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Phosphoramidates: Features of the Formation Mechanism and the Relationship Structure–Bioaction

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Abstract—Mechanism of the synthesis of phosphoramidates by Todd–Atherton reaction is based on the primary interaction of a polyhaloalkane with the highly basic amine to form a 1:1 associate. The subsequent attack by the associate on the hydrophosphoryl compound of "symmetric" structure leads to the formation of the target compounds in high yields. The test of the effect of dialkyl hexamethylene- and dialkyl(pyridin-2-yl)-phosphoramidates *in vitro* against the strains of a number of the producents of pathogenic bacteria and mycotoxins showed that the high level of biological activity of the target compounds is correlated well with the physicochemical parameters characterizing their structure.

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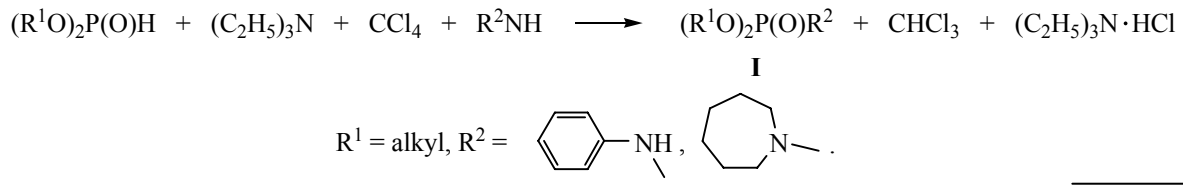
Organic phosphoramidates exhibit a wide spectrum of biological action, from pesticides to anticancer drugs. The reasons for such diversity are different, and include high alkylating ability of phosphoramidates, their propensity to both spontaneous and enzymatic hydrolysis, and the ability of molecules with a P–N bond to insert in the structure of natural nucleotides [1–3].

In this work we studied the mechanism of formation and biological action of some phosphoramidates:

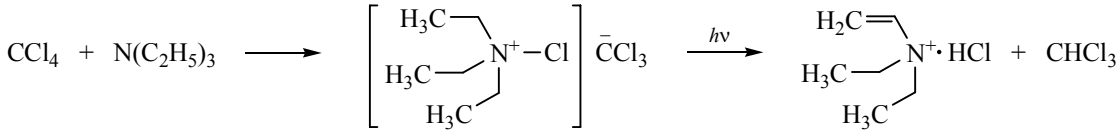


Methods of synthesis of phosphoramidates are well known. In particular, the classical four-component one-step Todd–Atherton reaction provides the target phosphoramidates of different structures under mild conditions and in a high yield. This reaction is based on the interaction of hydrogen phosphites or related compounds with amines in a polyhalogenated solvent. When the amine is not sufficiently basic, it is necessary to add an equivalent quantity of triethylamine or of other tertiary amine [4]. The popularity of Todd–Atherton method has played a major role in the fact that the chemistry of this process is known only in general terms. Strictly speaking, nobody attempted to prove it, and therefore into the textbooks a speculative scheme is included [5]. It is assumed that initially hydrophosphoryl compound interacts with triethylamine to produce either a P(III)-form, or the corresponding salt. Note that the cases of formation of salts between dialkyl phosphites and amines of different nature were described by Bakhtiyarova [6]. However, the results obtained cannot be unambiguously evaluated: the author wrongly claims that the formation of salts in the case of diethyl hydrogen phosphite is impossible due to the dominance in solution of "asymmetric" form $(C_2H_5O)_2P(O)H$, in contrast to other phosphites.

In the second stage of the proposed mechanism of Todd–Atherton reaction [5] the symmetrical hydrophosphoryl compound or a salt thereof reacted as a nucleophilic compound with carbon tetrachloride, acting as a donor of the positively charged chlorine. The latter statement is difficult to accept unreservedly. Very often many authors consider a molecule of

carbon tetrachloride as a source of ions or chlorine radicals and trichloromethyl groups. Unfortunately, the reason for the formation of such species is not cleared. Carbon tetrachloride is a sufficiently stable compound under normal conditions, and to become a source of charged particles it should interact with a nucleophilic reagent or a quantum of light, or be irradiated with other radiation source. Initially a transition state should appear with the charge redistributed between the parts of the molecule, in particular, the chlorine lone pair must move to the non-bonding σ orbital. This occurs in the case of the Kamai reaction [7].

