[CONTRIBUTION FROM THE PHYSICAL RESEARCH LABORATORY OF THE DOW CHEMICAL COMPANY]

Organic Polyphosphorus Compounds. II. Phosphorylations with Aliphatic Amido Polyphosphates and Polymetaphosphates

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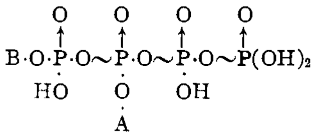
The thermal degradation of some aliphatic polyphosphoramides was investigated. It yields aliphatic pyrophosphoramide. The non-catalytic phosphorylation of hexamethylphosphoramide by aliphatic polyphosphoramides was investigated. They phosphorylate hexamethylphosphoramide thermally with formation of octamethylpyrophosphoramide. This type of non-enzymatic phosphorylation fundamentally is analogous to the enzymatic phosphorylation of mononucleotides by ATP, but evidently has a different reaction mechanism.

Introduction

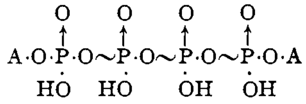
Adenosine-5′-triphosphate (ATP) is capable, among other biochemically important phosphorylation reactions, of converting certain monophosphates to pyrophosphates. Transfer of the terminal energy-rich phosphate group of ATP to adenosine monophosphate, in the presence of myokinase, yields the pyrophosphate ADP.[1] Reaction of the other high energy phosphate bond of ATP with nicotinamide ribose phosphate, riboflavin phosphate and pantethine phosphate yields, in the presence of specific enzymes, the pyrophosphate coenzymes DPN,[2] FAD[3] and A,[4,5] respectively. The by-product in each of the last three reactions is free pyrophosphoric acid.

The reversible character of these reactions and their dependence on the presence of magnesium ions suggests that the ATP reactions mentioned may involve the formation of certain tetraphosphate intermediates, like



$$B\cdot O\cdot\underset{HO}{\overset{\overset{O}{\uparrow}}{P}}\cdot O\sim\underset{\underset{A}{O}}{\overset{\overset{O}{\uparrow}}{P}}\cdot O\sim\underset{OH}{\overset{\overset{O}{\uparrow}}{P}}\cdot O\sim\overset{\overset{O}{\uparrow}}{P}(OH)_2$$

I



$$A\cdot O\cdot\underset{HO}{\overset{\overset{O}{\uparrow}}{P}}\cdot O\sim\underset{HO}{\overset{\overset{O}{\uparrow}}{P}}\cdot O\sim\underset{OH}{\overset{\overset{O}{\uparrow}}{P}}\cdot O\sim\underset{OH}{\overset{\overset{O}{\uparrow}}{P}}\cdot O\cdot A$$

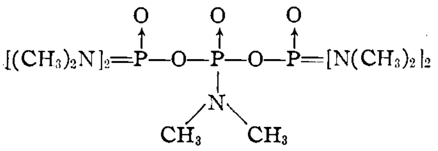
II

A = adenosine, B = nicotinamide ribose, riboflavin and pantethine, respectively

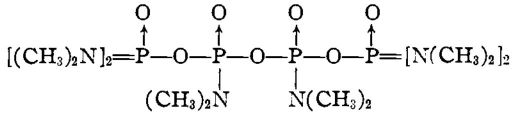
If two of the three energy-rich bonds shown in I and II are protected by chelation, hydrolytic cleavage of the remaining high energy bond may yield the reaction products, or again the reactants.

In view of the enzymatic ATP reactions cited it appeared interesting to investigate potential non-catalytic reactions of some organic polyphosphoramides with an organic monophosphoramide. The investigation described in the following was carried out with the compounds

