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Monomeric Metaphosphates

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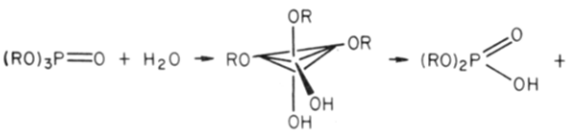
Introduction

Phosphate esters are among the most important intermediary metabolites, and phosphorylation is the key to biochemical synthesis. The immediate transducer of biochemical energy is adenosine triphosphate (ATP), which supplies the driving force for synthesis, muscle action, active transport, and nerve action. The mechanisms of the chemical reactions of ATP, as well as the enzymic ones, and in fact of all reactions that involve the formation and destruction of phosphate esters are vital to the understanding of living systems.

Two major mechanisms (and at least one minor one), together with some hybrid mechanisms, have been suggested for the hydrolysis of phosphate esters and anhydrides. In the more important of these, tetracoordinated phosphorus expands its coordination number to five; usually a pentacoordinated trigonal-bipyramidal intermediate is formed that then decomposes, with or without a "pseudorotation", to yield product[1-3] (eq 1).



ROH (1)

The reaction roughly parallels the normal hydrolysis of esters of carboxylic acids, where the coordination number of the ester carbon atom increases from three to four to produce a tetrahedral intermediate. In the second major mechanism for the hydrolysis of phosphate esters, the coordination number of phosphorus decreases from four to three, to produce a "metaphosphate", which then adds a nucleophile to yield product[4-10] (eq 2 and 3). This mechanism roughly parallels the acylium ion

$$ROPO_3H^- \rightarrow ROH + [PO_3^-] \quad (2)$$

$$[PO_3^-] + H_2O \rightarrow H_2PO_4^- \quad (3)$$

process for the hydrolysis of carboxylic acid esters. In addition to these "pure" mechanisms, concerted, borderline, or "merged" mechanisms come under consideration,[11-14] mechanisms where the transition state for the transfer of a phosphate residue is comprised of all the components of a displacement reaction but where the transition state could be described as a resonance hybrid to which a monomeric metaphosphate makes a sizeable contribution.

Frank H. Westheimer, Morris Loeb Professor of Chemistry at Harvard, received his bachelor's degree from Dartmouth in 1932 and Ph.D. from Harvard, where he worked with James Bryant Conant and E. P. Kohler, in 1935. After a year's postdoctoral research with L. P. Hammett at Columbia, he served from 1936 to 1953 (with time out during WWII) on the staff at the University of Chicago and then in the Chemistry Department at Harvard. His research in physical-organic and bioorganic chemistry includes a theory of electrostatic effects, molecular mechanics, mechanisms of chromic acid oxidation and of chemical and enzymic decarboxylations, discovery of direct and stereospecific transfer of hydrogen in enzymic oxidation–reductions, mechanisms of hydrolysis of phosphate esters, and the invention of photoaffinity labeling.

The hydrolysis of phosphate esters by way of hexacoordinated phosphorus compounds has also been suggested,[15-17] and verified in at least one instance;[18] the mechanism however, is probably a minor one. In any event it is not the subject of this review, the objective of which is to summarize recent evidence, especially from our laboratory, on the formation of monomeric metaphosphates and on their chemical properties.

Thermodynamics

Before, however, these data are presented, a few words about metaphosphates are needed. The equilibrium of eq 4 lies far to the right; Guthrie[19,20] has

$$[PO_3^-] + H_2O \rightleftharpoons H_2PO_4^- \quad (4)$$

estimated (by an indirect but reasonable method) that the standard free energy is −26 kcal/mol. By contrast, orthonitric acid is unknown; the hydrate of nitric acid

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has been identified by Raman spectroscopy[21] as hydronium nitrate (eq 5).

$$HNO_3 + H_2O \rightleftharpoons H_3O^+ + NO_3^- \rightleftharpoons H_3NO_4 \quad (5)$$

What is "wrong" with monomeric metaphosphate ion? Why isn't it stable? Why are only polymetaphosphates known?

A number of quantum mechanical calculations[22,23] have been made that purport to show that the monomeric metaphosphate ion is quite stable. [The statement in one paper that the kinetic barrier to the addition of a nucleophile to metaphosphate is low is at best irrelevant to the thermodynamic problem.] The answer lies, in all probability, not in the instability or reactivity of metaphosphate ion but in the stability of orthophosphate and in the corresponding lack of stability of orthonitrate.

The equilibrium between metaphosphate and orthophosphate depends on the relative strengths of single and double bonds between phosphorus and oxygen; the corresponding equilibrium in the nitrate series similarly depends on the relative strengths of single and double bonds between nitrogen and oxygen. Phosphorus to oxygen single bonds are certainly much stronger than those between nitrogen and oxygen.[24] Both nitric acid and orthonitric acid must have polar oxygen to nitrogen bonds, as in $HON(=O)^+O^-$ and $(HO)_3N^+—O^-$; no 2d orbitals are available to participate in the bonding. By contrast, the phosphorus to oxygen bonds in both meta- and orthophosphoric acid can be stabilized by major participation from the 3d orbitals on phosphorus.[25,26] Detailed calculations for these equilibria are not yet available, but will presumably verify the great relative stability of orthophosphate.

Preparations and Product Studies

PO_3^- in the Gas phase. Mass spectrometric investigations strongly implied the existence of monomeric metaphosphates in the gas phase. Mass spectral peaks corresponding to the mass number of various protonated metaphosphates have been reported, and in particular, masses corresponding to protonated metaphosphoric acid, $H_2PO_3^+$, and to protonated methyl metaphosphate, $CH_3OPO_2H^+$, were observed.[27-29] The structure of ions of these compositions are not, however, certain, since they might be hydrogen phosphonates and not metaphosphates. But in 1979, Hass, Bursey, Ramirez, Meyerson, and co-workers[30] observed PO_3^- in the negative ion mass spectrometer, and by determining the exact mass of the particle, clearly differentiated it from ions of the composition CH_4PO_2 that have the same mass number. Further, no structure other than metaphosphate is reasonable for PO_3^-.

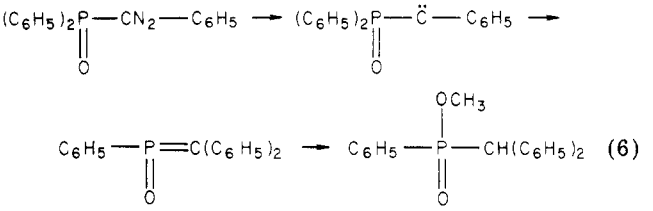
Nitrogen and Carbon Analogues of Monomeric Metaphosphates. Niecke and Flick and Scherer and Kuhn have prepared a nitrogen analogue of monomeric metaphosphate[31,32] (A) and Niecke et al. determined its

$$[(CH_3)_3Si]_2NP[=NSi(CH_3)_3]_2$$
A

X-ray structure.[33] The phosphorus and the three nitrogen atoms lie in a plane, as might be expected; the P=N "double bonds" average 1.49 Å in length. They also prepared an alkylidene analogue of metaphosphate with one phosphorus to nitrogen and one phosphorus to carbon "double bond",[34] as in B. This latter compound is a liquid; its structure has not yet been confirmed by X-ray methods.

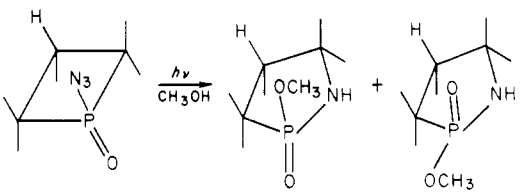
$$[(CH_3)_3Si]_2NP[=NSi(CH_3)_3][=C(CH_3)_2]$$
B

Many similar compounds have been postulated as intermediates in various chemical reactions. Regitz and his co-workers[35-39] have carried out the photochemical decomposition of diazophosphonates and obtained rearranged products best accounted for by postulating intermediates with tricoordinated pentacovalent phosphorus, e.g., eq 6.



(6)

In our own laboratory, Wiseman[40,41] carried out the photochemical rearrangement of the cyclic trans phosphinate azide shown in eq 7, and of the corresponding cis isomer. The ratio of cis to trans products, and ratio of yields of the principal byproducts were the same when either the cis or trans azide was photolyzed. Those results suggest a five-membered cyclic nitrogen analogue of metaphosphate with planar nitrogen as a common intermediate (eq 8).



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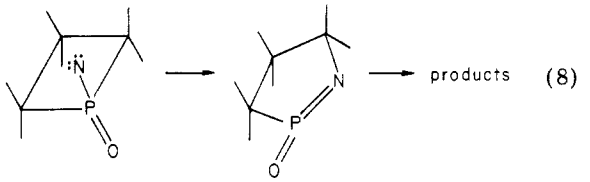

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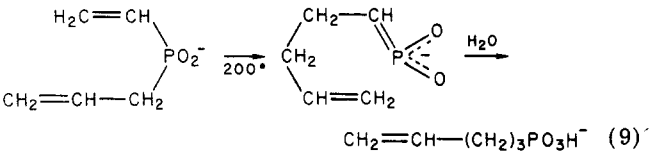
Another example from our laboratory comes from the phospho-Cope rearrangement discovered by Loweus;[42,43] the probable pathway for this process is shown in eq 9 and involves an alkylidene analogue of monomeric metaphosphate.



