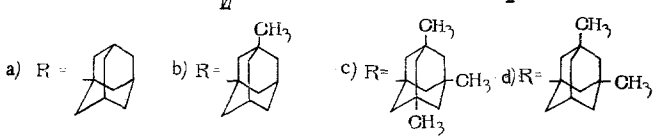
III IV I

b) R = CH_3, c) R = n-C_3H_7, d) R = iso-C_3H_7, e) R = n-C_4H_9, f) R = n-C_8H_{17}, a) R = H

It was indicated earlier that one of the most active radioprotectives of the series of mercaptoacetamidines is sodium 2-S-{N-[-(3,5-dimethyladamantyl)methyl]acetamidine} thiophosphate (IId). It seemed of interest to explore the influence of the adamantyl substituent and its methylated derivatives on radioprotective activity and toxicity. With this goal in mind, the sodium salt of the unsubstituted 2-S-[N-(1-adamantylmethyl)acetamidine] thiophosphoric acid (IIa), as well as its 3-methyl (IIb), and 3,5,7-trimethyl (IIc) derivatives were synthesized. Compounds IIa-c were synthesized by the action of the corresponding adamantylmethyl amine on ethyl 2-chloroacetimidate hydrochloride (V) with subsequent treatment of the resulting N-adamantylmethyl substituted chloroacetamidine hydrochloride (VI) with sodium thiophosphate. The properties of the compounds prepared are given in Table 2.



V VI II



a) R = b) R = c) R = d) R =

It is known that sterically hindered amines react only slowly with iminoesters [2]. Consequently, we undertook the activation of the amino group by means of silylation. For the interaction of the trimethylsilyl de-

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TABLE 1. Salts of 2-S-(1-amidinoalkyl)thiophosphoric Acid (I) and 2-Alkylsubstituted-2-bromoacetamidine Hydrochlorides (IV)

Compound	Yield, %	Melting point, °C	Found, %				Empirical formula	Calculated, %				R_f^*
			C	H	N	S		C	H	N	S	
Ib	87	134—6	12,8; 12,8	4,9; 5,1	10,0	11,9; 12,0	$C_3H_8N_2O_3PSNa \cdot 3,5H_2O$	13,39	5,61	10,41	11,92	0,65
Ic	79	115—6			10,0	11,6	$C_5H_{12}N_2O_3PSNa \cdot 2,5H_2O$†			10,03	11,48	
Id	44	121—3	21,9	6,1	10,3	11,5	$C_5H_{12}N_2O_3PSNa \cdot 2,5H_2O$‡	21,51	6,14	10,03	11,48	
Ie**	84	155—7	28,3; 28,6	7,8; 7,8			$C_6H_{16}N_3O_3PS \cdot 0,5H_2O$	28,56	7,59			0,64
If	95	105	37,5; 37,6	8,1; 8,2		9,6; 9,7	$C_{10}H_{22}N_2O_3PSNa \cdot H_2O$	37,26	7,51		9,95	0,69
IVb	91	125—8										
IVc	33	126—8			13,5		$C_5H_{12}ClBrN_2$			13,00		
IVd	62	127—30			13,4		$C_5H_{12}ClBrN_2$			13,00		
IVe	80	116—8										
IVf	80	126—8										

*In ethanol–29% ammonia–water (6 : 1 : 3)
† Water. Found, 15.8%. Calculated, 16.13%.
‡ Water. Found, 16.1%. Calculated, 16.13%.
**Ammonium salt.

TABLE 2. Salts of 2-S-{[N-(1-adamantyl)methyl]acetamidino} thiophosphoric Acid (II)

Compound	Yield, %	Melting point, °C (with decomposition)	Found, %			Empirical formula	Calculated, %		
			C	H	N		C	H	N
IIa	80	202—3	40,1	7,4	7,2	$C_{13}H_{22}N_2O_3PSNa \cdot 3H_2O$	39,59	7,16	7,10
IIb*	61	160—2	43,9	7,4	7,4	$C_{14}H_{24}N_2O_3PSNa \cdot 1,5H_2O$	44,09	7,14	7,35
IIb†	30	152—4	43,3	8,3	10,9	$C_{14}H_{28}N_3O_3PS \cdot 2H_2O$	43,62	8,35	10,90
IIc	70	172—7	44,9	7,7	7,0	$C_{16}H_{28}N_2O_3PSNa \cdot 2,5H_2O$	44,95	7,73	6,55

*Found: S 8.6%; Calculated: S 8.41%.
†Ammonium salt. Found: P 8.2%; Calculated: P 8.04%.

