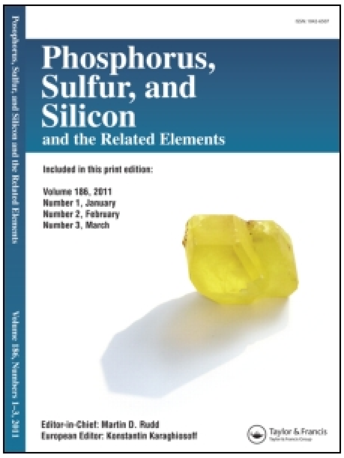

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CARBODIIMIDE-MEDIATED ESTERIFICATIONS AND CONDENSATIONS OF PHOSPHORIC ACIDS DISSOLVED IN VARIOUS ALCOHOLS

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CARBODIIMIDE-MEDIATED ESTERIFICATIONS AND CONDENSATIONS OF PHOSPHORIC ACIDS DISSOLVED IN VARIOUS ALCOHOLS

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Carbodiimide-mediated phosphorylations carried out with ortho-, pyro-, trimeta-, tetrameta-, and long-chain polyphosphoric acids dissolved in various alcohols, with and without an excess of tri-*n*-butylamine at 27° were found to consist of complicated reaction sequences involving various phosphoric acids, then esters and complexes formed between the carbodiimide and these acids or esters. In the case of the condensation of orthophosphoric acid, the process of stepwise esterification is seen to compete with the process of condensation to form condensed phosphoric acids and their esters. In general, esterification as opposed to condensation is promoted by (a) increased acidity, (b) smaller size, and (c) increased concentration of the alcohol, as well as by (d) increased concentration of dissociable protons in the solution. ^{31}P chemical-shift data are given for ortho, chain, and ring phosphoric acids and their esters dissolved in the corresponding alcohol. Typical kinetic curves and the distribution of products obtained upon the cessation of condensation are also presented.

INTRODUCTION

Although the phosphorylation of alcohols using carbodiimide condensing agents has been the subject of a number of papers[1-4] during a ten-year period starting in 1953, the then available experimental techniques did not adequately show the complexity of the reactions involved. In our more recent work we have successfully employed carbodiimide condensing agents to prepare novel, new condensed phosphoric acids[5,6] and related compounds.[7] We have also done some work towards elucidating the complicated reaction sequences which result from the use of condensing agents in phosphate chemistry.[8]

In a recent study,[9] we have shown by ^{31}P nmr that, when an alcohol is added to a condensed-phosphate mixture made by interacting orthophosphoric acid with a carbodiimide, the branched PO_4 groups disappear concomitantly with the formation of ester groups so that the "active phosphorylating agent" is seen to be the branch groups. In the work reported here, the ^{31}P-nmr technique has been employed to determine the various intermediates and products which are produced in appreciable amounts during the condensation and esterification reactions induced by a carbodiimide condensing agent in various alcohols as the solvent for the chosen phosphoric acid.

MATERIALS AND METHODS

Reagents. The preparation of crystalline inorganic ortho- and pyrophosphoric acids[10] and the sodium salts of pyro-,[11] tripoly-,[10] tetrapoly-,[12] trimeta-,[10] and tetrametaphosphoric[13] acids and a long chain ($\bar{n}$ = 100.8) polyphosphate glass[14] and their conversions by ion-exchange[15] into their free acids or their tri-*n*-butylammonium salts were carried out as previously described.

The various phosphate esters used as starting materials or as ^{31}P nmr reference compounds for the purpose of identifying resonance signals in the ^{31}P spectra were prepared from a thermally equilibrated syrupy ester mixture (made from orthophosphoric acid and the desired alcohol at a mole ratio of 1 : 2) by diluting it 10-fold with water, heating on a steam cone for 20 min, then cooling and titrating to pH 6.0 with BaOH. After centrifugation to remove precipitated inorganic barium orthophosphate, an equivolume of ethanol was added to precipitate the orthophosphate monoester, and this material was collected by centrifugation. The supernatant solution was treated with additional ethanol until the ^{31}P spectrum showed that all of the residual monoester had precipitated. After collecting the monoester, the alcohol concentration was then increased to 80%, and the precipitated orthophosphate diester collected. By further titration of the supernatant solution with absolute ethanol, the symmetrical diester of pyrophosphate was prepared. Each of these barium ester salts were then dried at 24° *in vacuo*. They were recrystallized from aqueous ethanol to effect final purification, and their free acids or alkylammonium salts were then prepared by ion-exchange using the hydrogen form of Dowex-50 resin.[15]

Methanol, ethanol, *n*-propanol, *n*-butanol, *n*-petanol, isopropanol, sec.-butanol, cyclohexanol, and t-butanol, were rendered anhydrous[16] in the standard manner as were the aproteic solvents, chloroform-d, and *N,N,N',N'*-tetramethylurea.[8] The carbodiimide employed was the dicyclohexyl derivative (Aldrich Chemical Co.), used without further purification.

Preparation of Reaction Mixtures. The reactions were carried out at 24° in 40-ml centrifuge tubes fitted with Teflon-lined caps. The chosen alcohol was usually employed as the solvent for the phosphate (0.001-1.0 M) and the amine, when used (usually in three-fold excess with respect to the phosphorus). The reaction was initiated by the addition of 20 moles of dicyclohexylcarbodiimide per mole of phosphorus, the tube capped, and the reaction centrifuged as necessary, after the initial heat of condensation had dissipated, to compact the precipitated urea. Aliquots of these reaction mixtures were then withdrawn as needed for ^{31}P analysis. For long-term observation (up to 6 months), the solutions were flame-sealed in 13-mm nmr sample tubes.

The chain and ring phosphates were studied at a 0.1 M total-phosphorus concentration by dissolving the anhydrous tetra-*n*-butylammonium salt of the chosen condensed phosphate in the solvent and subsequently acidifying the solution by adding crystalline tetramethylurea hydrochloride (prepared by saturating the anhydrous solvent with dry HCl gas and allowing crystallization to occur[17]). Orthophosphate preparations made by this procedure were indistinguishable with respect to products and kinetics from those made by dissolving crystalline orthosphoric acid. Since all of these reactions are very sensitive to moisture, extreme care was used in every step to keep out traces of water.[5]

The solid alcohols, phenol, 2,4-dinitrophenol, and 2',3'-isopropylidine adenosine, were studied in the liquid state by dissolving them in a small amount of *N,N,N',N'*-tetramethylurea at 50° and subsequently cooling the solution to 24°. This urea, which is an exceptionally good solvent for these alcohols, permitted the preparation of final isopropylidine, dinitrophenol, and phenol solutions containing respectively only 12, 8 and 4% by weight of tetramethyl urea. Reaction mixtures prepared from these solutions remained as a single phase (except for crystallization of the dicyclohexylurea hydration product of the carbodiimide) throughout each study.