(9)

Kinetics

Reaction Rates. Many of the kinetic studies that have been explained by recourse to monomeric metaphosphate or to its analogues have been discussed in some detail in previous publications and need therefore only be briefly mentioned here.[4-10] The pH–rate profiles for the hydrolysis of monoesters of phosphoric acid go through a prominent maximum[44-46] around pH 4. The concentration of monoester monoanions, $ROPO_3H^-$, peaks at that pH, and the profiles can be

accounted for on the assumption that the rate constant for hydrolysis of the monoester monoanions greatly exceeds those for the hydrolysis of the diacids or the dianions.[6] A reasonable mechanism for the hydrolysis is then[5,6]

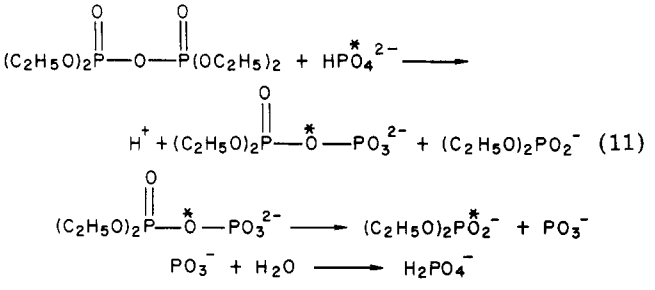
$$ROPO_3H^- \rightleftharpoons RO(H)^+—PO_3^{2-} \rightarrow ROH + [PO_3^-] \quad (10)$$

$$[PO_3^-] + H_2O \rightarrow H_2PO_4^- \quad (3)$$

The kinetics of the hydrolysis of the monoanions and (when appropriate) the dianions of acyl phosphates, phenyl phosphate, nitrophenyl phosphate, 2,4-dinitrophenyl phosphate, and phosphoramidates have been studied in detail.[47-56] In particular,[48] the entropy of activation for the hydrolysis of acetyl phosphate dianion and of acetyl phenyl phosphate monoanion are +3.8 and −28.8 eu, respectively; the former value is typical of unimolecular decompositions, whereas the latter corresponds to bimolecular displacements with water. The volume of activation for the hydrolysis of acetyl phosphate dianion is −1 cm^3/mol, whereas that for acetyl phenyl phosphate monoanion is −19 cm^3/mol; the relatively positive value for the dianion is again diagnostic for a unimolecular cleavage process rather than for a bimolecular reaction. Other data (isotope effects, structure–reactivity correlations, the formation of pyrophosphate from reaction in media with low water activity) again point to a monomeric metaphosphate mechanism for the hydrolysis of various dianions.

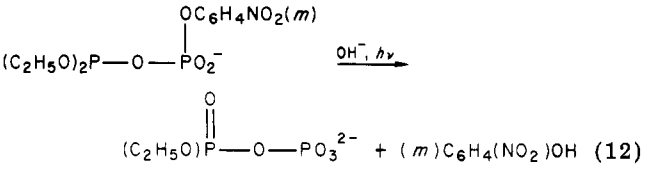
Not everything about these reactions, however, is clear. 2,4-Dinitrophenyl phosphate undergoes second-order hydrolysis[53] in the presence of tertiary amines. Although the second-order kinetics demands that the amine take part in the rate-limiting step, rate constants are independent of the basicity of the amines, from amines with pK of 0 to amines with pK of 10. The reaction then is a nucleophilic displacement that occurs at the same rate regardless of the nucleophilicity of the incoming reagent; thus the data almost seem to involve an internal contradiction. The reaction can probably best be considered an example of enforced catalysis, or a "merged" mechanism in the sense explained by Jencks.[11-14] Such processes are discussed in a later section of this review.

The hydrolysis of tetraethyl pyrophosphate in the presence of HPO_4^{2-} likewise presumably proceeds by way of monomeric metaphosphate ion. Brown and Hamer[57] found that the reaction is second order (first order in tetraethyl pyrophosphate and first order in HPO_4^{2-}). Subsequently, Samuel and Silver,[58] using monohydrogen phosphate dianion labeled with ^{18}O, showed that the reaction is accompanied by the transfer of one oxygen atom from the inorganic phosphate to a

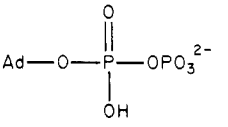


molecule of diethyl phosphate. These facts suggest the chemistry of eq 11.

This mechanism postulates unsymmetrical diethyl pyrophosphate as an intermediate. At the time, no example of an unsymmetrical disubstituted pyrophosphate was known, so that it was uncertain whether such a molecule would decompose rapidly enough to lie along the kinetic pathway for the catalyzed hydrolysis. Subsequently, Miller and Ukena[59] prepared unsymmetrical diethyl pyrophosphate by a photochemical alkaline hydrolysis modeled on the researches of Havinga[60,61] (eq 12). The unsymmetrical diester did in



fact hydrolyze spontaneously. The rate constant for the process was large enough to accommodate the results of Brown and Hamer; unsymmetrical diethyl pyrophosphate then could be an intermediate in the phosphate-catalyzed hydrolysis of the corresponding tetraethyl ester. Miller also measured the rate of hydrolysis of the monoacid dianion of ADP and similar compounds.[62] He then estimated the fraction of that molecule present as the unsymmetrical hydrogen pyrophosphate, C, where Ad stands for adenosine. The

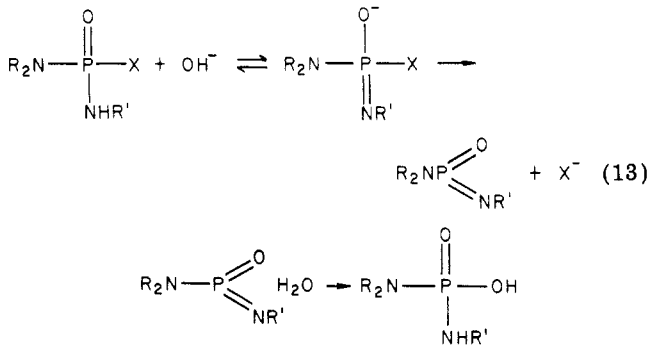


C

rate of acid hydrolysis could be accounted for quantitatively if one assumed that the ion C decomposes with the same rate as does unsymmetrical diethyl pyrophosphate. If, then, the latter hydrolyzes by a monomeric metaphosphate mechanism, presumably the acid-catalyzed chemical hydrolysis of ADP proceeds by this pathway as well.

Isotope Effects. The hydrolysis of a phosphate ester by way of a trigonal-bipyramidal intermediate need not involve the cleavage of a phosphorus–oxygen bond in the rate-limiting step. Consistent with this idea, Gorenstein et al.[63,64] found that the ^{18}O isotope effect in the hydrolysis of $(O_2N)_2C_6H_3{}^{18}OPO(OR)_2$ is less than 1%. By contrast, the hydrolysis of $(O_2N)_2C_6H_3{}^{18}OPO_3^{2-}$ is slowed, relative to the corresponding ^{16}O ester, by 2%. Although this effect is small, it appears that the cleavage of the P–O bond is rate limiting in the hydrolysis of the dinitrophenyl phosphate dianion; these data suggest, although they do not quite demand, that the cleavage takes place to liberate monomeric metaphosphate.

Rates of Hydrolysis of Phosphoramidates. Even more persuasive kinetic evidence for tricoordinated pentacovalent phosphorus compounds has been obtained for nitrogen analogues of monomeric metaphosphate. The alkaline hydrolysis of phosphoramidic fluorides[65] and chlorides[66-68] proceeds much more rapidly with amides of ammonia and of primary amines than with those of secondary amines. These data were interpreted[69] as indicating that the reaction of phosphoramides with one or more hydrogen atoms attached to nitrogen proceeds as shown in eq 13. A later kinetic study showed that the second-order rate constants for the reaction of various nitrogen nucleophiles (including

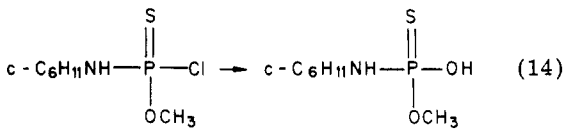


azide ion) with *N,N,N',N'*-tetramethylphosphorodiamidic chloride (D) were within a factor of 3 of those

$$\underset{\text{D}}{[(CH_3)_2N]_2POCl} \quad \cdot \quad \underset{\text{E}}{(C_3H_7NH)_2POCl}$$

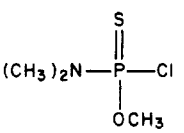
for the corresponding reactions with *N,N'*-dipropylphosphorodiamidic chloride (E) whereas the rate of hydrolysis of the latter with alkali exceeded that for the former by a factor of nearly 10 million.[70] Although the reactions of phosphoramidic chlorides are subject to steric effects,[71,72] these cannot be in question here; for example, the linear azide ion is not likely to have larger steric demands than hydroxide ion; in fact, considering solvation, azide is probably considerably smaller. The data require that a special mechanism obtain for the hydroxide ion catalyzed hydrolysis of *N,N'*-dipropylphosphorodiamidic chloride, a mechanism unavailable to the *N,N,N',N'*-tetramethyl derivative. The mechanism of eq 13, proceeding through a nitrogen analogue of monomeric metaphosphate, provides a reasonable explanation for the data.