Phosphate donors

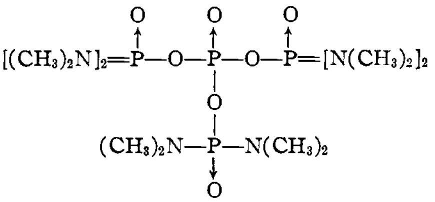


decamethyltriphosphoramide, III



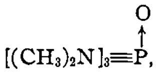
$$[(CH_3)_2N]_2{=}\overset{\overset{O}{\uparrow}}{P}{-}O{-}\underset{(CH_3)_2N}{\overset{\overset{O}{\uparrow}}{P}}{-}O{-}\underset{N(CH_3)_2}{\overset{\overset{O}{\uparrow}}{P}}{-}O{-}\overset{\overset{O}{\uparrow}}{P}{=}[N(CH_3)_2]_2$$

linear dodecamethyltetraphosphoramide, IV



pyramidal dodecamethyltetraphosphoramide, V

Phosphate acceptor

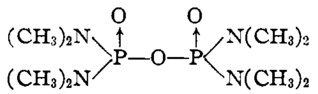


$$[(CH_3)_2N]_3{\equiv}\overset{\overset{O}{\uparrow}}{P}$$

hexamethylphosphoramide, VI

Before treating these phosphate donor–acceptor systems at higher temperature it was necessary to investigate the thermal behavior of the phosphate donors themselves.

Thermal Degradation of Polyphosphoramides.—The compounds III, IV and V decompose at temperatures not much higher than their formation temperature in thermal synthesis. This degradation was investigated at about 160° and the mixture of the degradation products separated by molecular distillation. The triphosphoramide III and the linear tetraphosphoramide IV were found to give a distillate consisting essentially of a single product, octamethylpyrophosphoramide (VII)



VII

It was obtained in a quantity of 0.31 and 0.28 mole per mole polyphosphate III and IV, respectively. No volatile products other than VII and a small amount of VI were found. Thus, it appears the thermal degradation of III and IV, respectively, essentially follows the equation

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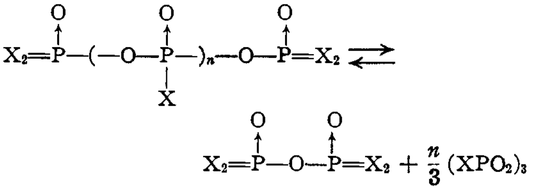
[1] H. M. Kalckar, *J. Biol. Chem.*, **148**, 127 (1943).

[2] A. Kornberg and W. E. Pricer, *ibid.*, **191**, 535 (1951).

[3] A. W. Schrecker and A. Kornberg, *ibid.*, **182**, 795 (1950).

[4] G. D. Novelli, J. D. Gregory, R. M. Flynn and F. J. Schmetz, Jr., *Federation Proc.*, **10**, 229 (1950).

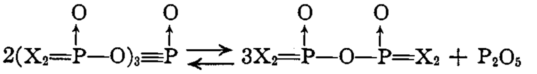
[5] L. Levintow and G. D. Novelli, paper presented at the 122nd A.C.S. Meeting, Atlantic City, N. J., Sept. 17, 1952.



$$X_2{=}\overset{\overset{O}{\uparrow}}{P}-(-O-\underset{\underset{X}{|}}{\overset{\overset{O}{\uparrow}}{P}}-)_n-O-\overset{\overset{O}{\uparrow}}{P}{=}X_2 \rightleftharpoons X_2{=}\overset{\overset{O}{\uparrow}}{P}-O-\overset{\overset{O}{\uparrow}}{P}{=}X_2 + \frac{n}{3}(XPO_2)_3 \quad (a)$$

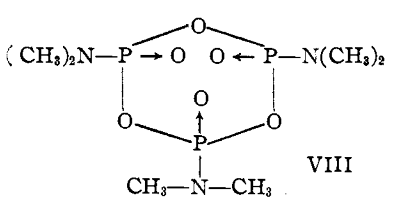
(X = dimethylamine radical, n = 1 or 2)

The thermal degradation of the pyramidal tetraphosphate V at 155° was found to give the same distillate VII in a slightly lower yield. The volatile products formed consisted of VII, a small amount of VI and traces of P_2O_5. In analogy to equation (a) the following over-all equilibrium equation appears suitable to characterize the degradation of V

