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Cyclic Metaphosphates from Hydrolysis of the Products from Phosphoric Acid Condensation with Carbodiimides

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When orthophosphoric acid in *N,N,N',N'*-tetramethylurea is condensed with an excess of a carbodiimide, RN=C=NR, a mixture consisting predominantly of cyclic ultraphosphates is formed. Controlled hydrolysis of this mixture gives the complete series of cyclic metaphosphates ranging from trimetaphosphate to decametaphosphate. Through the use of ion-exchange chromatography and other chemical operations, pure crystalline samples of the sodium salts of trimetaphosphate through nonametaphosphate have been prepared. Of these compounds, the penta-, hepta-, and nonametaphosphates—$(NaPO_3)_5$, $(NaPO_3)_7$, and $(NaPO_3)_9$—have not been isolated previously. The ^{31}P nmr chemical shifts of these compounds as well as long-chain polyphosphates have been determined in water and in anhydrous tetramethylurea. These data indicate that in aqueous solution and in the presence of sodium ion, the chains of the polyphosphate and the rings larger than the pentametaphosphate assume a helical conformation consisting of three PO_4 tetrahedra per turn of the helix.

Introduction

Although the tri- and tetrametaphosphates have been known for many years, the hexa-[1,2] and octametaphosphates[3] have been isolated only in the last few years. Indeed the first demonstration[4] of the existence of cyclic metaphosphates larger than the tetrameta- was made only 15 years ago, although the literature[5] of the prior century embodied many speculations as to the existence of such species. Carbodiimides have been widely used as condensing agents for phosphates in preparative biochemistry[6] but have found limited comparable use in inorganic chemistry.[7,8]

Experimental Section

Reagents.—Crystalline orthophosphoric acid[9] was made in the standard manner as were the tri-,[10] tetra-,[11] hexa-,[1] and octametaphosphates.[3] The long-chain phosphate reference compound was sodium Kurrol's salt,[12] which was dissolved by stirring in deionized water for 4 days at 4°. The tetra-*n*-butylammonium ions were placed upon the chosen phosphates when needed by immediately titrating solutions of the free acids, obtained from ion-exchange with Dowex 50 H^+ resin, with tetra-*n*-butylammonium hydroxide to pH 7 and freeze-drying the resulting salt solutions. Except for the pentametaphosphate, this procedure was shown by ^{31}P nuclear magnetic resonance (nmr) not to degrade the phosphates. The pentametaphosphate underwent hydrolysis to the pentapolyphosphate to the extent of about 10% of the total phosphorus.

Diisopropyl- and dicyclohexylcarbodiimide were purchased from the Aldrich Chemical Co. The anhydrous *N,N,N',N'*-tetramethylurea, an excellent solvent for anhydrous phosphoric acids, was prepared as previously described.[13] The solution of triethylammonium bicarbonate was made by allowing redistilled triethylamine to react with solid carbon dioxide in water at temperatures below 4° until the pH of the solution was 7.0. All other chemicals used in this study were commercial reagents.

Analytical Apparatus.—The ^{31}P nmr[14,15] measurements were carried out on a Bruker HFX-5 spectrometer at 36.43 MHz incorporating heteronuclear (^1H at 90.0 MHz) field-frequency stabilization and facilities for time averaging and ^{31}P indor. Column chromatography was carried out on diethylaminoethylcellulose (5 × 90 cm column, void volume 1840 ml) prepared in the bicarbonate form by washing with 4 l. of 1.0 *M* triethylammonium bicarbonate containing 10% tris(hydroxymethyl)-aminomethane. Linear-gradient elution of the phosphates was achieved with 18 l. of triethylammonium bicarbonate in water, using an eluent concentration ranging from 0.0 to 0.5 *M*. In a chromatographic run, 760 fractions of 23 ml each were collected at the rate of one fraction every 11 min to give a total running time of about 6 days.