TABLE 3. Radioprotective Activity and Toxicity of Thiophosphates I and II

Compound	Method of introduction	LD_{50}, mM/kg	Radioprotective action		
			dose, mM/kg	number of animals	
				total	% survival
Ia	Intraperitoneal	0,65	0,044	15	40
			0,11	15	60
			0,22	15	100
	Enteral	1,68	0,56	15	53
Ib	Intraperitoneal	0,66	0,041	15	47
			0,10	15	73
			0,21	15	100
	Enteral	2,50	0,83	15	47
Ic	Intraperitoneal	0,68	0,20	30	37
	Enteral	1,79	0,59	30	13
Id	Intraperitoneal	0,69	0,20	56	27
	Enteral	1,96	0,65	30	7
Ie	Intraperitoneal	0,35	0,10	20	5
If	"	0,06	0,009	20	0
			0,018	20	5
			0,036	20	0
IIa	"	0,120	0,058	19	79
	Enteral	0,290	0,116	20	40
IIb	Intraperitoneal	0,078	0,020	20	0
IIc	"	0,105	0,20	20	0
Control (physiological saline)				100	0

rivative of 3,5-dimethyl-1-aminomethyladamantane with V, however, a single product was obtained which was shown on isolation to be N-ethyl-3,5-dimethyl-1-aminomethyladamantane (VII). The trimethylsilyl derivative of piperidine reacted analogously.

The presence of high antiradiation activity (Table 3) by intraperitoneal and enteral introduction of compound Ia agrees well with the literature data [1]. Substitution of hydrogen atom on the α-carbon by a methyl group (compound Ib) practically does not change the toxicity and protective action by intraperitoneal introduction; by enteral introduction however, the toxicity decreased by approximately 1.5 times, and the effective protective dose increased by approximately 2 times.

It is interesting that by introduction of both n-propyl and isopropyl radicals on the α-carbon atom (Ic and d), the acute toxicity as a function of means of introduction is not changed, while the radioprotective effect is noticeably lowered by changing to the n-propyl and isopropyl derivatives.

Further elongation of the hydrocarbon chain resulted in a substantially increased toxicity (for the octyl derivative, approximately by a factor of 10) and loss of radioprotective action.

Comparison of the data obtained for the unsubstituted (Ia) and C-methyl (Ib) derivative confirms the conclusion [3] that the introduction of a methyl group into the hydrocarbon chain in a series of various substituted β-mercaptoethylamines has an ambiguous influence on the radioprotective activity and toxicity of these compounds.

Actually, in the present case, both show practically no change, but with the analogous structural change in β-aminoethylthiophosphoric acid, a lower toxicity for the preservation of the level of protective action is observed, and its aminopropyl derivative shows increased toxicity and decreased activity.

On examination of the results of the study of biological activity of the N-substituted compounds II (Table 3), it can be seen that introduction of the adamantyl group results in an increased biological activity; toxicity increases by more than five times.

Unexpectedly, it appears that in distinction from the active dimethyladamantyl derivatives (IId), the monomethyl (IIb) and trimethyl (IIc) homologs show no essential difference from the toxicity of IIa as well as lack of activity. This may be explainable by the low solubility of these substances in water. Alternatively, it is possible that there is a change in the mechanism of influence upon the dopaminergic receptors in the case of 1-aminoadamantane and its 1,3-dimethyl analog [4].

After a study of the acetamidine derivatives of thiophosphoric acid I and II, it is possible to compare the analogous β-aminoethyl thiophosphates. Replacement of the last two hydrogen atoms by the imino group leads to an appreciable increase (by approximately ten times) in biological activity, for example:

$H_3\overset{+}{N}-\underset{Ia}{C}=N\ HCH_2SPO_3^-Na$	$H_3\overset{+}{N}CH_2CH_2SPO_3^-Na$
LD_{50} (Intraperitoneal) 0.65 mM/kg	LD_{50} (Intraperitoneal) 4.3 mM/kg
ED_{100} (Intraperitoneal) 0.22 mm/kg	ED_{100} (Intraperitoneal) 2.0 mM/kg

Apparently this involves a peculiarity of the amidine group, which is significantly different from the amino function in its basicity and its stereochemistry. It is possible that compounds of the I and II series, in addition to activity on the organism of the usual type of aminothiol radioprotection, show an influence on the amidine receptors [5].