Even more convincing evidence for this mechamism has been offered by Gerrard and Hamer;[73,74] in addition, their work helps to defined the boundary between a monomeric metaphosphate mechanism and borderline or concerted processes. The authors resolved phosphoramidic derivatives with different leaving groups into their enantiomers and investigated the rates and stereochemistry of their hydrolyses. For the thiophosphoramidic chloride F, hydrolysis in neutral solution proceeded stereospecifically, whereas the reaction in alkali proceeded with almost complete racemization.



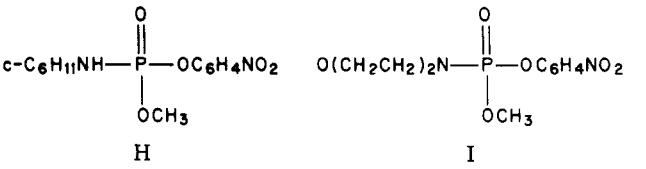
F

Further, the reaction of F in neutral solution proceeded only $^1/_{13}$th as fast as did that of its dimethyl analogue, G, whereas the rate for F in alkali was 45 000 times that for G.



G

By contrast, when *p*-nitrophenolate ion rather than chloride ion was the leaving group, even the reaction in alkali was (almost) stereospecific. The hydrolysis of the cyclohexylamidate H took place about 100 times as rapidly as did that of the corresponding morpholidate,

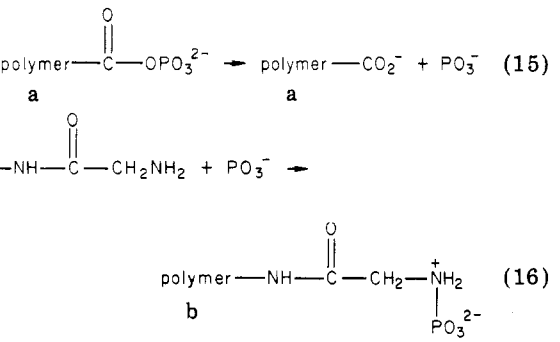


H I

I, but with little racemization. Racemization suggests that the alkaline hydrolysis of F proceeds according to the mechanism of eq 13, by way of a planar intermediate, J, c-$C_6H_{11}N{=}P(=S)OCH_3$, analogous to compound A.[33] The kinetic acceleration in the hydrolysis of H, although it is much less pronounced than that for E or F, suggests that the reaction proceeds by way of an analogue of monomeric metaphosphate; the stereochemical result, however, is that expected for an S_N2 process, with a pentacovalent intermediate (or transition state). Presumably the alkaline hydrolysis of H proceeds by a merged mechanism.

The Three-Phase Test. Rebek[75-78] has devised a general criterion for unstable intermediates, which he has designated as the "three-phase test". A system that generates (or is alleged to generate) an unstable intermediate is attached by covalent bonds to beads of modified polystyrene. A receptor for the unstable intermediate is similarly attached to a different batch of beads. The two sets of beads are then mixed in a solvent and chemical reaction is allowed to occur. Since a bimolecular reaction between the beads is virtually precluded, the appearance of the product of reaction of the intermediate with the receptor is evidence that an unstable intermediate was formed, and was transported through the solvent (perhaps by the solvent) from one set of beads to the other.

In particular, Rebek et al.[77] attached an acyl phosphate to one type of bead (by way of carboxyl groups on the aromatic rings of polystyrene), and glycine residues were attached by their carboxyl groups to the second type of polystyrene beads. The beads were mixed, and the acyl phosphate was allowed to decompose in dioxane as solvent. The amino group of the glycine residues were indeed phosphorylated (eq 16).



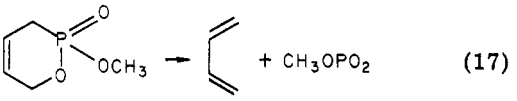
This proves that the PO_3^- residue migrates from one type of bead to the other through the solvent.

In a second experiment, the phosphorylating agent was a polystyrene-bound phosphoramidate. Here the leaving group, an *o*-nitrophenoxy residue, was covalently attached to the polymer; the phosphorus derivative was a bis(cyclohexylamide). Since the amide has hydrogen atoms attached to nitrogen, it is base sensitive (cf. eq 13). In the presence of Proton Sponge, this system transferred a phosphoramide group to an amino receptor on a different type of polystyrene bead in acetonitrile or dioxane as solvent; by contrast, a similar

phosphoramide without hydrogen atoms attached to nitrogen was stable, and did not serve as a phosphorylating agent. Again, a reactive intermediate must necessarily have been released from the original phosphoramidate on one type of bead and must have migrated through the solution, and then become attached at the receptor on a different type of bead. The question of the solvation of the intermediate remains to be explored; that a reactive intermediate (a metaphosphate, metaphosphoramidate, or solvated species) is involved can scarcely come into question.

Monomeric Methyl Metaphosphate

Pyrolysis. In 1974, Clapp[79] reported the preparation of methyl 2-butenylphostonate and its pyrolysis at 600 °C with a contact time of about 0.02 s in a carrier gas of nitrogen at a pressure of about 0.02 mm (eq 17). The

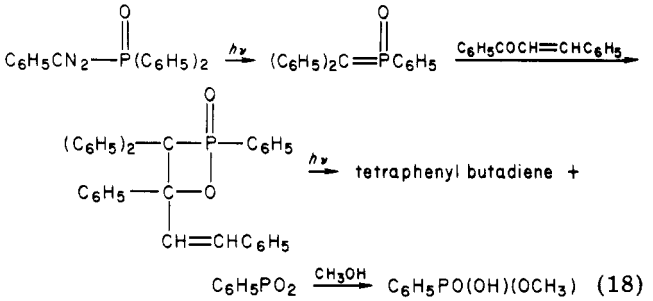


(17)

gas steam was allowed to impinge on the surface of a trap cooled in liquid nitrogen. A good yield of butadiene was obtained in the pyrolysis; the other material that collected on the glass surface at −195 °C was polymerized methyl metaphosphate. When the gas stream containing the products of pyrolysis of methyl 2-butenylphostonate (eq 17) was introduced into a rapidly stirred solution of *N*-methylaniline in butylbenzene as solvent at −78 °C, the major product[79] was the expected *N*-methylphosphoramidate.

$$CH_3OPO_2 + C_6H_5NHCH_3 \rightarrow C_6H_5NH(CH_3)^+PO_3^{2-}$$

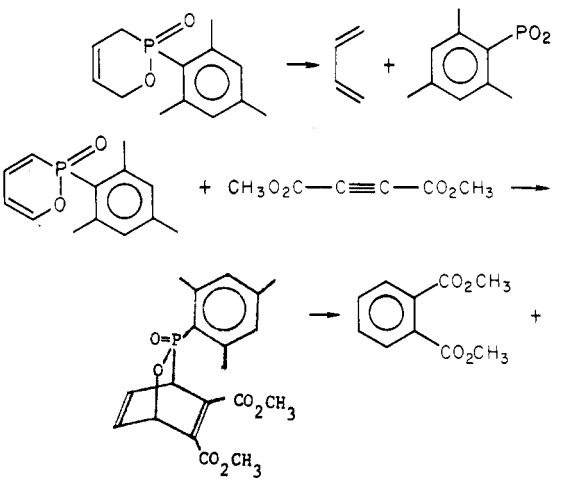
Phosphonobenzene is related to nitrobenzene as monomeric metaphosphate ion is related to nitrate ion. Phosphonobenzene was prepared by the pyrolysis of 1-phenyl-1-(2-butenyl)phostinate by the same technique and with the same sort of results as with the pyrolysis of methyl 2-butenylphostonate.[80] Further, Eckes and Regitz[81] have shown that the photolysis of diphenyl(1-diazo-1-phenylmethyl)phosphine oxide proceeds with rearrangement, presumably through a metaphosphate-type intermediate, to yield material that behaves as expected for phosphonobenzene (eq 18).



(18)

In our laboratories, Sigal and Loew tried to prepare phosphonomesitylene by the pyrolysis of the corresponding phostinate and by reaction of mesityl 2,4-butadienylphostinate with dienophiles,[82] as shown in eq 19 and 20, respectively.

Although the investigators had hoped that steric hindrance to hydration and to polymerization would



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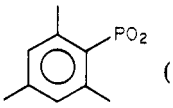
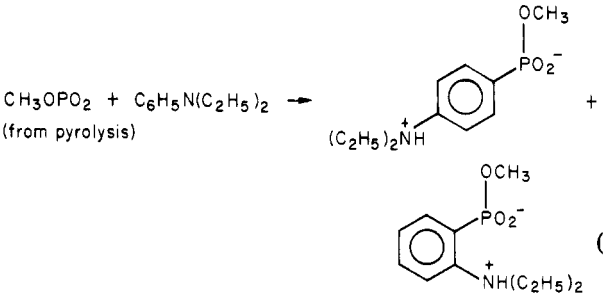


(20)

permit the isolation of phosphonomesitylene, the only products obtained were the polymer and mesitylenephosphonic acid.

Aromatic Substitution. The most exciting result of the experiment where monomeric methyl metaphosphate was introduced into a solution of methylaniline was the appearance of a minor product that could be separated by TLC from the phosphoramidate. The ^1H NMR spectrum of this product identified it as methyl *p*-(methylamino)benzenephosphonic acid, a product of the electrophilic attack of monomeric methyl metaphosphate on the aromatic ring.[83] When *N,N*-diethylaniline, rather than *N*-methylaniline, is used to trap the metaphosphate, both *o*- and *p*-(diethylamino)benzenephosphonic acids are formed (eq 21).