Thin layer chromatography was carried out according to the method of Tanzer, Krichevsky, and Chassy,[16] and each separate metaphosphate eluted from the ion-exchange column gave rise to a single spot. This technique was complemented by paper chromatography using the acid solvent of Ebel.[17] Total phosphorus was determined colorimetrically according to the method of Chen, Toribara, and Warner.[18]

Preparation of $(NaPO_3)_3$ to $(NaPO_3)_9$.—A solution of 206 g (1 mol) of dicyclohexylcarbodiimide and 200 ml of *N,N,N',N'*-tetramethylurea was vigorously stirred in a 2-l. three-necked flask, equipped with a thermometer and dropping funnel and vented to the air with a drying tube. Molten anhydrous orthophosphoric acid (40 g, 0.4 mol) was added slowly over a period of 6 hr so that the temperature of the reaction mixture never exceeded 40°. The mixture was then stirred until only middle and branch groups were observed in the ^{31}P nmr spectrum (about 2 days).

At this stage, 500 ml of anhydrous ether containing 56 ml (0.4 mol) of anhydrous triethylamine was added to the reaction slurry. Immediately thereafter, 500 ml of water was introduced, with the first few milliliters being added slowly so that a temperature of 30° was never exceeded throughout the entire hydrolysis. The resulting slurry was filtered to remove precipitated dicyclohexylurea and the lower liquid phase (aqueous) was separated and concentrated on a rotary evaporator at 25°. The concentrate (containing tetramethylurea and more precipitated dicyclohexylurea) was filtered and the filtrate was taken up in 1 l. of acetone. One liter of 1 *M* NaI in acetone was then added with stirring, and the thusly precipitated sodium phosphates were collected by filtration and then redissolved in 200 ml

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of water. Eighty grams of NaI was added, the pH adjusted to 7.0, and the solution filtered to remove additional precipitated dicyclohexylurea. After storage at 4° for 1 day, another filtration was carried out before adding 20 g of NaI to the filtrate. The pH was then adjusted to 7.0 and the solution stored at 4° for another day to crystallize sodium tetrametaphosphate. This process was repeated with additional 20-g quantities of NaI until the solution contained 160 g of NaI. Patience must be exercised during this crystallization procedure because if precipitation proceeds too rapidly, the tetrametaphosphate crystals will occlude substantial amounts of the other metaphosphates.

At this point the solution was concentrated on a rotary evaporator at 25° to a slurry which was taken up in 1 l. of acetone to extract the NaI and cause precipitation of the remaining sodium metaphosphates which were collected by centrifugation and washed three times with acetone (yield 16 g). Two grams of this precipitate was dissolved in 50 ml of a solution of 1 g of tris(hydroxymethyl)aminomethane in water. This was applied to the ion-exchange column and washed into the column with 2 l. of deionized water. In our column, elution was carried out for about 6 days at a rate of 2 ml/min using a linear gradient covering the range from 0.0 to 0.5 *M* triethylammonium bicarbonate. Appropriate fractions were collected (see Figure 1). Each fraction was then evaporated at 25° to a few milliliters of a thick syrup, 50 ml of methanol was added, and the resulting solution was evaporated to dryness. This addition of methanol and subsequent evaporation was repeated five times to purge the sample of the bicarbonate salt.

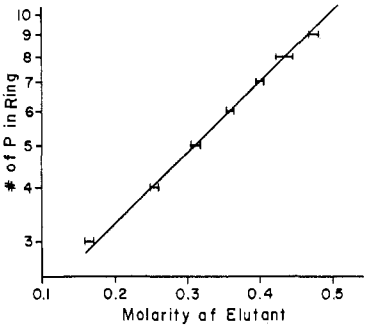
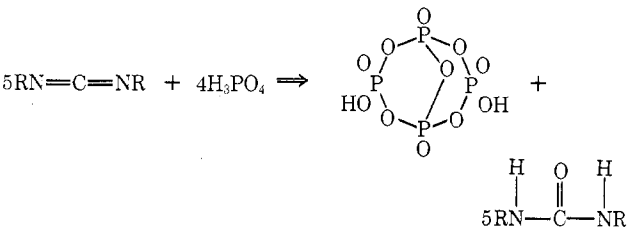


Figure 1.—The molarity of the eluent salt, triethylammonium bicarbonate, as a function of the logarithm of the number of phosphorus atoms present in each metaphosphate ring.