EXPERIMENTAL

The radioprotective effectiveness of the compounds was studied on hybrid F_1 (CBA × C_{57}BL) mice. An aqueous solution of the substance in a volume of 0.2 ml of water was introduced into the animals 15 min (intraperitoneal) or 30 min (enteral) before irradiation. The mice were irradiated by a gamma apparatus (ÉGO-2) with 950 R at a rate of 250-150 R/min. The toxicity of the substances was determined on white random-bred male mice weighing 22-25 g. LD_{50} was calculated by the standard method [6]. The results are presented in Table 3.

2-Bromopropionitrile (IIIB) and 2-Bromocapronitrile (IIIe). A mixture of 2-bromopropionamide (24 g, 0.154 mole) and 55 g (0.35 mole) of phosphoric anhydride was heated under vacuum (100 mm) in a stream of nitrogen and the nitrile formed was distilled. After distillation of 2 g of phosphoric anhydride, there was obtained 15.7 g of IIIb, bp 53°C (10 mm), yield 74%.

Found, %: N 10.7. C_3H_4BrN. Calculated, %: N 10.45.

Similarly, from 25 g (0.13 mole) of 2-bromocapronitrile and 32.5 g (0.23 mole) of phosphoric anhydride was obtained 19.67 g of IIIe, bp 95-110°C (12 mm), yield 86%.

Found, %: N 7.9. $C_6H_{10}BrN$. Calculated, %: N 7.95.

2-Bromopropionamidine Hydrochloride (IVb). To a methanol solution of sodium methylate, prepared from 0.46 g (0.02 mole) of sodium and 100 ml of absolute methanol, was added dropwise 27 g (0.2 mole) of 2-bromopropionitrile with cooling and stirring. The reaction mixture was maintained for 1 h at room temperature and 11.8 g (0.22 mole) of ammonium chloride were added. After stirring for 2 h and filtration, the solvent was removed under vacuum at 45°C. The residue was treated with dry ether. The precipitate was filtered off and washed on the filter with dry ether to give 33.4 g (91%), of IVb, mp 125-128°C.

The remaining compounds were prepared analogously (Table 1).

N-1-Adamantylmethyl-2-chloroacetamidine Hydrochloride (VIa). To a solution of 7.91 g (0.05 mole) of ethyl 2-chloroacetimidate hydrochloride in 50 ml of absolute ethanol was added a solution of 8.26 g (0.05 mole) of 1-adamantylmethylamine. The reaction mixture was kept for 36 h in a refrigerator. The white crystalline precipitate was filtered off, washed with alcohol, and dried to give 9.7 g (85%) of VIa, mp 260-262°C (decomp.).

Sodium S-2-(1-amidinovaleryl)thiophosphate (Ic). To a stirred solution of 5.22 g (0.025 mole) of 2-bromovaleramidine hydrochloride in 25 ml of water was added a solution of 8.8 g (0.22 mole) of trisodium phosphate dodecahydrate in 50 ml of water and the solution was stirred for 1.5 h (colorless qualitative test with silver nitrate). To the reaction mixture at 0.5°C slowly was added 350 ml of ethanol, and the mixture was maintained at this temperature for 2 h and filtered. The precipitate was washed with alcohol and air-dried to give 6.4 g (79%) of Ic, mp 115-116°C (decomp.).

A 0.2 g sample of Ic was dried in a drying pistol over phosphorus pentoxide at 60°C to constant weight. The weight loss was 15.8%, which corresponds to 2.5 moles of water in Ic.

The remaining compounds I and II were prepared analogously (Tables 1 and 2).

N-Ethyl-3,5-dimethyl-1-adamantylmethylamine Hydrochloride (VII). To 2.3 g (0.019 mole) of ethyl 2-chloroacetimidate (obtained from the corresponding hydrochloride and triethylamine) was added 5.07 g (0.019 mole) of N-trimethylsilyl-3,5-dimethyl-1-adamantylmethylamine (prepared from 3,5-dimethyl-1-adamantylmethylamine, trimethylchlorosilane and triethylamine), and the reaction mixture was stirred for 30 min. Five ml of methanol were added and the mixture was concentrated under vacuum. To the residue was added an ethereal solution of hydrogen chloride to pH 1.0. The white crystalline precipitate was filtered off, washed with ether and recrystallized from alcohol to give 3.6 g (74%) of VII, mp 280-282°C (decomp.).

Found, %: Cl 14.2. $C_{15}H_{27}N \cdot HCl$. Calculated %: Cl 13.75.

N-Ethylpiperidine hydrochloride was prepared in an analogous manner (yield 80%, mp 225°C), which did not depress the melting point of a sample prepared by a known method.

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