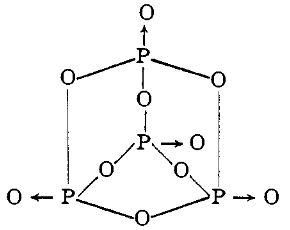


$$2(X_2{=}\overset{\overset{O}{\uparrow}}{P}-O)_3{\equiv}\overset{\overset{O}{\uparrow}}{P} \rightleftharpoons 3X_2{=}\overset{\overset{O}{\uparrow}}{P}-O-\overset{\overset{O}{\uparrow}}{P}{=}X_2 + P_2O_5 \quad (b)$$

The hypothetical by-products in the equations (a) and (b) have the structure[6,7]

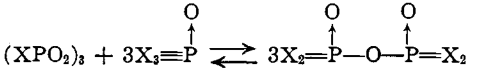


VIII



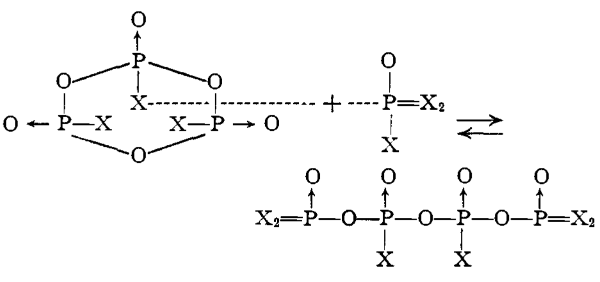
IX

Phosphorylations with Polyphosphoramides and Polymetaphosphates.—One mole of dimethylamidopolymetaphosphate (VIII) and one mole of hexamethylphosphoramide (VI) can, theoretically, form one mole of octamethylpyrophosphoramide (VII)



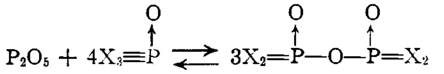
$$(XPO_2)_3 + 3X_3{\equiv}\overset{\overset{O}{\uparrow}}{P} \rightleftharpoons 3X_2{=}\overset{\overset{O}{\uparrow}}{P}-O-\overset{\overset{O}{\uparrow}}{P}{=}X_2 \quad (c)$$

The reaction of these components at 150° actually gave the expected compound VII in a yield of 30%. The first intermediate in this reaction probably is a linear tetraphosphoramide, already known (IV)



This intermediate forms the pyrophosphoramide VII according to equation (a).

The reaction of one mole of P_2O_5 and four moles of hexamethylphosphoramide could, theoretically, yield three moles of octamethylpyrophosphoramide



$$P_2O_5 + 4X_3{\equiv}\overset{\overset{O}{\uparrow}}{P} \rightleftharpoons 3X_2{=}\overset{\overset{O}{\uparrow}}{P}-O-\overset{\overset{O}{\uparrow}}{P}{=}X_2 \quad (d)$$

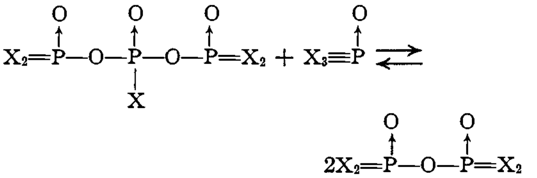
The product resulting from the thermal reaction of these components contained the pyrophosramide VII in an amounts corresponding to a yield of 25%. An analogous reaction with triethyl phosphate in place of hexamethylphosphoramide has been disclosed in the patent literature.[8] Although an exact proof was not possible, it appeared that the trimeric metaphosphate VIII is an intermediate in this reaction, *viz.*

$$P_4O_{10} + 2X_3PO \longrightarrow 2(XPO_2)_3$$

This cyclic metaphosphate VIII forms the pyrophosphoramide VII according to equation (c).

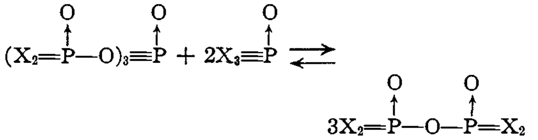
The reactions of the polyphosphoramides III and V, respectively, with the monophosphate VI gave the following results.