Each fraction was then taken up in a few milliliters of methanol, filtered to remove lint, and diluted to 30 ml in acetone; the phosphate was caused to precipitate as the sodium salt by dropwise addition of 1 *M* NaI in acetone. The resulting precipitates were collected by centrifugation, washed six times with acetone, and dried. If all of the crude metaphosphate mixture, obtained after selective crystallization of the tetrametaphosphate, is passed through the ion-exchange column (eight replicate runs) and subsequently worked up, the following yields are typical: trimeta, 2.63 g; tetrameta, 2.54 g; pentameta, 2.94 g; hexameta, 1.07 g; heptameta, 1.40 g; octameta, 1.05 g; nonameta, 0.29 g; deca and higher metaphosphates, 0.31 g.

Results and Discussion

As described in a previous communication,[7] an excess of either diisopropyl- or dicyclohexylcarbodiimide in tetramethylurea leads to the condensation of crystalline orthophosphoric acid to an ultraphosphate composition exhibiting relatively narrow nmr peaks in the branch-group region of the ^{31}P spectrum (+34 to +42 ppm upfield of 85% H_3PO_4) and in the middle-group region (+21 to +31 ppm). A formal equation showing the stoichiometry of this condensation process is given below for the case where orthophosphoric acid is condensed to 1,5-μ-oxo-tetrametaphosphoric acid. In these reactions the elements of water are transferred from the phosphoric acid to the carbodiimide resulting in the formation of a POP bond and the production of the corresponding urea.



Under the conditions employed there are no orthophosphate nor end-group resonances, although for a few hours after the start of the reaction there are several peaks in the neighborhood of +17 ppm. We have interpreted[19] these resonances as arising from complexes between the carbodiimide and metaphosphate ring structures, particularly the cyclic trimetaphosphate. Two reasonably typical ^{31}P nmr spectra of such reaction products have been published.[7]

A solution of the final reaction products in anhydrous ether containing anhydrous triethylamine was hydrolyzed by either the careful dropwise addition of water or the incorporation of wet ether. The triethylamine is necessary to prevent the buildup of acidity during the hydrolysis procedure. When hydrolysis was completed, additional water was added and the aqueous part of the resulting three-phase system (two liquid phases, one based on water and the other on ether, as well as a solid phase of the precipitated urea) was separated and concentrated at room temperature. After filtration, the ^{31}P nmr spectrum showed that about 75% of the total phosphate was present as the tetrametaphosphate, with the tri-, hexa-, and octametaphosphates accounting for about 9, 2, and 1% of the total phosphorus, respectively. Addition of the known cyclic metaphosphates to the sample showed that the peak assignments for these compounds were correct. In addition there were two resonances exhibiting the same signal characteristics as the known cyclic metaphosphates, which from their positions in the spectrum could tentatively be ascribed to the penta- (10%) and heptametaphosphate (3%). When the hydrolyses are carefully done, short-chain polyphosphates are not found to be present (less than 0.1% of the total phosphorus) even though extensive signal averaging of the end-phosphate region was carried out. The same was true of the orthophosphate, and there were no ultraphosphates nor was there any evidence for other phosphorus-containing species such as phosphoramidates.

As described in the Experimental Section, the individual sodium metaphosphates were isolated by utilizing fractional crystallization with sodium iodide, followed by column chromatography and recrystallization from acetone. As shown in Figure 1, the step involving column chromatography offers a proof of the structure of the two unknown metaphosphates observed in the ^{31}P nmr spectrum noted above. This figure gives the concentration of the eluting salt at which the maximum amount of each phosphate was observed as a function of the logarithm of the ring size. The two unknown phosphates appeared at the positions predicted for the penta- and the heptameta-

(19) T. C. Myers, R. Kleps, T. Glonek, and J. R. Van Wazer, submitted for publication in *J. Amer. Chem. Soc.*

phosphate; there was also a third phosphate fraction which appeared at the nonametaphosphate position, and a fourth phosphate fraction was eluted near the end of the salt gradient at about the position predicted for the decametaphosphate.