Nuclear Magnetic Resonance. The same Bruker spectrometer[18] and experimental techniques that were previously described[18-20] were employed in this work. Chemical shifts are reported with respect to the usual reference[21] of 85% orthophosphoric acid, with positive chemical shifts corresponding to the higher magnetic-field strengths, as has been customary in ^{31}P nmr.[22] The ^{31}P spectra of multiplet patterns arising from eight or less interacting spins and exhibiting complex-second or higher-order characteristics were simulated using the package of computer programs developed for this purpose by Swalen and Cooper.[23] In addition, a program which permits the composite simulation of weighted combinations of calculated multiplets was occasionally used in the quantitative analysis of mixtures exhibiting overlapping resonance groups.[5] Fortunately, this approach was not often required because of the wide range of observed shifts and the resulting lack of overlap of resonance multiplets from different phosphorus functional groups.

Other analytical methods employed for the purpose of identifying phosphorus-containing compounds were a general hydrolytic procedure based on aqueous triethylammonium bicarbonate[5] and thin-layer[24] and column chromatography.[25]

RESULTS AND INTERPRETATIONS

Phosphorus-Containing Molecules of the Reaction Systems. Because of the sensitivity of modern ^{31}P nmr spectroscopy and the complexity of phosphate-condensation reactions, a large number of discrete phosphorus-containing molecules were detected in the carbodiimide-mediated condensations described herein. These can be conveniently grouped into three families: The first family is composed of the unesterified and alkyl ortho- and pyrophosphates and the unesterified poly- and metaphosphates. These compounds were often found in the condensation reactions and they could be isolated, intact, by aqueous chemical procedures. Their nature was established with certainty by aqueous chromatography coupled with ^{31}P and ^{1}H nmr spectroscopy. The second family is composed of highly condensed cyclic alkyl meta- and ultraphosphates containing branch groups, compounds which were usually observed as transient intermediates in the course of the condensation reactions and which undergo rapid hydrolysis upon contact with water. These materials could not be isolated from their parent reaction mixtures; and this was also the case for the members of the third family of phosphates–the carbodiimide POC adducts. Thus, structure proofs for these last two families must depend solely upon the spectroscopic evidence.

The orthosphosphate region of the ^{31}P spectrum was almost always uncluttered, regardless of the nature of the alcohol undergoing phosphorylation; and, in virtually every case, it was possible to detect at some time in the course of the condensation resonances from each of the four possible orthophosphates which could have been generated, see Table I. With each system it was consistently found that the resonance at the lowest field in the orthophosphate region was that from the monoesterified orthophosphate while the highest-field signal in this region was due to the corresponding diester. The resonances from the triester and unesterified orthophosphate were usually found between these two extremes in these alcohol solvents; and, because the reactions were monitored frequently as they progressed, it was always possible to follow (and hence to distinguish) between their respective signals.

For all of the alkyl orthophosphates, the proton-coupled ^{31}P spectra yielded classic first-order multiplets which lent themselves to straight-forward interpretation. Thus, in the series of *n*-butyl orthophosphates, the triester yielded a septet, the diester a quintet, the monoester a triplet, and the inorganic moiety a singlet; with POCH coupling constants of 6 Hz typically being observed. Addition of the known material to the sample resulted in enhancement of the resonance multiplet of the phosphate in question. Similarly cochromatography of the reaction isolates and the known substance was also employed to identify these orthophosphates.

The pyrophosphates were observed in almost every reaction system; and, as with the corresponding orthophosphates, their ^{31}P spectra could be interpreted without undue difficulty. Thus the proton-decoupled signals from the symmetrical di- and tetraalkyl pyrophosphates and the unesterified pyrophosphate were single resonances, with the tetraalkyl compound coming into resonance at the highest field and the inorganic pyrophosphate ion at the lowest, see Table I. Of the three possible remaining pyrophosphates, only the monoester and triester were detected in these systems. The unsymmetrical diester was either not formed or was far too reactive to accumulate in measurable quantities as it had in previous work[7] with condensations employing stoichiometric quantities of alcohol. Both the mono- and triesters gave rise to second-order *ab* multiplets, with the monoester multiplet exhibiting *ax* character due to the rather large shift difference between the two phosphorus atoms, while the triester multiplet was classically *ab* in appearance. The POP coupling constants observed for these unsymmetrical pyrophosphates lay between 18 and 22 Hz, which are typical values for POP couplings.

The proton-coupled multiplets of all of the symmetrical pyrophosphate esters are complicated second-order patterns of the type $(x)_n aa'(x)'_n$ and cannot be interpreted with certainty without the aid of computer simulation. The proton-coupled multiplets of the unsymmetrical pyrophosphate esters, however, could usually be explained with a straightforward application of first-order multiplicity rules to the four-line, $^{31}PO^{31}P$ *ab* patterns. In either type of molecule, the POCH couplings for the methylene or methinyl groups were clustered about the value of 4 Hz. This value is smaller than the equivalent coupling observed for the corresponding orthophosphates; it is, however, typical for end-phosphate POCH couplings in simple alkylated polyphosphates.[7] The methyl-group POCH couplings were, as usual, larger, with $J = ca.$ 9 Hz.

All of these pyrophosphates could be detected in aqueous hydrolysates of the reaction mixtures, although the triester was readily hydrolyzed and did not persist in aqueous solutions at temperatures much above 4°. Thus the identity of many of these esters could be verified with ^{31}P nmr spectroscopy by the addition of known material to the aqueous hydrolysates. Chromatography was also used to identify these esters, except for the triester which almost always underwent hydrolysis during the chromatographic procedure.