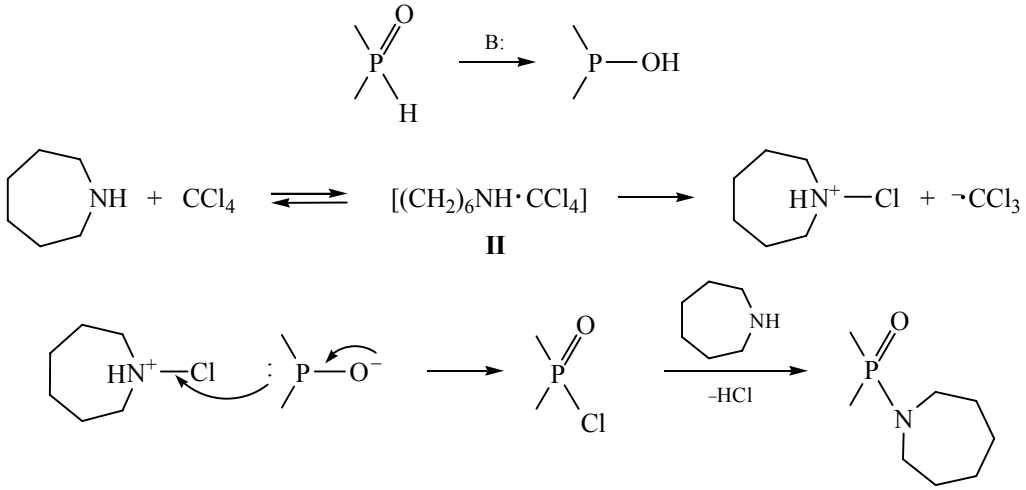
Analysis of published data and results of our experiments enable us to suggest two possible routes in the Todd–Atherton reaction. The first way (preliminary phosphite interaction with amine), as already noted, has been reviewed in [4]. The view expressed there is commonly accepted. However, in this case, it remains unclear why in the reaction mixture are not detected trihlormethylphosphonates, the typical products of the Kamai reaction. This discrepancy is explainable assuming an alternative view on the mechanism of the three-component interaction between hydrophosphoryl compound, carbon tetrachloride and highly basic amine. In our opinion, it is completely incorrect to restrict the role of triethylamine in the Todd–Atherton reaction as converting hydrophosphoryl compound into the "symmetric" form. The interaction between carbon tetrachloride and highly basic amine should not be ignored. Meanwhile, it is known [8] that amines and ordinary halogenated hydrocarbons form associates entering into the charge-transfer reactions:



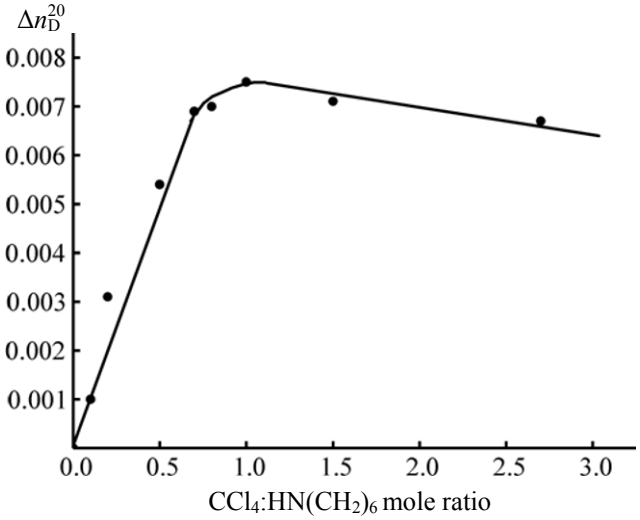
Therewith, it was proved that the dark and photochemical reactions of some amines with carbon tetrachloride proceed with a quantum yield greater than unity and, consequently, are the chain reactions. We believe that just the interaction of highly nucleophilic amine with carbon tetrachloride initiates the Todd–Atherton reaction.