The separated sodium phosphates were studied by an anion-exchange thin layer chromatographic technique developed[16] for separating condensed phosphates. The results are shown in Figure 2, and again the unknown phosphates exhibited R_f values corresponding to those which would be predicted for the penta-, hepta-, and nonametaphosphates. The phosphate fraction eluted from the column at the end of the gradient could not be resolved by either chromatographic procedure; however, there were phosphates present which exhibited R_f values lower than those of the octa- and nonametaphosphates. These phosphates were tentatively identified as the decameta and higher metaphosphates. Paper chromatography was also employed to identify the new metaphosphates, and the results from this technique were found to be in accord with the thin layer data as well as with previous studies on ring and chain phosphates[20] using paper chromatography.

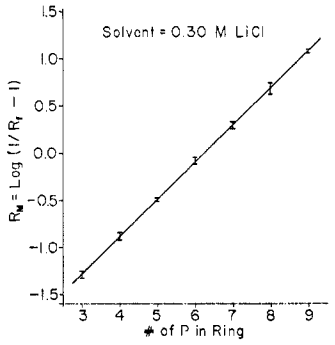


Figure 2.—A plot of R_M as a function of the number of phosphorus atoms present in each metaphosphate ring after the manner of Tanzer, Kirchevsky, and Chassy.[16] The developing solution was 0.30 M LiCl.

We have found that mixtures of chain and ring phosphates are easily characterized quantitatively by ^{31}P nmr spectra. In order to ensure precision in the chemical shift measurement it is desirable to work in relatively dilute solutions at constant pH (preferably neutral) and to add at least 1 mol of ethylenediaminetetraacetic acid (EDTA) per mole of phosphorus which serves greatly to reduce undesirable phosphatometal complexes. A ^{31}P nmr spectrum of a mixture of the recrystallized sodium cyclic metaphosphates, each present at 0.01 M in phosphorus, is shown in Figure 3. A previously published spectrum[21] showing tri-, tetra-, and hexametaphosphates surprisingly indicated that the chemical shift of the larger of these three lay between those of the two smaller compounds. This prior unexpected finding is in accord with the data of Figure 3, from which it can be seen that the chemical shifts lie in the following order proceeding upfield: tri < octa < hepta < hexa < tetra < penta.

Figure 4 shows a greatly expanded, highly resolved, ^{31}P nmr spectrum of the phosphate fraction eluted from the column at the end of the gradient containing a small amount of added octametaphosphate as a reference. The figure clearly shows three resonance signals: the reference compounds octametaphosphate, $(NaPO_3)_8$, and the nonametaphosphate, $(NaPO_3)_9$, which lies 0.01 ppm (0.5 Hz) upfield to that of $(NaPO_3)_8$; and a third signal lying 0.2 Hz to higher field of $(NaPO_3)_9$. It is unlikely that this high-field signal, exhibiting the narrow line width at half-height of 0.3 Hz, arises from long-chain polyphosphates because these generally give rise to signals of greater width.[22] Therefore, this signal is interpreted as arising from deca and higher cyclic metaphosphates. It is interesting to note that all three of the nmr resonance peaks of Figure 4 lie under the envelope of the middle-group resonances of the long-chain polyphosphate, when the measurements are taken under the same conditions.

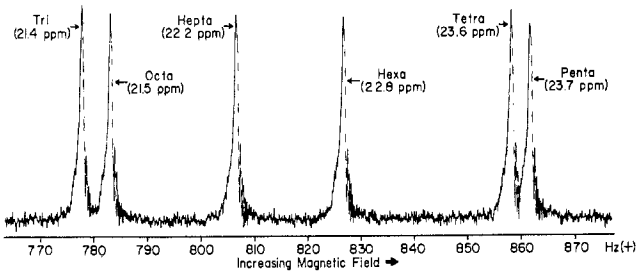


Figure 3.—The ^{31}P nmr spectrum taken at 36.43 MHz, of the middle-group phosphate region, showing resonances from the cyclic metaphosphates. Each component is present at 0.01 M in phosphorus, and the solvent is 0.1 M sodium ethylenediaminetetraacetate at pH 7.0. A 13-mm high-resolution spinning-sample tube was used containing 3 ml of solution. Heteronuclear (^1H) field-frequency stabilization (H_2O referenced) and signal averaging at a rate of 1.5 Hz/sec were employed to reduce the level of the background noise. The ppm scale is given relative to external 85% orthophosphoric acid.