One mole of the triphosphoramide III and 1.5 moles of hexamethylphosphoramide (VI) reacted at 155° with formation of octamethylpyrophosphoramide (VII). It could be isolated in a yield of 60%, based on the equation



$$X_2{=}\overset{\overset{O}{\uparrow}}{P}-O-\underset{\underset{X}{|}}{\overset{\overset{O}{\uparrow}}{P}}-O-\overset{\overset{O}{\uparrow}}{P}{=}X_2 + X_3{\equiv}\overset{\overset{O}{\uparrow}}{P} \rightleftharpoons 2X_2{=}\overset{\overset{O}{\uparrow}}{P}-O-\overset{\overset{O}{\uparrow}}{P}{=}X_2 \quad (e)$$

One mole of the pyramidal tetraphosphoramide V and two moles of hexamethylphosphoramide at about 150° also formed the pyrophosphate VII. Its yield amounted to 49%, based on the equation.



$$(X_2{=}\overset{\overset{O}{\uparrow}}{P}-O)_3{\equiv}\overset{\overset{O}{\uparrow}}{P} + 2X_3{\equiv}\overset{\overset{O}{\uparrow}}{P} \rightleftharpoons 3X_2{=}\overset{\overset{O}{\uparrow}}{P}-O-\overset{\overset{O}{\uparrow}}{P}{=}X_2 \quad (f)$$

Conclusions

The aliphatic amidopolyphosphates and polymetaphosphates investigated react with an amidomonophosphate in essentially the same manner as adenosine triphosphate and ribose monophosphates, *i.e.*, in either case an equilibrium reaction results in the formation of pyrophosphates. Non-catalytic as well as enzymatic transphosphorylations evidently represent the same type of reaction.

Enzymatic as well as non-catalytic transphosphorylations favor a redistribution of energy-rich phosphate bonds accumulated in the polyphosphate. This redistribution tendency is caused probably by opposing resonance and electrostatic repulsion at the site of the P–O–P bonds accumulated in the phosphate donor molecule.[9,10] This does not imply, however, the same reaction mechanism in either kind of transphosphorylation.

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(6) A. Michaelis, *Ann.*, **326**, 191 (1903).

(7) G. C. Hampson and A. J. Stosick, THIS JOURNAL, **60**, 1814 (1938).

(8) W. H. Woodstock, U. S. Patent 2,402,703 (1946).

(9) P. Oesper, *Arch. Biochem.*, **27**, 255 (1950).

(10) T. L. Hill and M. F. Morales, THIS JOURNAL, **73**, 1656 (1951).

Experimental

Starting products	$n^{20}D$	d^{20}_4	B.p. (5 mm.), °C.
Hexamethylphosphoramide $[(CH_3)_2N]_3PO$	1.4582	1.0253	102
O-Methyl dimethylamidochlorophosphate $(CH_3)_2NP(O)(OCH_3)Cl$	1.4430	1.2510	72
			Mol. wt.
Decamethyltriphosphoramide $\{[(CH_3)_2N]_2P(O)O\}_2P(O)N(CH_3)_2$	1.4644	1.1931	405
Linear dodecamethyltetraphosphoramide $\{[(CH_3)_2N]_2P(O)OP(O)N(CH_3)_2\}_2O$	1.4719	1.2561	510
Pyram. dodecamethyltetraphosphoramide $\{[(CH_3)_2N]_2P(O)O\}_3PO$	1.4681	1.2471	487

Thermal Degradation of Polyphosphates.—Fifteen grams of **decamethyltriphosphoramide** was heated for two hours at 155° with agitation. The weight and nitrogen content of the mixture (15.0 g. and 17.2%, respectively) did not change, but the molecular weight decreased from 405 to 371 and the refractive index (D line at 20°) increased from 1.4644 to 1.4680.

The infrared spectrum of the heated product was different from that of the starting product and indicated the presence of octamethylpyrophosphoramide.

The heated triphosphate (11.8 g.) was subjected to molecular distillation. The distillate weighed 3 g. and contained according to infrared analysis about 90% pyrophosphate VII (31.4%) besides hexamethylphosphoramide; weight of the distillation residue, 8.7 g.