Also included in the first family are two unesterified polyphosphates, tri- and tetrapolyphosphate, and

TABLE I

Observed ^{31}P chemical shifts (referenced in ppm to 85% H_3PO_4)

	Type of alcohol as solvent[a] and esterifying agent				
	Methyl	Primary	Secondary	Tertiary	Aromatic
Inorganic phosphates and phosphate esters					
Orthophosphates					
unesterified	−1.9 (–)	−2.6 (–)	−1.9 (–)	−1.7 (–)	−1.6 (–)
monoester	−3.4 (–)	−1.6 (–)	−0.5 (−2.3)	2.8 (1.4)	−2.7 (–)
diester	−3.3 (−2.5)	−0.8 (0.2)	−1.3 (1.9)	7.8 (5.6)	−0.2 (0.0)
triester	−2.7 (−3.3)	−0.4 (0.3)	0.2 (2.4)	– (2.5)	1.0 (1.1)
Pyrophosphates					
unesterified	– (–)	– (–)	9.1 (–)	9.4 (–)	– (–)
monoester	–; – (–; –)	–; – (9.1; 10.1)	9.4; 11.3 (–; –)	–; – (–; –)	–; – (–; –)
sym-diester	9.4 (–)	10.2 (10.2)	11.9 (10.2)	10.2 (8.0)	– (10.0)
triester	–; – (10.1; 10.8)	10.0; 12.3 (10.1; 12.0)	9.5; 10.0 (13.0; 14.0)	–; – (9.1; 11.9)	–; – (–; –)
tetraester	– (10.0)	10.3 (11.3)	11.0 (12.0)	15.3 (13.2)	10.4 (–)
Higher-chain Phosphates					
unesterified triphosphate	–; – (–; –)	9.6; 24.7 (–; –)	10.0; 24.9 (–; –)	11.1; 25.2 (–; –)	–; – (–; –)
unesterified tetraphosphate	–; – (–; –)	9.9; 26.4 (–; –)	10.0; 26.7 (–; –)	11.0; 27.0 (–; –)	–; – (–; –)
fully esterified tetraphosphate	–; – (10.7; 24.9)	–; – (–; –)	–; – (–; –)	–; – (–; –)	–; – (–; –)
Cyclic Metaphosphates					
unesterified trimeta	21.3 (21.4)	21.4 (20.9)	21.5 (21.2)	21.4 (26.4)	21.3 21.3)
unesterified tetrameta	23.9 (29.1)	23.8 (29.1)	23.7 (29.0)	23.8 (29.1)	22.9 (28.6)
monoesterified trimeta	24.9; 25.4 (24.9; 25.0)	23.3; 23.9 (23.8; 25.1)	24.0; 24.2 (–; –)	24.3; 24.9 (27.2; 27.9)	–; – (–; –)
fully esterified trimeta	– (21.4)	– (–)	– (–)	– (–)	– (–)
fully esterified tetrameta	– (25.8)	– (–)	– (–)	– (–)	– (–)

Orthophosphatyl Trimetaphosphate[6,7,29]					
unesterified	–; –; –	–; –; –	8.9; 24.0; 33.7	10.0; 26.8; 33.2	–; –; –
	(–; –; –)	(–; –; –;)	(–; –; –)	(–; –; –)	(–; –; –)
diesterified at the orthophosphate residue	–; –; –	10.9; 24.1; 33.6	8.1; 24.0; 33.7	–; –; –	–; –; –
	(10.3; 25.3; 33.8)	(11.8; 25.0; 35.2)	(13.9; 23.0; 35.1)	(21.6; 28.8; 33.6)	(–; –; –)
Carbodiimide adducts[6,7] *of the phosphates*					
Orthophosphate Adducts					
monoester	–	–	6.8	3.3	–
	(–)	(–)	(7.0)	(4.8)	(–)
diester	–	–	–	–	–
	(–)	(4.3)	(–)	(–)	(–)
N-substituted monoester	–	–	–4.2	–	–
	(–)	(–)	(–3.8)	(–2.1)	(–2.8)
N-substituted diester	–	–	–	–	–
	(–8.1)	(–)	(–)	(–)	(–)
Pyrophosphate Adducts					
monoester (adduct of the unesterified end)	–; –	–; –	9.4; 20.1	–; –	–; –
	(–; –)	(–; –)	(11.0; 15.0)	(–; –)	(–; –)
sym-diester (adduct on one end only)	–; –	10.5; 19.6	12.0; 20.5	15.1; 20.9	–; –
	(–; –)	(–; –)	(12.2; 20.1)	(–; –)	(–; –)
triester (adduct of the monoesterified end)	–; –	12.3; 23.4	12.0; 21.4	–; –	–; –
	(9.3; 20.1)	(12.0; 20.2)	(11.6; 20.2)	(13.0; 17.9)	(9.1; 16.1)
sym-diester (adduct on both ends)	–	15.8	16.1	–	–
	(–)	(–)	(–)	(21.8)	(–)
N-Substituted Trimetaphosphate Adduct	16.3; 24.2	16.4; 24.4	15.9; 24.6	25.8; 24.6	–; –
	(–; –)	(–; –)	(16.2; 25.2)	(16.0; 26.0)	(–; –)

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[a] Values shown in parentheses correspond to the shifts obtained when the free acid was used; and the other values correspond to the tri-n-butylammonium salts (3 moles of amine per mole of phosphorus). When two or three shift values are presented, they correspond to phosphorus atoms in chemically different sites.