Earlier [4] it has been proved experimentally that a stronger base provides a higher rate of the Todd–Atherton reaction action. Taking into account that the basicity of the hexahydroazepine is slightly higher than that of triethylamine (pK_{BH^+} are 11.1 and 10.87, respectively [9]), it can be assumed that between the molecules of carbon tetrachloride and hexahydroazepine should occur a specific interaction.

The affinity of the trichloromethyl radical to the proton produced at the dissociation of a "symmetric" form of phosphite is undoubtedly higher than to any electrophilic particle in solution, which results in the formation of a molecule of chloroform. Thus the lack of the products of the Kamai reaction in the reaction mixture is explained.



B is a base (any highly basic amine).



Refractometric titration curve in the system CCl_4–$HN(CH_2)_6$ indicating the formation of associate **II.**

The search for new conditions of the Todd–Atherton reaction continues to this day. In particular, a new method was developed recently of the synthesis of phosphoramidates using microwave irradiation [10, 11]. Studying the phosphorylation of the amino(aryl)-methylphosphonates in the Todd–Atherton reaction Minaeva et al. noted that the effect of microwave irradiation of the reaction mixture increased the reaction rate and the yield of the final compounds. We think that the fact is an indirect confirmation of the involvement of the charge transfer associates between the amine and carbon tetrachloride in the synthesis of phosphoramidates and their subsequent decomposition at action of the microwave energy, with the formation of desired products.

To prove the formation of associate **II**, we studied the interaction of tetrachlorometane with hexahydroazepine by the method of refractometric titration. The figure shows a curve characterizing the change in refraction index of the CCl_4–$HN(CH_2)_6$ mixture versus the molar ratio of components. The nature of the curve shows that carbon tetrachloride forms with hexahydroazepine a 1:1 charge transfer associate. We note that such a pattern was not observed in the case of 2-aminopyridine. This is due, most likely, to the low basicity of this amine (pK_{BH^+} = 6.86).

To prove that associate **II** lies on the reaction coordinate, we carried out the synthesis of dialkylphosphor(hexamethyleneamidates) **Ib** and **Ie** without the use of triethylamine, but with a twofold molar excess of hexahydroazepine. The reaction proceeded under mild conditions with a high yield, and hexahydroazepine hydrochloride formed almost quantitatively. In the synthesis of dialkyl (pyridine-2-yl)phosphoramidates **Ih–Ij** we used triethylamine as a component of the associate **II**, as in the absence of trietylamine we failed to isolate the target compounds from the reaction mixture. Table 1 lists the parameters of phosphoramidates **Ia–Ij**.

The phosphoramidates synthesized in this work were tested *in vitro* for antibacterial and fungicidal activity. Table 2 lists the data on the biological activity. Attention is drawn to the fact that all the target compounds showed significant antibacterial and fungicidal action. The level of activity in most cases is practically independent from the length of the alkoxy radicals at the phosphorus atom. The level of activity of various target compounds is approximately the same, which makes it impossible to optimize their structure for the synthesis of more effective drugs. An exception concerns prediction of the level of activity against the fungi *Fusarium graminearum*, the producer of mycotoxins.

The found inhibition of the *Fusarium graminearum* strain obeys the relation (1):

$$\log 1/C_{50} = 6.20–1.27\log p + 0.101(\log P)^2 + 0.112E_{hydr}, \quad (1)$$
$$r\ 0.924,\ s\ 0.71.$$

In this work at the optimization of the level of phosphoramidates, we chose the following characteristics: lipophilicity parameter log P, the value characterizing the ratio of solubility in water and lipids, and E_{hydr}, the energy of hydration of organic molecules. The choice of these characteristics is due to the ease of spontaneous and enzymatic hydrolysis of phosphoramidates.