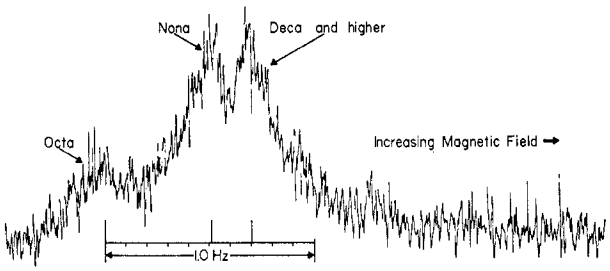


Figure 4.—A greatly expanded ^{31}P nmr spectrum of the middle-group phosphate region showing resonances from the sodium phosphates eluted from the chromatographic column at a concentration of triethylammonium bicarbonate of about 0.5 M. Nmr parameters were similar to those described for Figure 3 except that the sweep rate was reduced to 0.015 Hz/sec and the observing radiofrequency power was adjusted to a level 30% below that yielding the optimum signal-to-noise ratio.

The anomalous ordering of the chemical shifts of the cyclic sodium metaphosphates in aqueous solution, as shown in Figures 3 and 5, can be explained in terms of average molecular conformations. By analogy to solid-state structures determined by X-ray diffraction, it has been postulated[23] that the chain polyphosphates assume a helical conformation in solution with each turn of the helix corresponding to three PO_4 tetrahedra. This is the rationale[23] for the formation of trimetaphos-

(20) See ref 5, pp 663–664.

(21) M. M. Crutchfield, C. H. Dungan, J. H. Letcher, V. Mark, and J. R. Van Wazer, *Top. Phosphorus Chem.*, **5**, 67 (1967).

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(23) E. Thilo, *Advan. Inorg. Chem. Radiochem.*, **4**, 1 (1962).

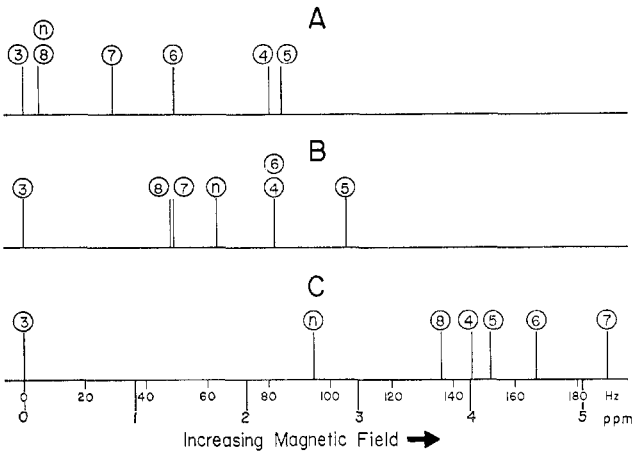

Figure 5.—The relative chemical shifts of the middle phosphate groups of long-chain poly- and metaphosphates in water and anhydrous tetramethylurea. Sequence A was determined in water at pH 7 in the presence of sodium ions and 0.07 *M* sodium ethylenediaminetetraacetate; B in water at pH 7 in the presence of tetra-*n*-butylammonium ions and 0.07 *M* tetra-*n*-butylammonium ethylenediaminetetraacetate; C in anhydrous tetramethylurea containing 10% diisopropylcarbodiimide as a desiccant and in the presence of tetra-*n*-butylammonium ions. Each sequence was determined with samples containing all of the phosphates, with each of these present at a phosphorus concentration of 0.01 *M:* trimeta, 3; tetrameta, 4; pentameta, 5; hexameta, 6; heptameta, 7; octameta, 8; long-chain polyphosphate, *n*. The ppm scale is given relative to internal trimetaphosphate.