TABLE II

Distribution of Phosphate Functional Groups After Condensation Had Ceased (*ca.* 1 week at 27°) in Reactions 0.1 M in P at 27°

Alcohol and starting phosphate	Moles of grouping per total moles of phosphorus[a]							
	Anhydride (POP) Linkages[b]	Esters	End-Group Esters	Middle-Group Esters	Total Esters	Carbodiimide PNC[c]	Adducts POC[d]	Unsubstituted Acidic OH
Methyl								
Ortho	0.015	0.971	0.029	–	1.000	–	–	1.970
	(–)	(2.166)	(–)	(–)	(2.166)	(0.018)	(–)	(0.816)
pyro	0.617	0.072	1.003	0.107	1.282	–	–	0.584
	(0.477)	(0.092)	(1.800)	(–)	(1.892)	(0.046)	(0.108)	(–)
trimeta	1.000	–	–	–	–	–	–	1.000
	(0.784)	(–)	(0.480)	(0.126)	(0.606)	(0.057)	(–)	(0.769)
tetrameta	1.000	–	–	0.313	0.313	–	–	0.687
	(0.600)	(0.411)	(0.886)	(0.378)	(1.675)	(–)	(–)	(0.125)
Primary								
ortho	0.114	1.474	0.400	0.002	1.876	–	0.007	0.889
	(0.154)	(1.629)	(0.295)	(–)	(1.924)	(–)	(0.063)	(0.705)
pyro	0.688	0.029	0.874	0.088	0.991	–	0.050	0.583
	(0.488)	(0.056)	(1.777)	(–)	(1.833)	(–)	(0.173)	(0.018)
trimeta	0.990	–	0.031	–	0.031	0.104	–	0.885
	(0.801)	(0.042)	(0.752)	(0.116)	(0.910)	(0.036)	(–)	(0.452)
tetrameta	1.000	–	–	0.321	0.321	–	–	0.679
	(0.938)	(0.124)	(–)	(0.246)	(0.370)	(–)	(–)	(0.754)
long chain poly	1.000	–	–	–	–	0.173	–	0.827
	(0.917)	(0.166)	(–)	(0.226)	(0.392)	(–)	(–)	(0.774)
Secondary								
ortho	0.550	0.627	0.315	0.033	0.975	–	0.004	0.921
	(0.205)	(1.644)	(0.845)	(–)	(2.489)	(–)	(0.006)	(0.095)
pyro	0.904	0.152	0.184	0.011	0.347	–	–	0.845
	(0.771)	(0.050)	(0.716)	(–)	(0.766)	(–)	(0.100)	(0.592)
trimeta	1.000	–	–	–	–	0.154	–	0.846
	(0.980)	(0.040)	(0.054)	(–)	(0.094)	(0.267)	(–)	(0.679)
tetrameta	1.000	–	–	0.009	0.009	–	–	0.991
	(0.990)	(0.020)	(–)	(0.242)	(0.262)	(–)	(–)	(0.758)

Tertiary							
ortho	0.714	0.442	0.176	0.045	0.663	–	–	0.909
	(0.650)	(0.447)	(0.193)	(0.023)	(0.663)	(0.271)	(0.043)	(0.723)
pyro	0.854	–	0.584	0.054	0.638	–	–	0.654
	(1.000)	(–)	(–)	(–)	(–)	(–)	(–)	(1.000)
trimeta	1.000	–	–	–	–	0.160	–	1.000
	(1.000)	(–)	(0.320)	(–)	(0.320)	(0.040)	(–)	(0.64)
tetrameta	1.000	–	–	–	–	–	–	1.000
	(0.982)	(0.036)	(0.031)	(0.116)	(0.185)	(–)	(–)	(0.853)
long chain poly	1.000	–	–	–	–	0.166	–	0.834
	(0.982)	(0.036)	(0.034)	(0.114)	(0.184)	(–)	(–)	(0.852)
Phenol								
ortho	0.071	1.634	0.282	–	1.916	–	–	0.942
	(0.278)	(1.290)	(0.776)	(–)	(2.066)	(0.045)	(0.333)	(–)
pyro	0.415	0.510	1.621	–	2.131	–	–	0.039
	(0.500)	(–)	(2.000)	(–)	(2.000)	(–)	(–)	(–)
trimeta[e]	1.000	–	–	–	–	–	–	1.000
tetrameta[e]	1.000	–	–	–	–	–	–	1.000

[a] The numbers in parentheses correspond to acidic condensations (no amine).

[b] Each mole of oxygen bridge serves as the functional group for 2 moles of phosphorus.

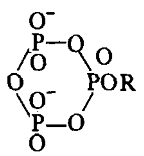
[c] The phosphorus is bonded to one of the carbodiimide nitrogen atom.

[d] The linkage is to the central carbodiimide through a phosphate oxygen atom.

[e] No data was obtained from acid systems because of solubility limitations.

two unesterified cyclic metaphosphates, tri- and tetrametaphosphate. These are the four most common members of the group of higher condensed inorganic phosphates and their ^{31}P nmr patterns have been well-characterized and reported in the literature.[24] They are also stable to the aqueous hydrolytic procedure used.

In these reaction systems, the most important condensed phosphates observed were the esterified and phosphate-substituted cyclic and tri- and tetrametaphosphate; and, of the numerous combinations of alkyl groups and phosphates which are possible in this family, only four molecules were found to accumulate to any degree. These were the monoesterified trimetaphosphate, I, the diesterified orthophosphatyl trimetaphosphate, II (both being observed when the condensation of tetrametaphosphate was conducted in alcohol as the solvent), and the fully esterified tri- and tetrametaphosphates, respectively III and IV (observed when an orthophosphate monoester was condensed in dilute methylene chloride or acetone solution in the absence of alcohols).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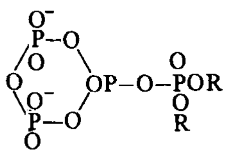

II

where R = alkyl or aryl

The proton-decoupled ^{31}P multiplet from I shows an ab_2 pattern which, because of the large POP coupling constants (J = 21-25 Hz) and relatively small chemical-shift difference between the a and b resonances ($\Delta\delta \approx 1.5$ ppm, 48 Hz) is quite sensitive to the precise values of the respective chemical shifts and coupling constants. An example of a ^{31}P spectrum from such a molecule (monoadenosine-5′-trimetaphosphate) and its computer simulation has been published.[26]

The molecular backbone of the esterified orthophosphatyl trimetaphosphate, II, and its unesterified analog is known in condensation and reorganization reactions involving highly condensed phosphates in relatively dilute solutions.[6,8] The proton-decoupled ^{31}P spectrum from this molecule is of the type a_2xb with no coupling between the a and b portions; hence, the spectra are pseudo-first-order in multiplicity and can be readily interpreted. Again the proton-coupled spectra show the usual small POCH end-group couplings, J = 3-4 Hz (see Ref. 5 for an example spectrum of this kind of molecule).