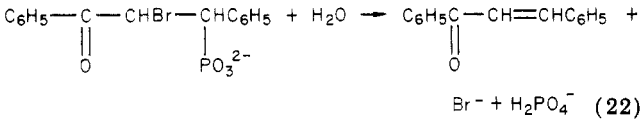
(21)

The yield of aromatic substitution products obtained in these experiments is low—only about 5%—but the identification is unambiguous.

Some question must necessarily arise as to the temperature at which the electrophilic substitution occurs. The gas stream is at 600 °C, but is at only 0.02-mm pressure, so its specific heat is minute. The butylbenzene solution is stirred at −78 °C, and of course has a high specific heat. The temperature of the complex between monomeric methyl metaphosphate and diethylaniline at the moment of reaction is not known and is not easy to determine. Quite possibly the reaction has taken place at a low temperature, but that cannot be guaranteed.

Conant–Swan Reaction. Monomeric methyl metaphosphate can, however, be prepared in solution under more precisely controlled conditions by the Conant–Swan fragmentation. In the 1920s Conant and his co-workers[84-87] synthesized several β-halophosphonates

and -phosphinates and found that their anions decompose in aqueous solution; for example,



$$Br^- + H_2PO_4^- \quad (22)$$

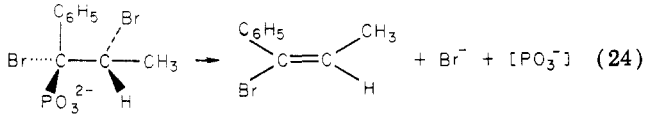
At the time, of course, the idea of monomeric metaphosphates or of phosphonobenzene as an intermediate did not arise.

In 1963, Maynard and Swan[88,89] rediscovered the fragmentation, as a general reaction of simpler β-halophosphonates, and recognized Conant's priority. An example of the work of the Australian investigators is the fragmentation of 2-chlorooctylphosphonate in the presence of *tert*-butyl alcohol to yield *tert*-butyl phosphate (eq 23). Phosphate esters of tertiary alcohols are

$$C_6H_{13}CHClCH_2PO_3^{2-} + (CH_3)_3COH \rightarrow C_6H_{13}CH{=}CH_2 + (CH_3)_3COPO_3H^- + Cl^- \quad (23)$$

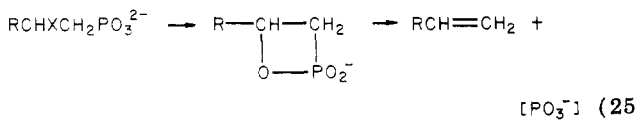
not readily prepared by conventional means, and the simplest explanation for the preparation is that it proceeds by way of a fragmentation of the β-halophosphonate to olefin, chloride ion, and PO_3^- followed by electrophilic attack of the metaphosphate ion on the alcohol. Maynard and Swan, however, looked upon the reaction as a bimolecular process where *tert*-butyl alcohol participates in the fragmentation. The research outlined in the following paragraphs strongly suggests that the metaphosphate is as free as possible for such compounds or at any rate that the transition state for the fragmentation is a resonance hybrid where the monomeric metaphosphate ion (or its ester, or phosphonobenzene, as appropriate) is a major contributor.

Kenyon later examined the stereochemistry of the Conant–Swan fragmentation. Both the threo and erythro isomers of 1,2-dibromo-1-phenylpropanephosphonic acid were prepared and allowed to undergo fragmentation in water or acetonitrile as solvent.[90] The stereochemistry of the dibromo compounds and of the resulting olefins were established:[90,91] the fragmentation, as expected, is trans; e.g.



$$+ Br^- + [PO_3^-] \quad (24)$$

This does not, of course, distinguish fragmentation from displacement at phosphorus, nor does it exclude a four-membered phostone as intermediate; if such an intermediate is formed, however, it presumably falls apart to monomeric metaphosphate; the reaction (if it occurs in this manner) would in some sense parallel the decomposition of the presumed four-membered intermediates in the Wittig reaction (eq 25). The fragmentation of β-bromo-α-styrenephosphonic acid,[87] however, even though it occurs with difficulty,[90] is unlikely to proceed by displacement of the vinylic halogen.



$$[PO_3^-] \quad (25)$$

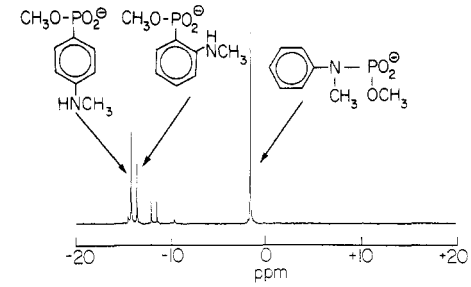


Figure 1. ^{31}P NMR spectrum of the crude reaction product from the fragmentation of the methyl ester of 1-phenyl-1,2-dibromopropylphosphonate in the presence of 2,2,6,6-tetramethylpiperidine in *N*-methylaniline as solvent.

Thus the pathway of eq 24 is more likely than that of eq 25.

The methyl ester of 1,2-dibromo-1-phenylpropane-1-phosphonic acid decomposes, in acetonitrile solution, in the presence of a base at 70 °C with a half-time of the order of 1.5 h. In our study, the base was 2,2,6,6-tetramethylpiperidine, a reasonably strong but sterically hindered amine. The decomposition of the anion (or perhaps ion pair) of the methyl ester of the phosphonic acid, like that of the corresponding acid, is stereospecific;[83] the erythro dibromide yields the *E* olefin, and the threo dibromide yields the *Z* olefin.

When the fragmentation is carried out in the presence of neat *N*-methylaniline, the major reaction products are the phosphoramidate and *o*- and *p*-(methylamino)benzenephosphonic acid. These compounds can be identified by ^{31}P NMR spectroscopy in the crude mixture and can be separated and purified chromatographically; the ^1H NMR spectra of the purified materials are identical with those of authentic synthetic samples. As already noted, aromatic substitution (both ortho and para) was observed when the gas stream from the pyrolysis of methyl 2-butenylphosphonate was introduced into a stirred solution of *N*,*N*-diethylaniline in butylbenzene at −80 °C; the same products were obtained when the methyl ester of either the threo- or the *erythro*-1-phenyl-1,2-dibromopropyl-1-phosphonate underwent fragmentation in diethylaniline. The ^{31}P NMR spectrum of a crude reaction mixture from a fragmentation in *N*-methylaniline is shown in Figure 1, and the ^1H NMR spectra of the products from pyrolysis and from fragmentation in the presence of *N*,*N*-diethylaniline are compared in Figure 2 with that of the synthetic methyl ester of o-(diethylamino)-benzenephosphonic acid.

The ^{31}P NMR spectrum of the usual reaction mixture from *N*-methylaniline shows, in addition to peaks for the phosphoramidate and for the *o*- and *p*-(methylamino)phosphonic acids,[92] numerous other peaks that presumably arise from pyrophosphates or mixed anhydrides of phosphoric acid with the phosphonic acid used as starting material, plus other byproducts discussed in the section on phosphonates. When, however, about 3–5% of methanol is added to the reaction mixture, the many peaks for the pyrophosphates, etc., are essentially eliminated, while a peak for dimethyl phosphate appears in the spectrum of the products. Methanol thus greatly reduces the complexity of the spectra and facilitates their interpretation.

We have proposed that electrophilic attack on an aromatic ring—even a highly activated aromatic ring

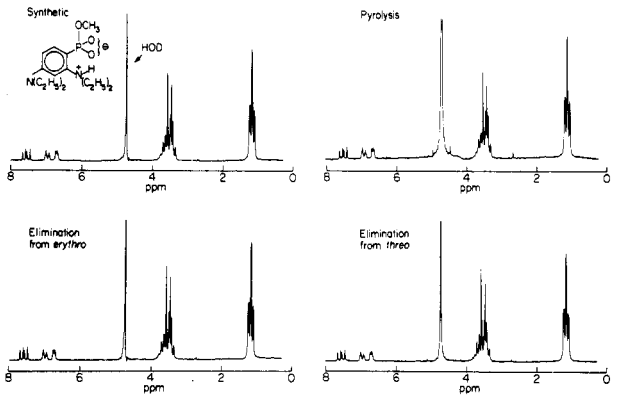


Figure 2. 100-MHz ^1H NMR spectra of four samples of methyl hydrogen 2-(diethylamino)-4-(diethylamino)benzenephosphonate in D_2O; the chemical shifts are given relative to that of sodium 4,4-dimethyl-4-silapentane-1-sulfonate. The spectrum at the upper left is that of product obtained by synthesis; the other three spectra are those of product produced on trapping monomeric methyl metaphosphate. The source of the monomeric methyl metaphosphate (pyrolysis or fragmentation in solution) is shown in the figure. The pyrolysis is that of methyl 2-butenylphostonate; erythro and threo refer to the diastereomers of methyl hydrogen 1-phenyl-1,2-dibromopropylphosphonate.

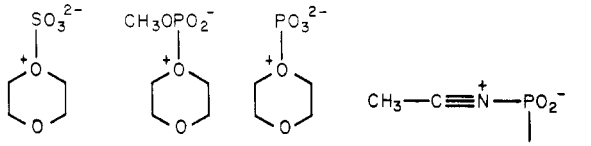
TABLE I. Aromatic Substitution on *N*-Methylaniline by "Monomeric Methyl Metaphosphate"

diluent 9:1 v/v	% aromatic substitution	% phosphoramidate
none	35	50
$CDCl_3$	29	67
CD_3CN	3	85
dioxane	≤2	85

such as that in a substituted aniline—requires the intervention of a strong electrophile and therefore constitutes a diagnostic test for monomeric methyl metaphosphate.

