SYNTHESIS AND BIOLOGICAL PROPERTIES OF CERTAIN CHLOROORGANOPHOSPHORUS COMPOUNDS

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UDC 542.91+661.718.1+541.69+546.13

It was reported in previous work [1, 2] that hypophosphorous acid condenses with chloral. Moreover the reaction proceeds in stages, initially a 1:1 adduct, 1-hydroxy-2,2,2-trichloroethylphosphonous acid, is formed. The reaction of this with a second molecule of chloral leads to bis-(1-hydroxy-2,2,2-trichloroethyl)phosphinic acid. These adducts and the acyl derivatives of bis-(1-hydroxy-2,2,2-trichloroethyl)phosphinic acid possess biological activity [2].

In this paper, which is a continuation of the study, we have established that the adduct of hypophosphorous acid and chloral, i.e., 1-hydroxy-2,2,2-trichloroethylphosphonous acid, is capable of condensing not only with chloral but also with other aldehydes and ketones. Thus (1-hydroxy-2,2,2-trichloroethyl)-1-hydroxyisopropylphosphinic acid is obtained on reacting 1-hydroxy-2,2,2-trichloroethylphosphonous acid with acetone. The adducts with benzaldehyde and methyl ethyl ketone were prepared similarly.

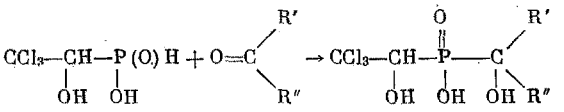
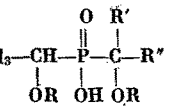


TABLE 1 

Compound No.	R	R′	R″	Yield, %	Mp, °C	Found/calculated, %	
						P	Cl
X	H	CH_3	CH_3	50,3	156—157 (with decomp.)	11,14 / 11,42	39,80 / 39,22
XI	CH_3CO	CH_3	CH_3	50,8	170—171	8,56 / 8,72	29,37 / 29,95
XII	C_2H_5CO	CH_3	CH_3	34,0	145—146	8,23 / 8,08	27,81 / 27,70
XIII	H	H	C_6H_5	65,7	172 (with decomp.	9,76 / 9,70	32,88 / 33,33
XIV	CH_3CO	H	C_6H_5	56,8	181—183	7,76 / 7,68	26,90 / 26,39
XV	H	C_2H_5	CH_3	35,1	153—154 (with decomp.)	10,97 / 10,85	37,74 / 37,30
XVI	$CH_3(CH_2)_2CO$	CH_3	CH_3	49,3	122—123	7,29 / 7,53	25,65 / 25,88

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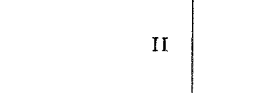
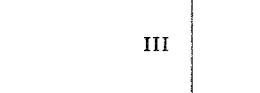
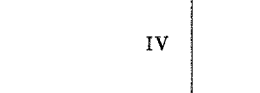
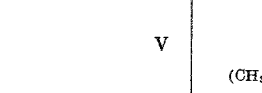
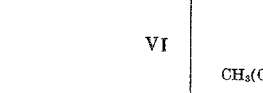
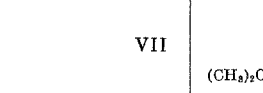
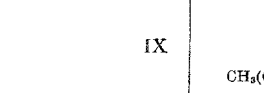
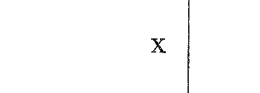
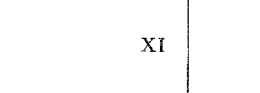
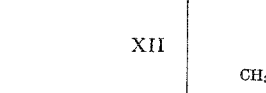
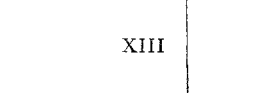
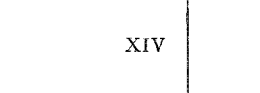
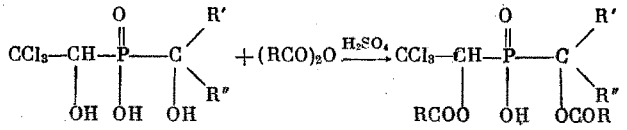
Translated from Izvestiya Akademii Nauk SSSR, Seriya Khimicheskaya, No. 10, pp. 2241-2249, October, 1969. Original article submitted July 12, 1968.