Both fully esterified cyclic metaphosphates yield a single ^{31}P resonance signal when the spectra are recorded under ^{1}H broad-band decoupling conditions. Their respective chemical shifts follow a pattern observed before for their inorganic relatives,[15] with the trimeta derivative coming into resonance at the low end of the middle-phosphate region of the ^{31}P spectrum and the tetrameta derivative coming into resonance at the high end. That these single ^{1}H-decoupled ^{31}P resonances were, in fact, signals from the fully esterified cyclic metaphosphates was established through an examination of the respective proton-coupled spectra of the esters of the secondary alcohols, isopropanol and sec.-butanol. In these cases, the POCH coupling was found to involve only one proton per phosphorus atom, so that the respective spin systems ought to be based on only six and eight interacting nuclei for the tri- and tetrameta derivatives. These numbers of interacting spins are small enough so that the spectra can be simulated mathematically, and this was done for isopropyl and sec.-butyl derivatives of the tri- and tetrametaphosphates. (An example of the spectrum simulation for such a spin system is given in Ref. 5 for the analogous symmetrically substituted diisopropylurea derivative of trimetaphosphate.) The POCH coupling constants measured were the smallest yet observed for couplings of this type, J = 1.5-2 Hz, and are consistent with the general trend wherein the observed POCH coupling constant decreases in magnitude as the phosphorus atom of the ester grouping is involved in progressively increasing numbers of anhydride linkages.

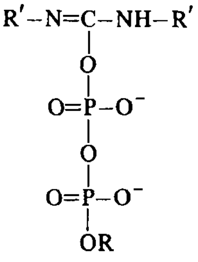
It should be noted that minor amounts of other highly condensed phosphates were observed in some of the condensation reactions. For example, when monoisopropylphosphoric acid is condensed in acetone, a third fully esterified metaphosphate accounting for about 3% of the total phosphorus is observed. From its chemical shift (which is only 6 Hz to higher field than the tetrameta compound), its small relative amount, and the fact that the proton-coupled multiplet observed in the ^{31}P spectrum collapses to a single resonance signal upon ^{1}H irradiation, it is concluded that this phosphate is quite likely the fully esterified pentametaphosphate. However, the ^{31}P multiplet contains too many unresolved transitions to permit any sort of a rigorous mathematical analysis. Two other phosphates observed in these systems deserve mention. In the condensation of inorganic orthophosphoric acid in methanol, the proton-decoupled spectra clearly showed the second-order pattern of a tetrapolyphosphate structure, while the corresponding trimeta derivative was not observed. The end- and middle-group multiplets of this pattern were ^1H coupled; and the continuous-wave ^1H decoupling showed that only the methyl protons of the methoxyl group were involved, suggesting that the molecule giving rise to the resonances was the fully esterified compound, hexamethyl tetrapolyphosphate, or perhaps the symmetrically esterified tetramethyl ester.

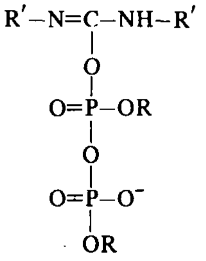
In the condensation of any inorganic phosphate in t-butanol, a resonance singlet is observed in the branch-group spectral region at +41.8 ppm after all condensation had ceased and the sample has come to thermodynamic equilibrium (usually in about 2 months at 24°). The signal, which only accounts for about 1.5% of the total phosphorus, shows no evidence for coupling to protons. Further, this same signal can be seen in reaction systems composed only of phosphorus pentoxide in t-butanol; once again, only after equilibrium was established. There can, of course, be a number of interpretations for such a signal; but the one which appears most likely to us is that the resonance arises from the bird-cage, P_4O_{10}, modification of phosphorus pentoxide in solution in t-butanol (see the second citation under Ref. 5 for a related structure). The mathematically simulated spectrum, assuming values of 20 Hz for the POP coupling constants, does, in fact, show that this cyclic four-spin system will give rise to a single sharp resonance signal, even though the signal itself is composed of numerous transitions with the same shift values. The observed signal is extremely narrow, $\nu_{\frac{1}{2}} < 0.2$ Hz, which is consistent with a compact, small, and highly symmetrical molecule.

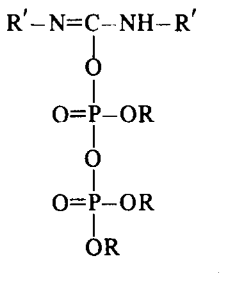
In addition to resonances from the above described inorganic and alkyl ortho-, poly-, and metaphosphates, the reaction mixtures also showed resonances from phosphorus-containing molecules which were of a distinctly different nature. The compounds giving rise to these resonances almost always appeared as transient species in the time-course of the reaction, rarely accumulated at any point in the reaction to amounts accounting for more than 10% of the total phosphorus, and were always converted to simple alkyl phosphates upon contact with water.

The resonances in the orthophosphate region of the ^{31}P spectrum appeared at fields atypically high for simple alkyl orthophosphates (3.5-8.0 ppm). Another set of resonances appeared about midway between the esterified end-phosphate-group resonance band and that of the middle-group phosphates. These resonances were shown by ^{31}P-^{31}P homonuclear INDOR to be coupled to conventional esterified end-group phosphates. Typical POP couplings of between 17 and 25 Hz were observed, and the proton decoupled ^{31}P multiplets were simple pseudo-first-order *ax* patterns exhibiting classical doublet-doublet structure. The proton-coupled spectra showed no additional complexity above that attributable to the alkyl groups of the esterified alcohol moieties. This latter observation was easily made when a secondary or tertiary alcohol was esterified to the phosphate. In the first case, only one proton per esterified alkyl group interacts with the phosphate, and the resultant combination of doublets pattern can always be resolved and interpreted. In the second case, coupling of the type $^{31}POC^1H$ is not possible, and none was observed. In addition, for methyl, primary and secondary alkyl groups, continuous-wave homonuclear ^1H decoupling was employed selectively to collapse the $^{31}POC^1H$ multiplets arising from the coupled protons of the esterified alkyl groups. In every case, no residual ^1H coupling was observed. Further, ^1H-^{31}P heteronuclear INDOR, taken while observing the ^1H resonance spectrum, showed that the only resonances coupled to phosphorus were those from the esterified alkyl groups.