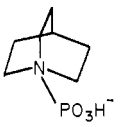
Solvent Effects. The argument in favor of the monomeric metaphosphate intermediate is strengthened by a study of the effect of solvents on aromatic substitution. Satterthwait's data for the attack of monomeric methyl metaphosphate on *N*-methylaniline are shown in Table I. When *N*-methylaniline itself is used as solvent, the fraction of aromatic substitution (*o*- and *p*-phosphonates combined) is about 35%, with most of the rest as phosphoramidate.

The yield of aromatic substitution product is slightly diminished by dilution of the reaction mixture with chloroform but drastically diminished by dilution with acetonitrile or dioxane. Presumably a strong electrophile such as a monomeric metaphosphate will add to the unshared electron pairs of solvents to produce less active phosphorylating or phosphonylating agents. Monomeric metaphosphate ion is isoelectronic with SO_3 and would be expected to add to an oxygen atom of dioxane or to the nitrogen atom of acetonitrile just as SO_3 does; the adduct of SO_3 to dioxane is a well-known, crystalline zwitterion and mild sulfonating reagent.[93,94] The structures would be those of K–N.



K L M N

Although the evidence for such zwitterionic "solvates" of metaphosphate with weak bases is indirect,[55] Ramirez and Maracek[95] have observed the ^{31}P NMR spectrum of the adduct of monomeric metaphosphate and quinuclidine. The solvolysis of 2,4-dinitrophenyl phosphate in the presence of that base gave rise to an unstable intermediate, with ^{31}P δ 10.2, that almost certainly is the zwitterionic species P.



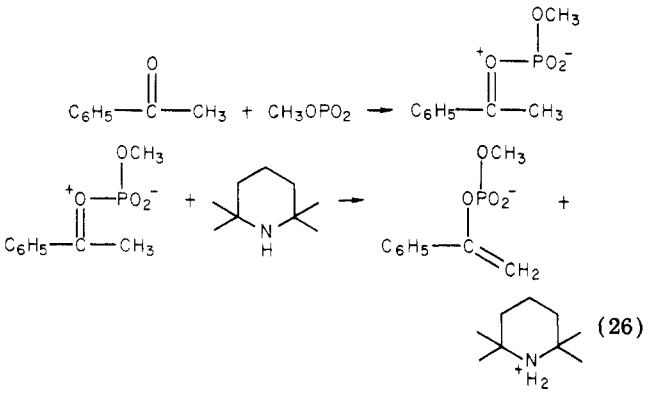
P

In the presence of chloroform, the "solvation" of metaphosphates may involve weak attachment of metaphosphates to the chlorine atoms; such attachment would presumably be readily reversible. The addition of monomeric methyl metaphosphate to dioxane should also be reversible, so the question necessarily arises as to whether phosphorylation in the presence of dioxane occurs by way of a rapid reaction with the minute concentration of monomeric methyl metaphosphate that is free in solution or by way of a slower reaction with the zwitterionic adduct. The answer depends upon the relative magnitude of rate and equilibrium constants that are not at present known. Still, in the presence of dioxane phosphorylation occurs preferentially on the nucleophilic nitrogen atom of the amine, rather than on the aromatic ring, whereas in the absence of dioxane the reaction is remarkably nonselective (Table I). These data then suggest that, in the presence of dioxane, the adduct rather than free metaphosphate is the phosphorylating agent. Monomeric metaphosphate and its nitrogen analogues produced in solution by the "three-phase test"[75–78] are presumably similarly solvated and not "free".

Obviously, all the problems that pertain to the solvation of protons in solution also surface with respect to the solvation of other strong electrophiles, and the backward state of physical chemistry with respect to solvation in general leads to the cicumlocutions here devised as the best current description of the condition of monomeric metaphosphates in solution.

Reaction with Carbonyl Compounds. The reaction of methyl metaphosphate with the unshared electron pairs of the ether oxygen atoms of dioxane suggested that the reagent might also react with the unshared electron pairs of carbonyl groups. This expectation proved fully justified. Satterthwait found that the fragmentation of the methyl ester of 1,2-dibromo-1-phenylpropylphosphonic acid in actophenone[96] in the presence of 2,2,6,6-tetramethylpiperidine led to the formation in good yield of the methyl ester of the corresponding enol phosphate; the reaction presumably takes place according to eq 26.

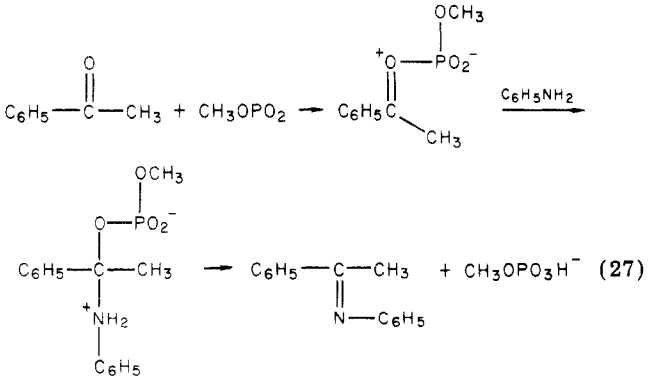
An alternative pathway that must be considered involves the reaction of monomeric methyl metaphosphate with the small amount of enol present in equilibrium with the keto form of acetophenone. Since, however, the amount of enol in equilibrium with monoketones is minute,[97–99] this reaction could occur only if the metaphosphate were highly selective, which is precisely contrary to the facts. Furthermore, if such were the case, presumably the metaphosphate would



(26)

also react with alcohols that mimic the enol. However, when 5% (relative to acetophenone) of either phenol or phenethyl alcohol was added to the reaction mixture, the predominant product was still the enol phosphate. This result was obtained despite the fact that these models for the enol were present in enormous excess over the minute amount of enol in solution and must have swamped any reaction with the enol itself. The pathway in eq 26 is therefore reasonably secure.

When aniline was also added to the reaction mixture, the product was the Schiff base of acetophenone. The yield, however, exceeded that expected stoichiometrically on the basis of the amount of monomeric methyl metaphosphate generated; further, at 70 °C, aniline slowly reacts directly with acetophenone in the presence of 2,2,6,6-tetramethylpiperidinium bromide to yield the Schiff base. Although the metaphosphate-promoted and direct reactions can be dissected, the point can be made more cleanly by using *o*-(trifluoromethyl)aniline as the nucleophile.[96] Under the conditions of the experiment, this weak and sterically hindered amine does not undergo appreciable direct reaction to yield the corresponding Schiff base, but in the presence of the system that generates monomeric methyl metaphosphate the Schiff base is formed in essentially quantitative yield and at the same rate at which monomethyl phosphate is formed. These data point to the mechanism shown in eq 27. The addition of monom-



(27)

eric methyl metaphosphate to the carbonyl group of acetophenone introduces a positive charge on the carbonyl group, analogous to that created in acid catalysis, and activates both the methyl group to loss of a proton and the carbonyl group to nucleophilic attack. The result can be described as acid catalysis in basic solution.

A similar reaction can be carried out with ethyl benzoate and aniline in the presence of the fragmentation system that generates monomeric methyl meta-

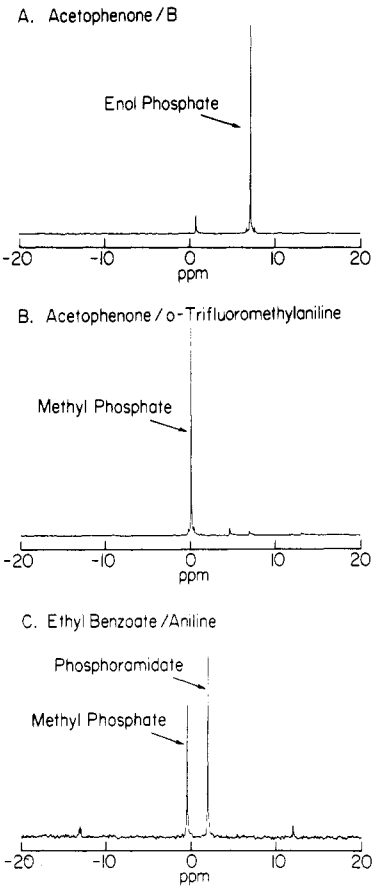
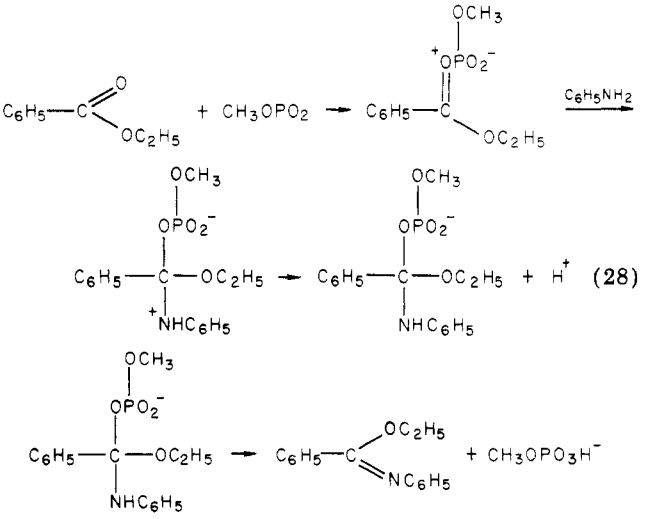


Figure 3. 40.5-MHz ^{31}P NMR spectra of products formed from the fragmentation of 25 mg of methyl hydrogen *erythro*-1-phenyl-1,2-dibromopropylphosphonic acid (I) at 70 °C in (A) 3.0 mL of acetophenone mixed with 50 μL of 2,2,6,6-tetramethylpiperidine, (B) 3.0 mL of acetophenone mixed with 0.30 mL of *o*-(trifluoromethyl)aniline and 50 μL of 2,2,6,6-tetramethylpiperidine, and (C) 3.0 mL of ethyl benzoate mixed with 0.30 mL of aniline and 50 μL of 2,2,6,6-tetramethylpiperidine. Chemical shifts are relative to an external standard of 85% phosphoric acid; upfield shifts are assigned a negative sign.

phosphate. Here the product is ethyl *N*-phenylbenzimidate. The reaction almost certainly takes place as shown in eq 28: direct reaction between ethyl benzoate



(28)

and aniline yields benzanilide, not the benzimidate. Again, the reaction involves the activation of the carbonyl group; the rate of formation of the benzimidate is the same as that for the formation of methyl phosphate.