A significant improvement should be noted in the correlation characteristics when the chemical shift of compounds **I** in the ^{31}P NMR spectra was used as an additional correlation parameter [Eqs. (2) and (3)]. The choice of δ_P is defined by the fact that this quantity is an independent experimental value that largely depends on the electronic effect of substituents at the phosphorus atom. The latter circumstance makes it more advantageous than the widely used Kabachnik constants. Use of the latter is often limited due to the impossibility of assessing the contribution of inductive and resonance components.

$$\log 1/C_{50} = 1.86 + 1.89\log p – 0.189(\log P)^2 – 0.134E_{hydr} – 0.203\delta_P;\ r\ 0.967,\ s\ 0.12, \quad (2)$$

$$\log 1/C_{50} = 4.26 + 0.172\log p – 0.0327(\log P)^2 0.0961\delta_P;\ r\ 0.955,\ s\ 0.11. \quad (3)$$

Table 1. Yields and characteristics of phosphoramidates **I**

Comp. no.	R^1	R^2	Yield, %	n_D^{20}	d_4^{20}	bp, °C (mm Hg) or mp, °C	Found, %			Formula	Calculated, %		
							C	H	N		C	H	N
Ia	CH_3O	$N(CH_2)_6$	61	1.4619	1.059	140(10)	46.1	8.39	6.69	$C_8H_{18}NO_3P$	46.4	8.69	6.76
Ib	C_2H_5O	$N(CH_2)_6$	73[a]	1.4525	1.063	144(19)	51.2	9.16	5.76	$C_{10}H_{22}NO_3P$	51.1	9.36	5.96
Ic	C_3H_7O	$N(CH_2)_6$	56	1.4498	1.055	150(9)	54.7	9.81	5.19	$C_{12}H_{26}NO_3P$	54.8	9.88	5.32
Id	*i*-C_3H_7O	$N(CH_2)_6$	41	1.4469	1.064	100(2)	54.7	9.85	5.12	$C_{12}H_{26}NO_3P$	54.8	9.88	5.32
Ie	*i*-C_4H_9O	$N(CH_2)_6$	85[a]	1.4549	1.008	157(4)	57.5	10.1	4.69	$C_{14}H_{30}NO_3P$	57.7	10.3	4.81
If	$C_6H_{13}O$	$N(CH_2)_6$	49	1.454	1.009	153(4)	62.2	10.7	4.01	$C_{18}H_{38}NO_3P$	62.2	10.9	4.03
Ig	$C_8H_{17}O$	$N(CH_2)_6$	52	1.4301	1.018	160(6)	66.6	11.4	3.19	$C_{22}H_{46}NO_3P$	66.5	11.4	3.47
Ih	CH_3O	2-Amino-pyridin-2-yl	74	–	–	89	41.3	5.89	13.8	$C_7H_{12}N_2O_3P$	41.4	5.91	13.8
Ii	*i*-C_4H_9O	2-Amino-pyridin-2-yl	59	–	–	70	54.4	8.12	9.65	$C_{13}H_{24}N_2O_3P$	54.4	8.36	9.75
Ij	$C_6H_{13}O$	2-Amino-pyridin-2-yl	44	–	–	190(6)	58.4	10.4	8.1	$C_{17}H_{37}N_2O_3P$	58.6	10.6	8.04

[a] Compounds were obtained in two ways, the given yield corresponds to the method *b*.

Table 2. Antibacterial activity of phosphoramidates and correlation characteristics