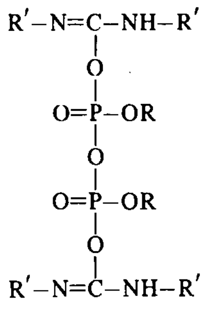
The ^{31}P shift and multiplicity of these resonances and the hydrolytic behavior of the compounds giving rise to them suggests that these signals are related to analogous resonances seen in similar condensations of inorganic orthophosphate in anhydrous tetramethylurea,[8] resonances which were attributed to carbodiimide POC adducts of ortho- and trimetaphosphoric acids. By analogy, the high-field orthophosphate resonances observed here are assigned to the corresponding carbodiimide POC adducts of mono- or diesterified orthophosphoric acid. The resonances lying midway between the end and middle phosphate regions are similarly thought to arise from the corresponding carbodiimide POC-substituted end phosphates. The molecules containing these POC-substituted end-phosphate functional groups were always simple pyrophosphates; and, of the five pyrophosphates observed in these systems, only the mono adducts of the mono- (V), di- (VI), and triester (VII) accumulated to measurable levels. The sym-pyrophosphate diester also gave rise to measurable quantities of a sym-diadduct. The tetraalkylpyrophosphate, of course, could not give rise to such adducts.


V


VI



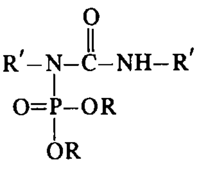
VII



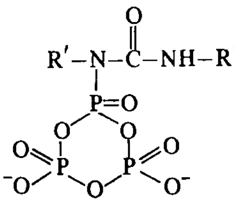
VIII

(The adducts are shown unprotonated; R = an alkyl or aryl group and $R' = C_6H_{11}-$.)

In aged reaction mixtures, carbodiimide PN adducts[5] can also be observed. The orthophosphate PN adducts come into resonance at low magnetic fields (−8 to −2 ppm), and similarly the *N*-substituted phosphate middle group comes into resonance at low magnetic fields for a phosphate middle group (16 ppm). In these systems carbodiimide PN adducts were observed for the orthophosphate mono- and diester (IX). The other PN adduct, which is quite important in all carbodiimide-mediated condensations of inorganic phosphates, is the *N*-phosphoryl-urea of trimetaphosphate (X). The nature of this molecule has been extensively discussed.[5,8]



IX



X

Kinetic Studies. A series of kinetic studies were carried out on the carbodiimide-mediated reactions of orthophosphoric acid when dissolved in the various alcohols in the presence of amine at 27°. Typical results are shown in Figure 1 for normal and tertiary butanol. It should be noted in Figure 1 that there are two early reactions: one involving the esterification of the orthophosphoric acid (curve a in Figure 1A) with the alcohol to give its monoester (curve b); and the other, the condensation of two molecules of orthophosphoric acid to give pyrophosphoric acid (curve g). Since there are roughly 10^2 moles of alcohol per mole of starting orthophosphoric acid, the fact that the concentration of monoester increases in the initial part of the reaction curve for *n*-butanol so as to remain consistently about 10 times more concentrated than the pyrophosphoric acid, indicates that the rate constant for esterification by *n*-butanol is roughly 10 times greater than that for the condensation to pyrophosphoric acid. Once formed, the monoester produces an adduct with the condensing agent (curve c) and then this adduct probably reacts further with alcohol to form the diester of orthophosphoric acid (curve d) which also then produces an adduct (curve e) which presumably reacts further with the alcohol to form the triester of orthophosphoric acid (curve f). Obviously the symmetrical pyrophosphoric diester (curve h) could result from P-O-P condensation of the monoester of phosphoric acid or from a double esterification of the pyrophosphoric acid. This symmetrical diester of pyrophosphoric acid also forms a carbodiimide adduct (curve i); and this adduct presumably reacts further to give the tetraester of pyrophosphoric acid (curve j). It should be noted that the mono- and triesters of pyrophosphoric acid or their adducts were not observed; and we interpret this to mean that both of these species are highly reactive so that they are used up about as fast as they are formed. In view of our prior work, the presence of trimetaphosphoric acid (curve k) as well as its monoester (curve l) and the ortho-group diester of orthophosphatyl trimetaphosphoric acid (curve m) indicates that there must be several condensed phosphoric acids and their adducts which also react about as rapidly as they are formed so that they are not seen. For example, neither tripolyphosphoric acid or tetrametaphosphoric acid were observed, although either of them may serve[8,9] as an intermediate for the condensation of pyrophosphoric acid into trimetaphosphoric acid.

It is important to note that when tertiary instead of normal butanol is employed as the alcohol (see Figure 1B), the pyrophosphoric acid is produced in the initial part of the reaction sequence at about 1.5-fold higher concentration than is the monoester, a finding which is readily explained by steric hindrance of the esterification for the tertiary as compared to the normal butanol. With t-butanol, curve h describing the concentration of the symmetrical diester of pyrophosphoric acid is about fifteen times higher than in the case of the *n*-butanol; and these results are probably attributable to the much more rapid formation of pyrophosphoric acid in the case of the tertiary as compared to the normal alcohol. The curves corresponding to normal and tertiary butanol were shown in Figure 1 since they rather well represent the two extremes of kinetic behavior for all of the alcohol studies, with the sec.-butanol being intermediate.