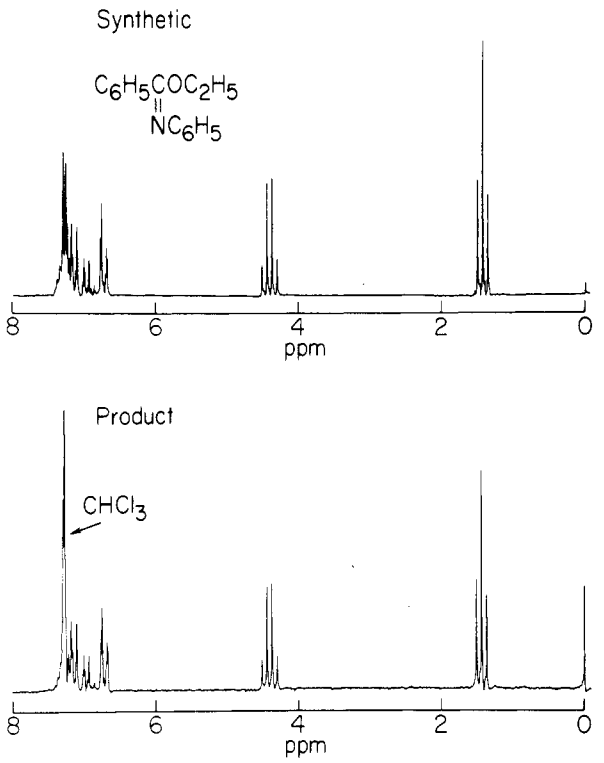


Figure 4. 100-MHz ^1H NMR spectrum of two samples of ethyl *N*-phenylbenzimidate in chloroform-*d*; Me_4Si is at 0 ppm. The upper spectrum is that of an authentic sample of ethyl *N*-phenylbenzimidate. The lower spectrum is that of product purified from fragmentation of 25 mg of methyl hydrogen *erythro*-1-phenyl-1,2-dibromopropylphosphonic acid (I) in 3.00 mL of ethyl benzoate mixed with 0.30 mL of aniline and 50 μL of 2,2,6,6-tetramethylpiperidine.

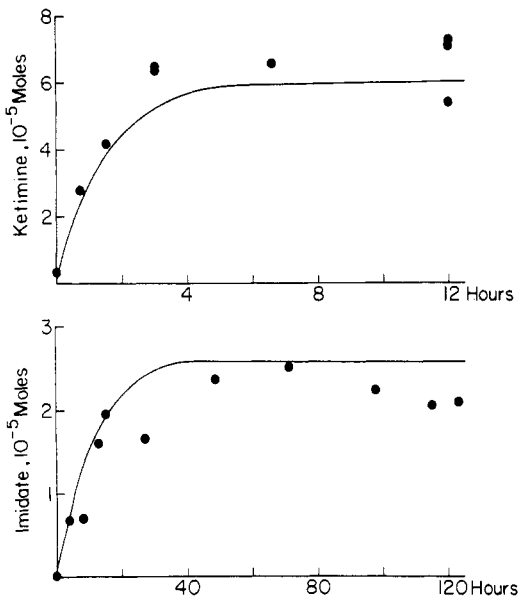


Figure 5. (Upper) Comparison of the rates of formation of methyl phosphate (calculated solid line) and *N*-(1-methylbenzylidene)-2-aminobenzotrifluoride (●) from fragmentation of 25 mg of methyl hydrogen *erythro*-1,2-dibromopropylphosphonate (I) at 70 °C in 3.0 mL of acetophenone mixed with 0.30 mL of *o*-(trifluoromethyl)aniline and 50 μL of 2,2,6,6-tetramethylpiperidine. (Lower) Comparison of the rates of formation of methyl phosphate (calculated solid line) and ethyl *N*-phenylbenzimidate (●) from fragmentation of 25 mg of methyl hydrogen *erythro*-1-phenyl-1,2-dibromopropylphosphonate at 70 °C in 3.0 mL of ethyl benzoate mixed with 0.30 mL of aniline and 50 μL of 2,2,6,6-tetramethylpiperidine. The calculated lines are based on half times of methyl phosphate formation and the final yields.

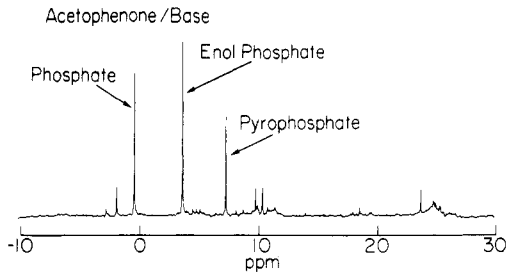


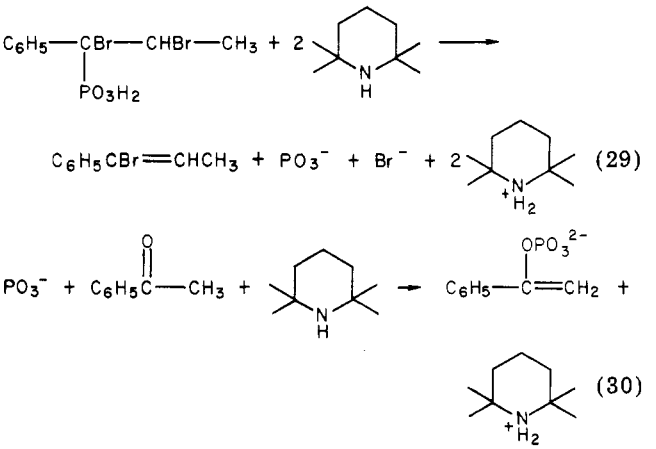
Figure 6. 40.5-MHz ^{31}P NMR spectra of products formed from the fragmentation of 25 mg of dihydrogen *threo*-1-phenyl-1,2-dibromopropylphosphonate (I) at room temperature in 3.0 mL of acetophenone mixed with 1.0 mL of 2,2,6,6-tetramethylpiperidine. Chemical shifts are relative to an external standard of 85% phosphoric acid; upfield shifts are assigned a negative sign.

The ^{31}P NMR spectra of the crude reaction mixtures from fragmentations in the presence of acetophenone, acetophenone plus *o*-(trifluoromethyl)aniline, and ethyl benzoate plus aniline are shown in Figure 3; the signals identify the phosphorus-containing products. The other products were isolated by chromatography and identified by comparison with authentic samples; the ^1H NMR spectrum of authentic *O*-ethyl *N*-phenylbenzimidate is compared in Figure 4 with that of the reaction product obtained in the reaction promoted by methyl metaphosphate. Some of the data that demonstrate that the rate of formation of methyl phosphate equals that for the formation of Schiff base, or of benzimidate, are shown in Figure 5.

Monomeric Metaphosphate Ion

Chemistry. Although the properties of monomeric methyl metaphosphate are of interest, the biochemical reactions of ATP, if they involve monomeric metaphosphate at all, must take place by way of PO_3^- itself. Satterthwait has now made a beginning in describing the chemistry of monomeric metaphosphate ion.[100]

When 2,2,6,6-tetramethylpiperidine is added to 1,2-dibromo-1-phenylpropylphosphonic acid in acetophenone as solvent, the major products are enol phosphate, phosphate, and pyrophosphate (eq 29 and 30).



The ^{31}P NMR spectrum of the crude reaction mixture from the fragmentation of the dianion of 1-phenyl-1,2-dibromopropylphosphonate in acetophenone is shown in Figure 6. The identification of the enol phosphate, purified by chromatography, is demonstrated by the ^1H NMR spectra in Figure 7.