Comp. no.	E_{hydr}	log *P*	δ_P, ppm	log $1/C_{50}$					
				Xant. malv.	*Scl. sol.*	*Fus. gram.*	*Vent. in.*	*Hel. sat.*	*Rhiz. sol.*
Ia	–13.2	1.27	12.4	3.62	3.89	3.18	3.76	3.74	3.18
Ib	–5.36	1.95	9.3	3.19	3.83	3.71	3.80	3.71	–
Ic	3.10	2.89	8.1	3.54	3.99	3.59	3.87	3.47	3.29
Id	2.76	2.78	7.2	3.85	3.99	3.76	3.71	3.81	3.59
Ie	6.28	3.70	9.3	3.29	3.81	3.63	3.91	3.89	3.81
If	9.83	5.27	8.6	3.97	–	3.41	3.51	3.93	3.71
Ig	16.3	6.85	–1.7	3.91	4.07	4.07	3.89	3.95	3.95
Ih	–34.6	1.82	2.0	3.43	–	3.17	3.28	3.73	3.47
Ii	–10.2	4.24	1.0	3.28	4.03	3.80	3.90	3.91	3.80
Ij	–6.69	5.82	–1.1	3.84	3.88	3.70	4.02	3.59	3.40

From these correlations a preliminary conclusion can be made: for obtaining more effective fungicides it is necessary to introduce into the structure of the phosphoramidate electronegative groups and fragments that ensure good solubility of the substance in the lipids and water.

EXPERIMENTAL

The assessment of biological activity of the synthesized compounds was carried out in the Krasnodar Research Institute of Biological Plant Protection, and Russian Research Institute of the Plant Protection Chemicals.

Individuality of the synthesized compounds was proved by GLC (an LKhM-8MD chromatograph, stationary phase XE-60 10% on Chezasorb or SE-30 5% on Inerton, detector katharometer, carrier gas helium, 40 ml min^{-1}) (for liquid products) and TLC (the plates Silufol UV 254, eluent acetone–hexane, 1:4) for crystalline substances. The ^{31}P NMR spectra were recorded on an instrument developed at

St. Petersburg Technological Institute (operating frequency 16.2 MHz, external reference 85% H_3PO_4). Elemental analysis was performed on a Hewlett Packard B-185 analyzer.

The values of hydration energy and lipophilicity parameter log *P* of the target compounds were calculated using the software package HyperChem™ Release 8.08, after geometric optimization by the Fletcher–Reeves method. Refractometric titration was performed on an instrument of the URL type. The processing of the titration results was performed using the Origin Pro 8 SRO software package.

Diethyl *N*,*N*-hexamethylenephosphoramidate (Ib). *a*. In a reactor was placed 6.4 g of triethylamine, 9.8 g of carbon tetrachloride, and 6.3 g of hexahydroazepine. The reaction mixture was cooled to 0°C, and then 8.8 g of diethyl phosphite was added dropwise while stirring and maintaining the reaction mixture temperature within 0–5°C. Then the reaction mixture was stirred at room temperature for 2 h. The triethylamine hydrochloride formed was filtered off, from the filtrate chloroform and carbon tetrachloride excess were distilled off, the residual oil was distilled in a vacuum at 144°C (19 mm Hg). Yield 58%. In a similar manner were obtained other phosphoramidates.

b. In a reactor was placed 10.3 g of hexahydroazepine and 16 g of carbon tetrachloride. To the mixture was added dropwise over 0.5 h 7.2 g of diethyl phosphite while stirring and cooling to 0–5ºC. The reaction mixture was stirred for 2 h at room temperature. The resulting hexahydroazepine hydrochloride precipitate was filtered off (6.6 g, 94%). From the filtrate were distilled off the volatile components and then by fractional distillation at 140°C (14 mm Hg) 8.8 g (73%) of target amidate was isolated. The compound parameters coincided with those of the compound **Ib** obtained by the above technique.

Dimethyl (*N*-pyridine-2-yl)phosphoramidates (Ih). The reactor was charged with 2 g of triethylamine, 20 ml of carbon tetrachloride, and a solution of 1.9 g of 2-aminopyridine in diethyl ether. To the resulting mixture was added dropwise at room temperature 2.2 g of dimethyl phosphite while stirring. The reaction mixture was stirred for 3 h at 40°C. After cooling to room temperature, triethylamine hydrochloride formed was filtered off, and from the filtrate volatile components were distilled off. The residual oil was washed with diethyl ether. A colorless crystalline substance formed. After filtration and drying 3 g (74%) of the product was obtained.

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