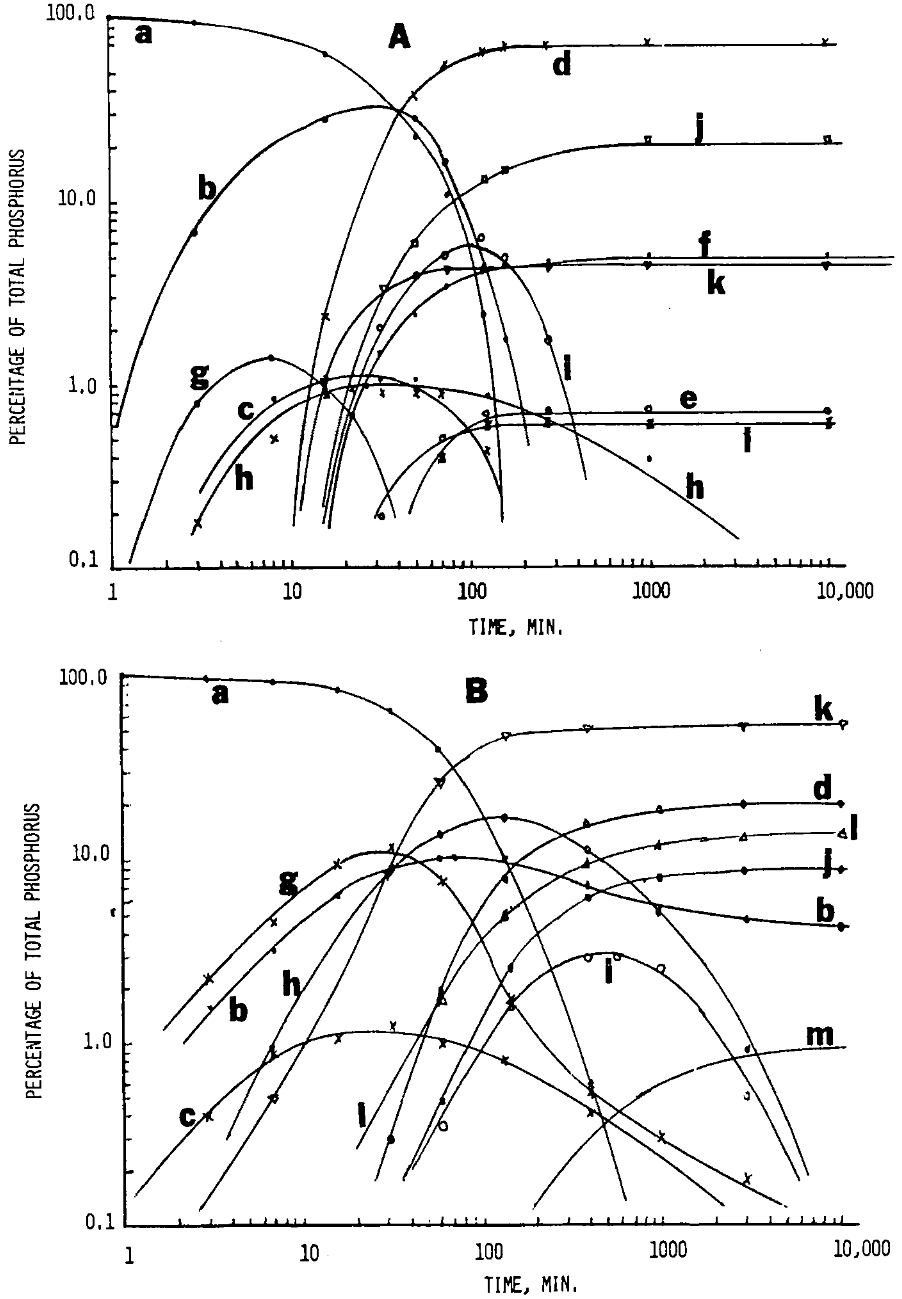


FIGURE 1 Log-log plots of the time-course for the dicyclohexylcarbodiimide-mediated condensations at 27° of orthophosphoric acid dissolved in two alcohols: (A) *n*-butanol and (B) t-butanol. In both instances, the initial reaction mixtures were 0.1 M in H_3PO_4, 0.3 M in the tri-*n*-butylamine, and 2.0 M in the condensing agent. The curves are identified as follows: a, the starting H_3PO_4; b, monoesterified orthophosphoric acid; c, the carbodiimide adduct of monoesterified orthophosphoric acid; d, diesterified orthophosphoric acid; e, the carbodiimide adduct of diesterified orthophosphoric acid; f, triesterified orthophosphate; g, pyrophosphoric acid; h, symmetrically diesterified pyrophosphoric acid; i, the adduct formed between one molecule of the condensing agent and one end of the symmetrical diester of pyrophosphoric acid; j, tetraesterified pyrophosphate; k, trimetaphosphoric acid; l, monoesterified trimetaphosphoric acid; and m, orthophosphatyl trimetaphosphoric acid diesterified at the orthophosphate residue. The compounds corresponding to curves e and f were not detected in the t-butanol system, and that corresponding to m was not detected in the *n*-butanol system.

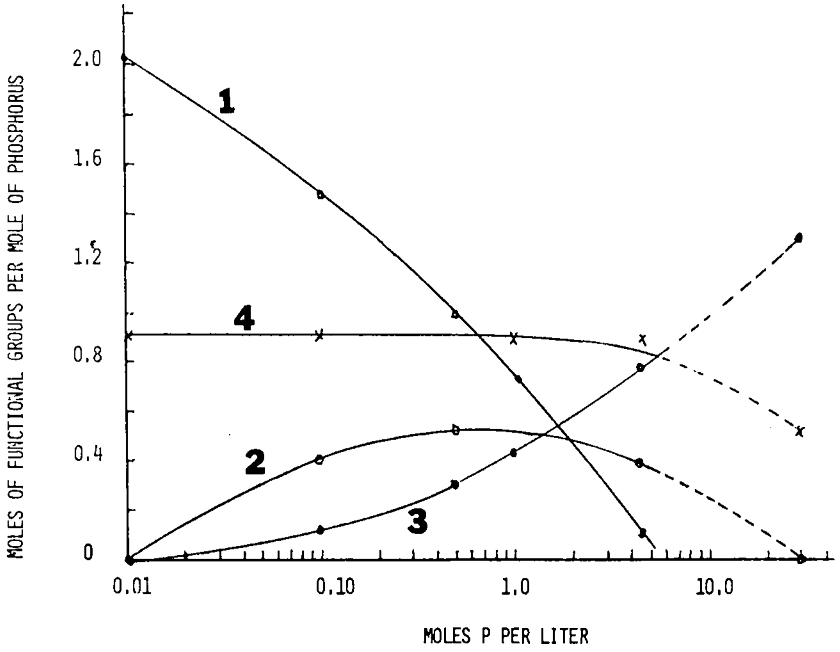


FIGURE 2 Final pattern of the functional groups generated by the condensation of orthophosphoric acid in isopropanol with excess amine and excess dicyclohexylcarbodiimide at 27°; where 1 represents the ester groups on the orthophosphate moiety; 2, the ester groups on phosphate end groups; 3, the POP linkages; and 4, the acidic OH groups.