TABLE 2

Compound		LD_{50} by intravenous injection with treatment of the data according to Miller and Tenter, mg/kg	LD_{50} by subcutaneous injection with treatment of the data according to Miller and Tenter, mg/kg	Minimum effective concentration in frog heart according to Straub, M
No.	formula			
I		515±48,8	720±142,9	10^{-4}
II		—*	—	10^{-8}
III		—	—	10^{-8}
IV		652±70,6	—	10^{-5}
V		—	—	10^{-5}
VI		272±34,4	—	10^{-7}
VII		—	—	10^{-4}
VIII		—	—	10^{-5}
IX		—	—	10^{-8}
X		356±52,6	—	10^{-4}
XI		605±126,9	—	10^{-4}
XII		400±88,2	—	10^{-4}
XIII		339±70,2	—	10^{-5}
XIV		560±70,2	—	10^{-5}
	Chlorophos	380±31,9	530±140,9	

* The LD_{50} was not successfully determined since on introducing the compound at the limiting solubility deterioration of the animals did not commence.

These adducts give the corresponding acyl derivatives with anhydrides of carboxylic acids in the presence of catalytic quantities of concentrated H_2SO_4.



The compounds obtained and their physicochemical properties are given in Table 1.

The biological activity both of the compounds recently synthesized (X)-(XIV) and also the compounds described previously (I)-(IX) [2] has been studied. The toxicity was determined in white mice for intravenous and subcutaneous methods of injection. With intravenous injection, deterioration of the animals commenced in the first minute after introducing the compound with symptoms of asphyxia following short-lived clonic and clonico-tonic spasms. With subcutaneous injection of the compounds the toxic effect showed as a depressed state. Deterioration commenced after a day with the same toxic picture. The LD_{50} values for the derivatives of trichloroethylphosphinic acid are given in Table 2. Acylation of the hydroxyl groups decreases somewhat the toxic properties of the compounds. Thus, the LD_{50} of compounds (XI) and (XIV) is almost half the LD_{50} of compounds (X) and (XIII). However, lengthening the acyl group leads again to an increase in toxicity, the LD_{50} of compound (XII) is somewhat higher than the LD_{50} of compound (XI). The condensation products of 1-hydroxy-2,2,2-trichloroethylphosphonous acid with acetone and benzaldehyde are almost indistinguishable in their toxic properties [cf. the LD_{50} of compounds (X) and (XIII) and (XI) and (XIV)]. Chlorophos was used as a standard compound for the comparison of toxicities. As is evident from Table 2 the compounds synthesized by us possess toxicity of the same order as chlorophos.

Chloral, of which the compounds under study are derivatives, is used in the hydrated form as a soporific. Many derivatives of chloral possess soporific, narcotic, and antispasmodic activity [3]. However, the reaction product of chloral and amphetamine is a stimulant [4]. Chlorophos also exerts an effect on the functional state of the cortex of the brain, this appears as an increase of the process of active internal inhibition [5]. According to the data of certain authors, chlorophos produces a decrease of cardiac activity [6]. Since the compounds under study are also organophosphorus derivatives of chloral it was of interest to investigate the effect of these compounds on cardiac activity and on the central nervous system.