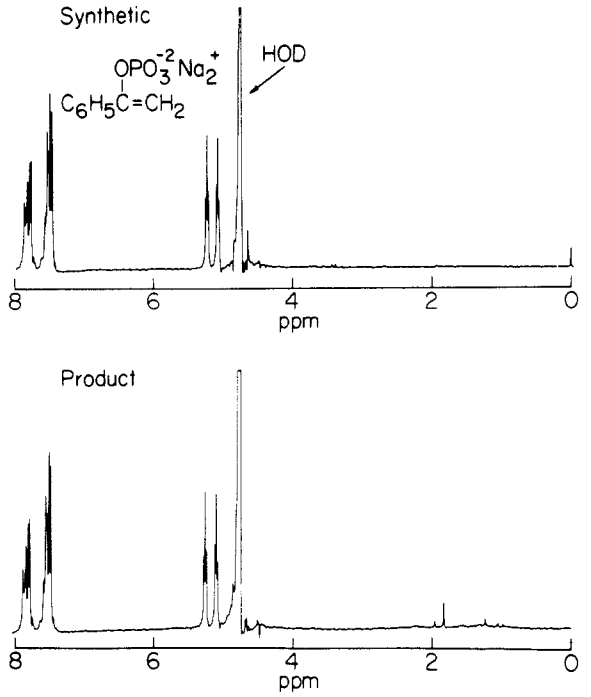


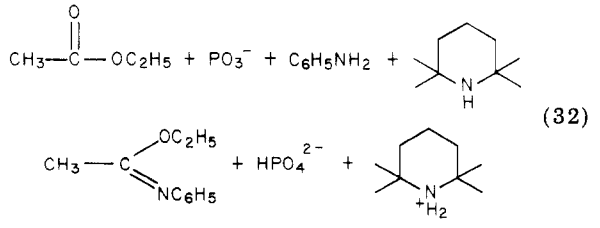
Figure 7. Comparison of the 100-MHz ^1H NMR spectra of synthetic disodium 1-phenylvinylphosphate with that prepared with monomeric metaphosphate anion. Minor impurities between δ 1 and 2 are present in the product. Chemical shifts are in ppm relative to DDS (upper spectrum).

When aniline is present in the reaction mixture, the major products are the Schiff base and *N*-phenylphosphoramidate (eq 31). In this instance, the Schiff

$$PO_3^- + C_6H_5\overset{O}{\overset{\|}{C}}-CH_3 + C_6H_5NH_2 \rightarrow C_6H_5-\overset{}{\underset{\|}{C}}-CH_3 + \underset{N-C_6H_5}{} \quad H_2PO_4^- \qquad (31)$$

base formation is obviously metaphosphate promoted; at 25 °C the direct reaction between acetophenone and aniline does not occur to an appreciable extent in the few seconds needed for the process of eq 31.

The reaction between metaphosphate ion and ethyl benzoate in the presence of aniline failed, but the reaction with ethyl acetate afforded *O*-ethyl *N*-phenylacetimidate in moderate yield (eq 32).



(32)

The reason ethyl acetate reacts to yield the corresponding imidate, whereas ethyl benzoate does not, has not yet been established, although several hypotheses are under consideration. No attempt has yet been made to exploit these reactions for preparative chemistry.

Rate. The distinguishing feature of the reactions shown in eq 29–32 is their speed. They are all extremely fast.[100] The fragmentation of 1,1-dibromo-1-phenylpropylphosphonic dianion in water at 25 °C has a half-time of the order of 0.1 s, as contrasted to a half-time for the fragmentation of the corresponding methyl ester monoanion in acetonitrile of about 90 min at 70 °C. Further, although the rates of the reactions of PO_3^- with carbonyl compounds have not yet been measured, they are also fast, i.e., complete in a few seconds. The evidence for this statement is as follows: If 5% of methanol is added to the reaction mixture before the base (2,2,6,6-tetramethylpiperidine) is added, the yield of enol phosphate or of Schiff base or of amidate is small. (This contrasts with the corresponding experiment that generates methyl metaphosphate, where a few percent of methanol does not drastically affect yields, but simplifies the product mixture.) When, however, the base is mixed with the phosphonic acid and, say, acetophenone, and methanol added a couple of seconds later, the yields of products are the same as those obtained when no methanol is added. The metaphosphate reaction obviously goes to completion prior to the addition of methanol, that is to say, within a few seconds.

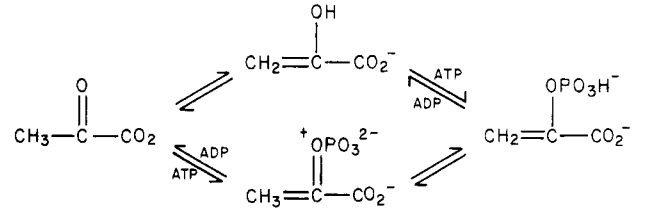
These rapid rates reinforce the conclusion that monomeric metaphosphate is indeed formed and that the reactions under consideration are not primarily nucleophilic attacks, e.g., of acetophenone, on the phosphonate dianion. The attack of a nucleophile on a phosphate ester is strongly suppressed by negative charge; rates are enormously greater (factors of the order of 10^2–10^6) for attack of hydroxide ion on a triester than on the corresponding diester monoanion.[73,101,102] If the reaction under consideration involved a nucleophilic attack, it should proceed much more rapidly with the monoester monoanion than with the dianion of the phosphonic acid. Just the reverse, however, is the fact: reaction of the dianion is much more rapid; with a reasonable guess as to the temperature coefficients for the reactions (to permit extrapolation from 70 to 25 °C), one calculates that the reaction of the dianion is 10^5 times more rapid than that of the monoanion. This, of course, accords with expectation for fragmentation. Furthermore, the rate factors are large enough to be meaningful.

Biochemistry

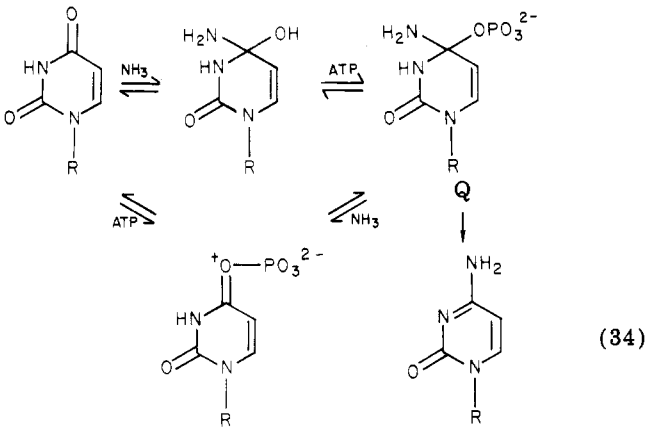
Analogies. The reaction of acetophenone with metaphosphates to yield enol phosphate is formally analogous to the formation of phosphoenolpyruvate from pyruvate and ATP;[103,104] the exchange of a carbonyl group for double-bonded nitrogen in the metaphosphate-promoted reaction between an ester and aniline is formally analogous to a number of biochemical reactions such as the conversion of uridine triphosphate to cytidine triphosphate[105] and the amidation of *N*-formylglycinamidoribose phosphate.[106] The work with our model system raises the possibility that the enzymic reactions of ATP with carbonyl compounds may proceed by way of the initial phosphorylation of the carbonyl group. It must be stressed that this possibility is independent of the question of whether the reactions occur by way of monomeric metaphosphate ion. Phosphorylation of the carbonyl group, if it occurs in enzymic processes, could result from direct, nucleophilic attack of the carbonyl group on the terminal phosphorus atom of ATP or by way of the dissociation of ATP to yield PO_3^-, followed by its attack on the carbonyl compound, or by a merged mechanism. Alter-

natively, the various biochemical processes that involve carbonyl compounds could proceed by a pathway that avoids attack on the carbonyl group and involves the phosphorylation of an hydroxyl group; for example, in the formation of phosphoenolpyruvate (PEP), the first step might be the enolization of pyruvate, followed by phosphorylation of the enol. The subsequent phosphorylation could proceed through the formation of PO_3^- or by direct nucleophilic attack of the hydroxylic compound on the terminal phosphorus of ATP or by a merged mechanism.

Alternative pathways for the formation of PEP from ATP and pyruvate and for the formation of cytidine triphosphate from uridine triphosphate are shown by way of illustration in eq 33 and 34. Any, all, or none



(33)



(34)

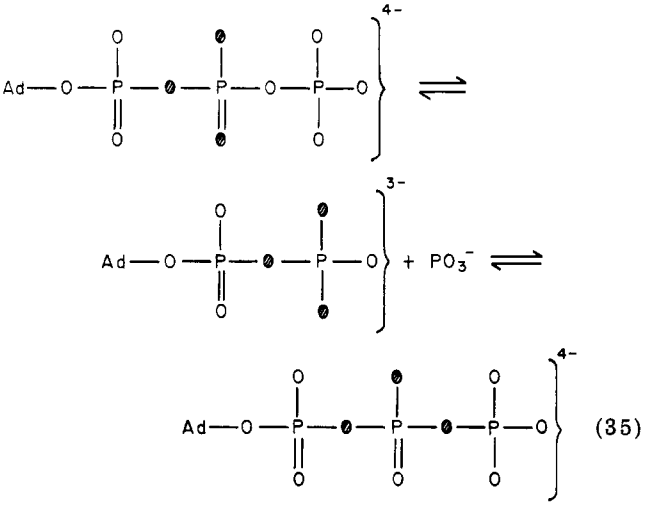
of the phosphorylation reactions involving ATP may occur by way of monomeric metaphosphate.

Rose and his co-workers[103,104] have adduced strong evidence that the reaction between phosphoenol pyruvate and ADP (by reverse of the formation of the enol phosphate) proceeds by way of the phosphorylation of ADP by PEP, with concomitant formation of enol pyruvate, which subsequently ketonizes. This, of course, requires that the reverse process begin with the enolization of pyruvate rather than with attack of monomeric metaphosphate on the carbonyl group. It leaves open the detailed mechanism of the phosphorylation of the enol.