When the condensation is carried out in the absence of amine, the same range of products is formed, only in somewhat different proportions. The main difference is that both the esterification and the P-O-P condensation proceed to a greater degree before condensation ceases. Generally speaking, the rate of formation of the various species without added amine is from 10 to 100 times faster than when amine is present in excess. This means that, without amine, it is difficult to obtain rate curves (similar to those of Figure 1). In Figure 2, the final gross composition resulting when the condensation processes have been completed is shown as a function of the overall concentration of phosphorus in a system obtained by condensing orthophosphoric acid in isopropanol with a molar excess of amine and sufficient dicyclohexylcarbodiimide so that some is left when condensation is over. The points at the far right side of this graph correspond to the composition of a solvent-free condensed-phosphoric-acid glass exhibiting an H_2O/P_2O_5 mole ratio of 0.5. This means that the data on the carbodiimide-mediated condensation in the presence of amine extrapolates to the composition of a neat condensed phosphoric acid having the same empirical composition as 1,5-μ-oxo-tetrametaphosphoric acid, which is practically the sole product that is formed when fairly concentrated solutions (2-5 M in P) of phosphoric acid are condensed with carbodiimide. It is important to note in Figure 2 that, as the concentration increases, the esterification of the orthophosphate moiety decreases while that of the end group passes through a maximum. As would be expected, the final degree of phosphate condensation (P-O-P linkages) increases continuously while the number of acidic OH groups per phosphorus atom remains essentially constant throughout the experimentally approachable region corresponding to the carbodiimide-mediated condensation.

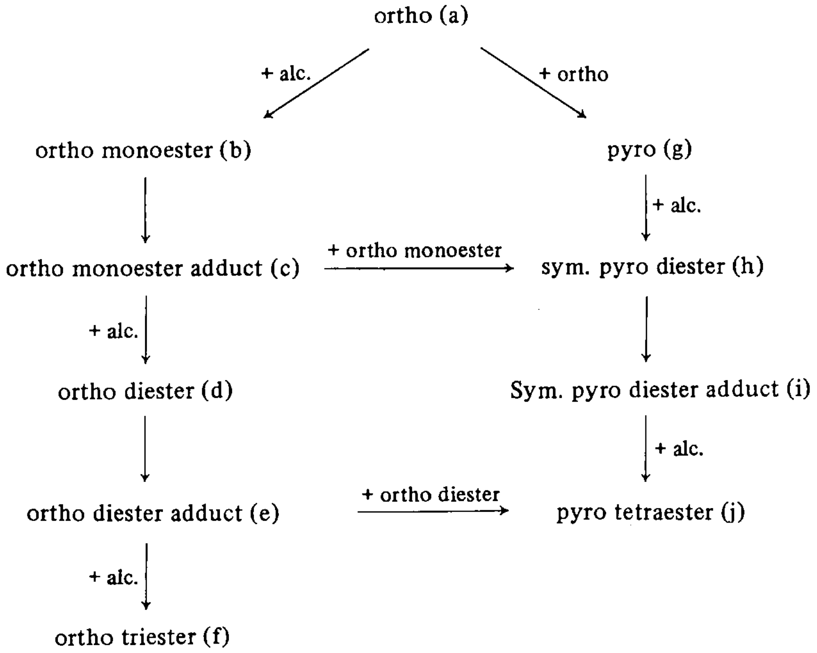
When the monoester is employed instead of phosphoric acid as the starting phosphate in a solution of its alcohol, no inorganic condensed pyrophosphate is formed but all of the other species are observed. However, with amine in an inert solvent, such as acetone, methylene chloride, or *N,N,N',N'*-tetramethylurea, the monoester is found to condense to only the symmetrical diester of pyrophosphoric acid and the reaction then stops. In the absence of amine, the sequence of events consists of nearly instantaneous condensation to form the tetraalkyl tetrametaphosphate, which then disappears within 6–8 min at 27° to give initially the fully esterified trimetaphosphate. This compound also disappears in about 2 hr to form a complex series of inorganic and esterified condensed phosphates and *N*-substituted urea derivatives. The fact that the fully esterified trimetaphosphate results from condensation from the orthophosphate monoester in an inert solvent and is not seen when the alcohol is used as the solvent is in accord with the conclusion[4] by Khorana's group that the fully esterified trimetaphosphate is a phosphorylating agent for alcohol.

The distribution of phosphate functional groups after condensation had ceased is shown in Table II for various starting phosphates in different alcohols. Since all of the primary (or secondary, or tertiary) alcohols (except for methanol) behaved very similarly, the results on them are lumped together.

Reaction Sequences. In the case of carbodiimide-mediated condensation of orthophosphoric acid dissolved in either an alcohol or a phenol, with or without added amine, the same general range of products is always found, corresponding to the reaction scheme shown at the bottom of this page (where the letters in parentheses refer to the corresponding rate curves in Figure 1).

In addition to the molecular structures based on the ortho- and pyrophosphates shown above, a number of more condensed species are also formed but the reaction pathways at these more advanced stages in the condensation may well correspond to a number of alternative routes and cannot be clearly delineated for the systems studied here. However, in our prior work,[9] we have shown that carbodiimide-induced condensation of pyrophosphoric acid gives the tetrametaphosphate anion and that this further rearranges to form the orthophosphatyl trimetaphosphate structure which apparently then forms a mixture of the trimeta- and orthophosphate anions. Furthermore, the work reported here along with our prior work[9] on the reaction of alcohols with condensed phosphates has shown that branch groups in phosphate structures are reactive centers for esterification reactions with alcohol.

In general, esterification (as opposed to phosphate condensation) is promoted by the following factors: increased acidity of the alcohol, smaller size of the alcohol and hence less steric hindrance, increased alcohol concentration, and increased concentration of the dissociable protons in the solution. If the reaction scheme presented above is started with one of the products from the carbodiimide-mediated condensation of orthophosphoric acid in alcohol, it appears that the reaction path carries on from the chosen starting point. Thus, by starting with the ortho monoester one obtains the ortho diester, the ortho triester, as well as the symmetrical pyro diester and the pyro tetraester as well as the indicated adducts; whereas by starting with the ortho diester, the products obtained are the ortho triester and the pyro tetraester. Likewise, by starting with pyro- instead of orthophosphoric acid, esters of orthophosphoric acid are nor produced until



quite late in the reaction. The fact that they are produced at all indicates that there is scrambling and rearrangement as well as condensation in these complicated reaction sequences.

ACKNOWLEDGMENTS

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