On studying the effect of the compounds on isolated frog heart by Straub's method it was clear that they all caused a reduction of the amplitude of the contractions in retaining the rhythm of the systoles. Both the action on the heart and the toxicity were increased on lengthening the carbon chain in the acyl radical (q.v. Table 2). The presence of iso-radicals reduced the toxic effect of the compounds on isolated frog heart. Thus, the depressive action of compounds (V) and (VII) is much more weakly expressed than for compounds (VI) and (VIII). The compounds with the larger number of carbon atoms in the acyl radical (up to C_4) cause an interruption in the diastole of the heart at a concentration of $1 \cdot 10^{-3}$ M while the remainder of the compounds at the same concentration only reduce the amplitude of the systoles, this is spontaneously recoverable over 15-20 min. In order to explain the mechanism of the action on isolated frog heart of the compounds under study it was of interest to elucidate the relationship of these substances to atropine, adrenaline, and ATP by reducing the metabolism of the substances in the myocardium. Compound (VI) in which an intense negative inotropic action is combined with a satisfactory solubility was selected for this purpose. Atropine did not alter the reaction of isolated heart to the introduction of the compounds. Consequently, the stimulation of the M-cholino reactive structures does not underlie a negative inotropic action. The compound completely prevented or clearly weakened the reaction of the heart to adrenaline. The decrease in amplitude of the systoles caused by compound (VI) completely reduced the ATP and urea. On the bais of this the presence of an adrenolytic action and effects on the metabolic processes of the myocardium can be assumed.

The majority of investigations link the mechanism of the action of organophosphorus compounds with their anticholinesterase activity. The presence of adrenolytic properties for organophosphorus compounds together with weakly expressed anticholinesterase properties drew attention to the compounds under study. Compound (I), for which the depressive action shows significantly in the subcutaneous method of injection, was selected for the study of the influence of the compounds on the central nervous system. Experiments were conducted in which the compound and agents exerting an influence on the central nervous system, i.e., central nervous system soporifics and stimulants, were introduced. Urethane and Barbamil were the soporifics used. Corazole, camphor, and phenamine were the central nervous system stimulants used. The

TABLE 3 $CCl_3-CH(OR)-P(=O)(OH)-C(R')(OR)-R''$ (X)

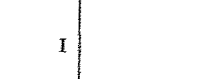
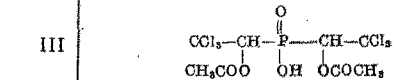
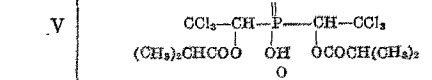
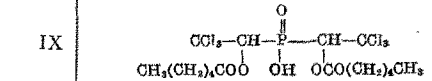
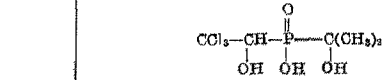
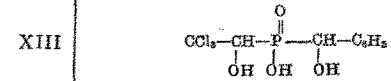
Compound No.	R	R′	R″	X	Solution concentration, M	Percentage loss of mice
I	H	$-C(R')(OR)-R''=H$		—	0,025 0,05 0,1	31 53 22
III	CH_3CO	CCl_3	H	—	0,025 0,05 0,1	0 0 2
IV	C_2H_5CO	CCl_3	H	—	0,025 0,05 0,1	4 6 4
V	$(CH_3)_2CHCO$	CCl_3	H	—	0,025 0,05 0,1	0 3 3
VI	C_3H_7CO	CCl_3	H	—	0,025 0,05 0,1	0 0 2
VII	$(CH_3)_2CHCH_2CO$	CCl_3	H	—	0,025 0,05 0,1	0 0 3
VIII	C_4H_9CO	CCl_3	H	—	0,025 0,05 0,1	0 16 10
IX	$C_5H_{11}CO$	CCl_3	H	—	0,025 0,05 0,1	21 25 33
X	H	CH_3	CH_3	—	0,025 0,05 0,1	44 50 25
XI	CH_3CO	CH_3	CH_3	—	0,025 0,05 0,1	48 49 55
XII	C_2H_5CO	CH_3	CH_3	—	0,025 0,05 0,1	34 42 29
XVII	CH_3CO	CCl_3	H	$N(C_2H_5)_3$	0,025 0,05 0,1	34 33 23

compound had no effect on the length of the Barbamil sleep ($P > 0.05$) but lengthened the urethane sleep 6.3 times with $P = 0.02$. On introducing a subthreshold dose of urethane into the experimental series 20% of the mice assumed the secondary condition. The compound under study did not prevent the spasms caused by camphor and corazole but it reduced the intensity of the camphor spasms. The compound did not prevent the loss of the white mice associated with the introduction of camphor and corazole and it did not influence the life duration of the animals but it secured the survival of 50% of the white mice from the introduction of a lethal dose of phenamine within the 10 min following its introduction whereas a 100% loss of animals was observed in the control.