phate during the hydrolysis[24] of long-chain phosphates. Inspection of molecular models shows that while the tetra- and pentametaphosphates cannot be twisted into a conformation involving a helix of three PO_4 groups, this can be done for the larger cyclic metaphosphates. Since it appears that five of the PO_4 groups of the hexametaphosphate, six of the hepta, and eight of the octa can participate in this kind of helix formation, we would expect that the sequence of chemical shift of this series might well approach that found for the trimetaphosphate itself because the trimetaphosphate is a close approximation to a helix based on three PO_4 tetrahedra. In other words, we ascribe the anomalous set of shifts for the sodium cyclic metaphosphates in aqueous solution to a strong tendency for the polymerized PO_4 tetrahedra to assume the same kind of helical conformation which has been proposed for the chain phosphates—a conformation which resembles a stack of trimetaphosphate molecules.

However, it is apparent from the data of Figure 5 that the above rationale must be modified if the countercation in aqueous solution is the large tetra-*n*-butylammonium ion. Although the ordering of the metaphosphates remains about the same, their chemical shifts, relative to that of the trimetaphosphate, are altered substantially, and the resonance position of the long-chain polyphosphate is moved upfield from trimeta- to a position between hepta- and hexametaphosphate. In comparing sequence B of Figure 5 to sequence A, it is observed that, in general, the change in chemical shift of the various metaphosphates relative to the trimetaphosphate increases as the ring size increases being least for the tetrametaphosphate ($\Delta\delta$ = 0.55 ppm) and greatest for the octametaphosphate ($\Delta\delta$ = 1.21 ppm). The shift of the chain polyphosphate shows the greatest change of all ($\Delta\delta$ = 1.62 ppm) upon going from the sodium to the tetra-*n*-butylammonium cation.

These data can be interpreted if it is assumed that the tetra-*n*-butylammonium ion cannot be closely associated with the various condensed phosphates when they are tightly coiled into a trimeta-like helix. Thus, in the presence of this ion, the conformation of the condensed phosphates is interpreted to be somewhat distended relative to their conformation in the presence of sodium ion. The cyclic molecule having the greatest capability for coiling would thus be expected to exhibit the largest change in chemical shift upon changing from the sodium to the tetra-*n*-butylammonium cation, as indeed seems to be the case.

A comparison of sequence C of Figure 5 wherein the solvent is the less polar, aprotic, anhydrous tetramethylurea to sequence B wherein the solvent is the highly polar, protic water reveals that the solvent also greatly influences the relative chemical shifts of the various condensed phosphates. In anhydrous tetramethylurea, the tetra- through octametaphosphates are all shifted upfield with respect to the trimetaphosphate by the significantly large amount of *ca.* 4 ppm and are closely grouped within a range of 1.5 ppm. The sequence of the chemical shifts of the tetra- through heptametaphosphates proceeding upfield is tetra < penta < hexa < hepta. Further, as the ring size in this series increases, the chemical shift difference existing between them increases. The octametaphosphate, however, occurs 0.25 ppm to lower field of the tetrametaphosphate while the long-chain polyphosphate comes into resonance at a position 1.13 ppm to lower field of the octametaphosphate. The long-chain polyphosphate, nevertheless, appears 2.61 ppm upfield from the trimetaphosphate. The ordering of the chemical shifts of the condensed phosphates in anhydrous tetramethylurea is then tri <<< poly << octa < tetra < penta < hexa < hepta.

We interpret this behavior to mean that, in general, cyclic metaphosphate salts in anhydrous tetramethylurea in contrast to aqueous solutions do not tend to exhibit a preferential molecular conformation but instead randomly assume the various available conformational states.[25] However, the anomalous occurrence of the resonance position of the octametaphosphate and long-chain polyphosphate indicates that even in anhydrous tetramethylurea, there remains some tendency for the larger condensed phosphates to assume a preferred conformation.

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(25) R. M. Levy and J. R. Van Wazer, *J. Chem. Phys.*, **45**, 1824 (1966).