Although the equilibrium constant for the formation of enol pyruvate from pyruvate is unknown,[97-99] the equilibrium obviously strongly favors the keto acid. The negative free energy for the formation of ATP and pyruvate from ADP and PEP certainly includes a substantial contribution from the ketonization of the enol. This, of course, does not in any way preclude the pathway offered by Robinson and Rose, since the equilibrium between pyruvate and its enol may be much more favorable to enolization on the surface of the enzyme than in solution.[107-109] In other words, enol pyruvate may be bound much more tightly to the enzyme than is ketopyruvate.

The evidence offered by Rose and his collaborators is of two kinds. First, Robinson and Rose[104] showed that tritiated PEP does not exchange its hydrogen atoms with solvent in the presence of pyruvate kinase, or more precisely, that the rate of such reaction, if it occurs at all, is slow relative to that in the presence of ADP. If the reaction involved a phosphorylated carbonyl compound (as shown in the lower pathway of eq 33), then the exchange could probably occur in the absence of ADP, although one must allow for the possibility that the reaction is, in one sense or another, concerted. Second, Rose et al. showed[110] that alkaline or acid phosphate will hydrolyze PEP to the enol phosphate and that the enol phosphate only slowly ketonizes. (A similar demonstration of a metastable enol of an α-keto acid came from the author's laboratory.)[111] Pyruvate kinase was then found to catalyze the ketonization of enol pyruvate. This catalysis shows that enol pyruvate is a substrate for the enzyme, and so supports the upper pathway of eq 33.

In another study, Lowe and Sproat[112] prepared ATP labeled with ^{18}O as shown in eq 35. The position of



(35)

●, ^{18}O; Ad, adenosine

the heavy oxygen atoms in the ATP can be determined by ^{31}P NMR spectroscopy,[113,114] where the chemical shift of the signal from the phosphorus atom depends (to a small but detectable extent) on the isotopic substitution around phosphorus and on the bonding of the ^{18}O atoms. With specifically labeled ATP in hand, Lowe performed a "scrambling" experiment modeled on that previously carried out on a different system by Midelfort and Rose.[115,116] Lowe reported that, in the absence of pyruvate, pyruvate kinase catalyzes the scrambling of the oxygen atoms of ATP. The simplest explanation for this finding is that, on the surface of the enzyme, ATP dissociates reversibly to ADP and PO_3^-, although the reaction could involve the reversible phosphorylation of a group in the enzyme or even the reversible phosphorylation of water.

Stereochemistry. Another aspect of the mechanism of action of pyruvate kinase has been illustrated by the stereochemical research of Knowles and his collaborators.[117-125] If metaphosphate is free, then the transfer of the elements of metaphosphate from a chiral unit should proceed with complete racemization, whereas if the reaction is a displacement at phosphorus, it should proceed with inversion. But still another possibility

exists. Jencks[11-14] has pointed out that a number of chemical reactions proceed by way of intermediates so unstable that they react, frequently with solvent, as fast as they are formed. The solvating molecule is required for reaction; the chemistry can be described as "enforced" catalysis. This may be part of a detailed description of the well-known fact that the formation of ions (and other polar species) requires solvation and again represents our ignorance concerning the details of solvation processes. A reaction may occur by way of a complex where one reaction partner is already present and in position, although the reaction bears all the other characteristics of one that proceeds through an unstable intermediate. In such cases, the reaction will necessarily have the stereochemical consequences of inversion, even though it is not truly a displacement. A substrate for an enzyme is certainly the limiting case of a "spectator molecule" that not only is present but is held in the correct geometrical position for reaction. Such processes partake of the major features of concerted reactions, and inversion of configuration can be expected. It follows then that, in an enzymic process of phosphate transfer, racemization would provide positive evidence for a free metaphosphate as intermediate, but inversion is ambiguous.

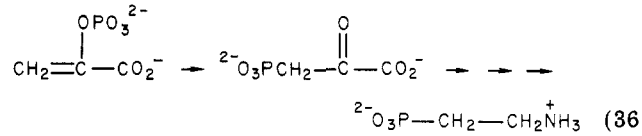
These ideas have been illustrated in a chemical example, already described; Gerrard and Hamer[73,74] showed that the chemical transfer of a nitrogen analogue of metaphosphate involves racemization only with an excellent leaving group and that generally the transfer takes place almost stereospecifically even though the transition state is presumably a resonance hybrid with a large contribution from a structure with metaphosphate character. The hydrolysis of Gerrard and Hamer's compound H is presumably an example of Jencks' merged mechanisms. Similarly, the kinetics of amine catalyzed hydrolysis of 2,4-dinitrophenyl phosphate[53] requires a similar explanation.

In the case of the enzyme, pyruvate kinase, Knowles and his co-workers have found, by research with chiral thiophosphates and with chiral [^{16}O,^{17}O,^{18}O]phosphate, that the transfer of the elements of metaphosphate from PEP to ADP is accompanied by stereochemical inversion at phosphorus. As they noted, although this result is compatible with a displacement reaction, it could also result from enforced catalysis, where all the components of the reaction are present, and geometrically arranged for reaction, in the transition state.

The detailed mechanism of the amidation of UTP to CTP is currently under investigation. The reaction may occur by either of the pathways shown in eq 34 or by a concerted process, and phosphorylation either may or may not proceed by a full or partial monomeric metaphosphate mechanism. Since one oxygen atom is transferred,[105] during the enzymic reaction, from UTP to inorganic phosphate, the intervention of Q as an intermediate is reasonably secure.

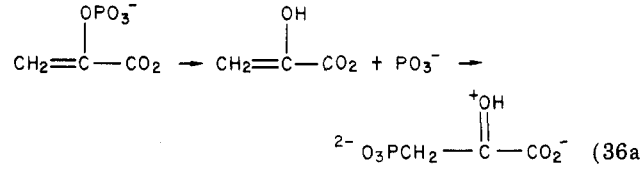
Phosphonates

Although phosphate esters are by far the most important phosphorus derivatives in biochemistry, a modest number of phosphonates have been found in nature;[127-129] in some organisms they are the dominant phosphorus compounds.[130] One of the most common of the phosphonates is 2-aminoethylphosphonate, which sometimes replaces phosphoethanolamine in phospholipids. The biosynthesis of this compound[131] proceeds from PEP (eq 36).



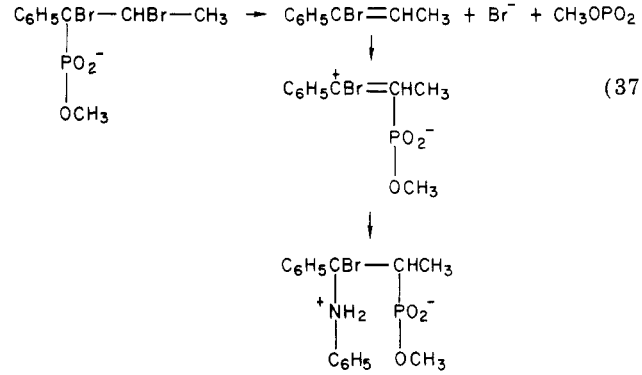
(36)

The detailed mechanism for the transformation of PEP to β-phosphopyruvate is not known, but one possibility is that it involves the attack of PO_3^- on the double bond of enolpyruvate (eq 36a).

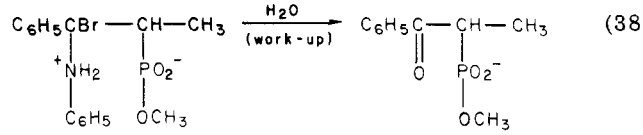


(36a)

A reaction similar to this one presumably has been observed in the reactions of monomeric methyl metaphosphate. One of the minor byproducts of the decomposition of the methyl ester of 1,2-dibromo-1-phenylpropylphosphonic acid in the presence of aniline is the methyl ester of 1-benzoylethylphosphonic acid.[132] (See Figure 3 for a ^{31}P NMR spectrum showing byproducts.) The formation of this product, like the transformation of PEP to phosphoropyruvic acid, involves the migration of the elements of a metaphosphate; a possible pathway for the overall transformation is shown in eq 37 and 38.



(37)



(38)

This chemical analogue to the biochemical transformation suggests the possibility that monomeric metaphosphate may be involved in the metabolic formation of phosphonates.

Conclusion

The possibility that monomeric metaphosphates play a role in the hydrolysis of phosphates and that similar transformations proceed through nitrogen and carbon analogues of metaphosphate has a long history. Recent work from our laboratory exploits the Conant–Swan reaction to produce highly reactive electrophiles that behave as expected for monomeric methyl meta-

phosphate and monomeric metaphosphate anion. The methyl ester phosphorylates the ring of aromatic amines, and this electrophilic reaction is quenched by weak bases such as dioxane. The reagents convert acetophenone to its enol phosphate and in the presence of aniline convert ethyl esters to *O*-ethyl *N*-phenylimidates. These reactions mimic important biochemical transformations and raise the possibility that monomeric metaphosphates are involved in the enzymic reactions.

Almost four decades ago, Lipmann[133] showed how the thermodynamically favorable hydrolysis of ATP could be coupled with, and drive, energetically unfavorable processes. The present work introduces the possibility that ATP can phosphorylate carbonyl groups to activate them; if this mechanism is correct, then ATP will prove to have a kinetic as well as a thermodynamic role in intermediary metabolism.

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