The antagonism of phenamine, the potentiation of the cortical soporific urethane and the absence of influence on the effect of the nerve cord soporific Barbamil, and the reduction of camphor spasms and the absence of changes in corazole spasms lead one to assume a deprimenting effect on the cortex of the brain for the compound under study.

The insecticidal action of the compounds prepared was studied by topical application of equimolar acetone solutions (0.025; 0.05; 0.1 M) of the compounds to the middle back of flies of 4-6 days growth with a calibrated microloop. The volume of the microloop was 0.6 μl. Two controls were carried out: in the solvent and with 0.025 M chlorophos solution for comparison of the insecticidal action of the compounds being tested. The compounds under study appeared to show a much weaker action than chlorophos. The loss of insects fluctuated from 0 to 55% over 24 h (Table 3) while in the control 100% loss of insects was observed in 1-2 h after application of the chlorophos solution.

TABLE 4

Compound		dilution	Fungus							
			Stachybotrys olternaus				Fusarium sporotrichiella			
			liquid medium		solid medium		liquid medium		solid medium	
No.	formula		10-day	20-day	10-day	20-day	10-day	20-day	10-day	20-day
I		1 : 500	—	—	±	±	—	—	±	±
		1 : 1000	—	—	±	±	+	+++	±	+++
III		1 : 500	—	—	±	±	+	+++	±	±
		1 : 1000	++	+++	±	+++	+++	++++	±	+++
V		1 : 500	—	—	±	±	—	—	±	±
		1 : 1000	—	—	±	++++	—	++	±	++++
IX		1 : 500	—	—	±	±	—	—	—	—
		1 : 1000	—	—	±	+++	—	—	±	++++
		1 : 500	+	++	±	±	—	—	±	±
		1 : 1000	+	+++	±	++++	+	+	++++	++++
XIII		1 : 500	—	—	±	±	—	—	±	±
		1 : 1000	—	—	±	±	+	+++	±	++++
	Chlorophos	1 : 500	++	++	++++	++++	++	++++	++++	++++
		1 : 1000	+++	++++	++++	++++	++++	++++	++++	++++
	Formaldehyde	1 : 500	—	—	—	—	—	—	—	±
		1 : 1000	—	—	—	—	—	—	±	±
	Control		++++	++++	++++	++++	++++	++++	++++	++++

Note: "-" is absence of growth; "±" is delay in growth; "+" is weak growth; "++" is good growth but colony not united; "+++" is strong growth with colony united but having gaps; "++++" is continuous growth.

Corn infected with the fungi Stachybotrys olternaus and Fusarium sporotrichiella Birai and 30 day cultures of these fungi grown on the surface of Czapek agar were used as test materials for the study of the fungistatic activity. Innoculations of the test materials on the same media but without the addition of the chemical compounds served as a control. Analogous experiments with chlorophos, as the substance closest in chemical structure to these compounds, and formaldehyde, as a substance possessing most marked fungistatic properties, were carried out in order to compare the fungistatic activity of the compounds being tested. The data obtained is given in Table 4. It is evident from Table 4 that all the compounds possess fungistatic properties relative to the fungi S. olternaus and F. sporotrichiella. It appeared that the fungi possessed a different stability to the action of the chemical substances. The fungistatic activity of the compounds is dependent on the chemical structure. Lengthening the acyl radical leads to an increase in fungistatic properties [compounds (III) and (IX)]. Compound (V) with an iso-radical also possesses some fungistatic activity. The presence of the 1-hydroxyisopropyl radical [compound (X)] probably does not reduce the activity of the compounds relative to the fungi studied. The presence of the hydroxybenzyl radical reduces somewhat the fungistatic activity of the compounds [compound (XIII)]. The compounds studied by us proved to be much more active than chlorophos, compound (IX) approaches the activity of formaldehyde in its fungistatic properties.