Eighteen grams of **linear dodecamethyltetraphosphoramide** was heated for 1.5 hours at 160°. The weight and nitrogen content were not altered (18.0 g., 16.2%, respectively) but the molecular weight decreased from 510 to 397. The refractive index remained fairly constant ($n^{20}D$ 1.4719 → 1.4712). Infrared analysis of the heated product: result analogous to that of the heated decamethyltriphosphoramide.

Molecular distillation of 14.5 g. of the heated tetraphosphate gave 2.9 g. of a distillate which contained about 80% pyrophosphate VII besides hexamethylphosphoramide, according to infrared (yield 28%); weight of the distillation residue, 11.4 g.

Seventy-five grams of **pyramidal dodecamethyltetraphosphoramide** was heated for 2.5 hours at 155°. Weight and nitrogen content were found to be unchanged by this treatment. The molecular weight was 487 before and 376 after heating. The infrared pattern of the heated product pointed to the presence of the pyrophosphate VII. The insecticidal activity of the heated product was found to be different from that of the unheated one.

Molecular distillation of 45 g. of the heat-treated product yielded 11.5 g. of a distillate containing 75% of pyrophosphate VII, besides hexamethylphosphoramide and traces of P_2O_5, according to infrared analysis; pyrophosphate yield 22.3%; weight of the distillation residue, 32.8 g., conditions of the molecular distillations carried out: pressure 1.0 to 0.5 mm., temperature 70 to 120°.

Phosphorylations with the Polymetaphosphates VIII and IX.—To investigate the reaction between the trimeric **dimethylamido polymetaphosphate** $[(CH_3)_2NPO_2]_3$ (VIII) and hexamethylphosphoramide (VI), according to equation c, the compound $(CH_3)_2NPO(OCH_3)Cl$ was used. According to Michaelis,[6] this amido chlorophosphate forms the trimer $[(CH_3)_2NPO_2]_3$ plus methyl chloride when heated.

A mixture of 13.5 g. (0.0857 mole) of O-methyl dimethylamidochlorophosphate with 15.4 g. (0.086 mole) of hexamethylphosphoramide (VI) was heated with agitation for two hours at 150°. Amount of methyl chloride released, 3.8 g. (87.5%), molecular weight: found 291, theory 286. Infrared analysis of the dark brown, liquid reaction product: 30% of pyrophosphate VII present. An insecticidal test of this crude material showed an activity similar to that of commercial octamethylpyrophosphoramide.

Four grams (0.0282 mole) of **phosphorus pentoxide** was added to 20.3 g. (0.113 mole) of hexamethylphosphoramide (VI). This mixture was heated at 145° with agitation. After about 50 minutes the insoluble pentoxide had dissolved. The homogeneous mixture was heated an additional hour at 160°; product yield, 24.3 g. (theory 24.3 g.). Infrared analysis of the dark brown liquid: about 25% of pyrophosphate VII present; molecular weight found, 304 (theory 286.3). The insecticidal activity of this product was found to be only slightly different from that of commercial octamethylpyrophosphoramide.

Phosphorylations with the Polyphosphates III and V.—A mixture of 78.7 g. (0.02 mole) of **decamethyltriphosphoramide** (III) with 53.8 g. (0.03 mole) of hexamethylphosphoramide (VI) was heated for five hours with agitation at 150°. The dark brown reaction mixture obtained was separated by vacuum distillation. The residue consisted of 32 g. of an unidentifiable polyphosphate mixture. The distillate weighed 98 g. and contained according to infrared analysis 70% of the pyrophosphate VII. This represented a yield of 60%.

A mixture of 60 g. (0.12 mole) of **pyramidal dodecamethyltetraphosphoramide** (V) with 43 g. (0.24 mole) of hexamethylphosphoramide was heated 2.5 hours with agitation at 155°, separated and analyzed as already described; residue, 26 g. The distillate (75 g.) contained 67.5% of pyrophosphate VII, by infrared; product yield, 49%. Conditions of the vacuum distillations carried out: pressure 2 to 1.0 mm., temperature 85 to 130°.

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