EXPERIMENTAL

1-Hydroxy-2,2,2-trichloroethyl-(1-hydroxyisopropyl)phosphinic Acid. 1-Hydroxy-2,2,2-trichloroethylphosphonous acid (15 g) was heated for 12 h with a large excess of acetone in a flask fitted with a reflux condenser. After removal of the acetone the residue was washed with ether. This gave 9.6 g (50.3% of theoretical) of a product with mp 156-157°C (with decomp.). Found: P 11.14; 11.14; Cl 39.72; 39.89%. $C_5H_{10}Cl_3O_4P$. Calculated: P 11.42; Cl 39.22%.

1-Hydroxy-2,2,2-trichloroethyl-(1-hydroxy-sec-butyl)phosphinic Acid. A mixture of 10.7 g of 1-hydroxy-2,2,2-trichloroethylphosphonous acid and 7.2 g of methyl ethyl ketone was boiled in a flask fitted with a reflux condenser for 5 h. After this the suspension was filtered off and the excess of ketone removed, the residue was recrystallized from CH_3OH. This gave 5 g (35.1% of theoretical) of a product with mp 153-154°C (with decomp.). Found: P 11.00; 10.95; Cl 37.77; 37.71%. $C_6H_{12}Cl_3O_4P$. Calculated: P 10.85; Cl 37.30%.

1-Hydroxy-2,2,2-trichloroethyl-(1-hydroxybenzyl)phosphinic Acid. A mixture of 10.6 g of 1-hydroxy-2,2,2-trichloroethylphosphonous acid and 12 g of benzaldehyde in benzene was heated to boiling for 2 h. All gradually dissolved and then a bluish precipitate separated. After recrystallization from $(CH_3)_2CHOH$ 10.5 g (65.7% of theoretical) of product was obtained with mp 172°C (with decomp.). Found: P 9.71; 9.82; Cl 32.98; 32.77%. $C_9H_{10}Cl_3O_4P$. Calculated: P 9.70; Cl 33.33%.

1-Acetoxy-2,2,2-trichloroethyl-(1-acetoxyisopropyl)phosphinic Acid. 1-Hydroxy-2,2,2-trichloroethyl-(1-hydroxyisopropyl)phosphinic acid (13.6 g) was placed in a four-necked flask fitted with a stirrer, reflux condenser, thermometer, and dropping funnel. At room temperature 20.4 g (100% excess) of $(CH_3CO)_2O$ to which 10 drops of concentrated H_2SO_4 was applied as catalyst was added with stirring. A slight exothermic reaction was observed. Then 30 ml of ether was added and the mixture heated for 1-2 h. After recrystallization from ether 9 g (50.8% of theoretical) of product was obtained with mp 170-171°C. Found: P 8.54; 8.58; Cl 29.35; 29.38%. $C_9H_{14}Cl_3O_6P$. Calculated: P 8.72; Cl 29.95%.

Compounds (XII), (XIV), and (XVII) (q.v. Table 1) were prepared in a similar manner.

CONCLUSIONS

1. The reaction of 1-hydroxy-2,2,2-trichloroethylphosphonous acid with aldehydes and ketones yields the corresponding adducts. These adducts on reaction with anhydrides of carboxylic acids give acyl derivatives.

2. The biological activity of the condensation products of hypophosphorous acid with chloral and aldehydes and ketones has been studied; an assumption is expressed concerning the presence of an adrenolytic action and effect of these compounds on the metabolic processes of myocardium; a study of the effect of organophosphorus derivatives of chloral on the central nervous system led to a suggestion concerning the deprimenting action of the compounds on brain cortex.

3. A study of the insecticidal properties of the compounds synthesized indicated that they are weak insecticides.

4. All of the compounds synthesized show fungistatic activity.

5. A correlation is traced between the chemical structure of the compounds and their biological action.

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