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## Phosphorus-31 Nuclear Magnetic Resonance Studies on Condensed Phosphates.<sup>1,2</sup> III. Polyphosphate Spectra

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Straight-chain phosphates exhibiting up to 10 phosphorus atoms per chain have been prepared in pure form by column chromatography of a suitable sodium phosphate glass. The <sup>31</sup>P NMR spectra of the resulting sodium polyphosphates, Na<sub>n+2</sub>P<sub>n</sub>O<sub>3n+1</sub> for n < 9, have been obtained and the spectra for n < 8 have been fully analyzed for their respective shielding parameters and coupling constants. The trends in these values are discussed with emphasis on the curious variation with chain length of the middle-group chemical shifts.

### Introduction

The existence of a series of polyphosphates was postulated<sup>3</sup> over a century ago, and the smallest and next larger molecule (the orthophosphate and the pyro- or, alternatively, diphosphate) in this series have been known in crystalline form since alchemical times. The presence of the next two larger species (the tri- and tetrapolyphosphates) has been demonstrated<sup>4,5</sup> in phase-diagram studies only within the last 35 years and the longer-chain phosphates in pure form are still laboratory curiosities. Although naturally occurring mixtures of the longer straight-chain phosphates have been a subject of considerable interest<sup>6,7</sup> for 25 years, there has only been one report<sup>8</sup> of the isolation of the individual members of this series of molecule ions up through the ten-unit chain. The procedure used in that isolation<sup>8</sup> was a difficult and laborious one involving fractional crystallization of the acridinium salts.

In the more recent work on mixtures of polyphosphate molecule ions, <sup>31</sup>P nuclear magnetic resonance (NMR) has proved to be an especially powerful analytical tool.<sup>9</sup> However, the fact that chain phosphates having more than three phosphorus atoms per molecule exhibit second-order spectra of considerable complexity has been a great hindrance to the full utilization of this technique in the study of phosphates. Unfortunately, the structure of the spectra are so involved that any systematic spectral analysis usually has been precluded in mixtures which include the longer straight-chain phosphates. Therefore, it seemed desirable to develop an improved method for isolating the longer-chain polyphosphates and to obtain their NMR spectra under optimum conditions. This is the substance of this report.

### Experimental Section

**Polyphosphate Separations.** A separation was effected on a sodium phosphate glass<sup>10</sup> exhibiting a number-average chain length,  $\bar{n}$ , of 5.5 as determined by end-group titration,<sup>11</sup> as well as by the zinc-oxide gravimetric determination of water<sup>12</sup> coupled with the Na<sub>2</sub>O/PO<sub>5</sub> ratio of the reagent mixture from which the glass was made. In order to obtain samples containing as much as 0.2 g of the individual polyphosphate, it was necessary to carry out a two-stage

fractionation. In the first stage a concentrated solution of the glass was applied to the top of a column (80 cm length, 5.0 cm diameter) packed with diethylaminoethyl cellulose in the bicarbonate form.<sup>13</sup> The phosphates were eluted using an 18-l. linear-gradient (0.2 to 1.0 M) triethylammonium bicarbonate solution to give 100-ml cuts which were analyzed for total phosphorus.<sup>14</sup> Upon pooling the appropriate fractions and concentrating in a rotary evaporator at 25°, the bicarbonate-purged triethylammonium phosphates were precipitated from methanol solution by sodium iodide in acetone.<sup>13</sup>

These samples obtained from the crude cuts were further separated by an analogous procedure, using a similarly packed column (240 cm length, 2.5 cm diameter) again with 18 l. of the same eluent. In the initial run with this long, narrow column, 10-ml cuts were employed with an elution rate of 1.0 ml/min. During the elution procedures, it is important always to keep the receiver vials covered, since we have observed that small particles of dust may catalyze the hydrolysis of an individual fraction. Since, under the best conditions, aqueous solutions of phosphates undergo appreciably rapid hydrolysis, the dry sodium salts should be prepared as expeditiously as possible. Each preparation from this procedure yielded NMR spectra showing no unaccountable resonances in the <sup>31</sup>P spectrum and also exhibited a clean solitary spot upon thin-layer chromatographic analysis.<sup>15,16</sup> The tetra-*n*-butylammonium salts were prepared by immediately titrating the solution of the free polyphosphoric acid (obtained by passing the Na salt through a Dowex 50 H<sup>+</sup> column) with tetra-*n*-butylammonium hydroxide to the desired pH.

**NMR Studies.** The <sup>31</sup>P NMR spectra<sup>17,18</sup> were obtained on a Bruker HFX-5 spectrometer operating at 36.4 MHz for <sup>31</sup>P and 90.0 MHz for the <sup>1</sup>H lock. The samples were studied at 25° in freshly prepared aqueous solutions containing 0.1 M of total phosphorus, using 5- or 13-mm spinning sample tubes. The pH was held at 10.2 and the solution was 0.2 M in ethylenediaminetetraacetic acid (EDTA), which was added in order to sequester any trace of multiply charged metal ions since these ions form complexes with the polyphosphates and thereby may cause rather large changes in their <sup>31</sup>P NMR parameters. In each run, the scanning speed and observing rf power were carefully adjusted under the signal-averaging conditions employed to ensure that signal saturation was not taking place. All ref-

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erencing was, of course, carried out with respect to the proton lock signal, but the chemical shifts are formally reported with respect to 85% orthophosphoric acid (externally situated), with positive shifts being upfield.<sup>18</sup>

The spectra were analyzed using a computer package developed by Swalen<sup>19</sup> consisting of three separate programs (NMRIT, NMREN2, and NMRPLT). In this study, it was possible to fit the experimental spectra of the tetra-, penta-, and hexopolyphosphates, using an iterative analytical approach (involving NMREN2 and NMRIT), since the number of observed transitions were not excessively large and were fairly well resolved so that their positions could be assigned with reasonable certainty. Due to the excessive number of transitions involved, the spectra of the hepta- and octopolyphosphates had to be analyzed by cut-and-try methods (using NMRIT and NMRPLT) to obtain a set of NMR parameters which were concordant with those obtained for the shorter-chain phosphates. In order to get a good fit between the calculated and experimental data, 35 separate sets of calculations were needed for the heptopolyphosphate and 13 for the octopolyphosphate molecule ion.

## Results and Interpretations

By running a small sample of the original sodium phosphate glass ( $\bar{n} = 5.5$ ) through the 240-cm column employed for the final fractionation of the NMR samples, the elution curve shown in Figure 1 was obtained. Note that this curve approximates the linear elution pattern which should be expected for a regular series of related compounds. The smallest members of the family (the pyro- and tripolyphosphates) deviate from a straight-line curve, as might be anticipated. A similar type of dependence has been observed<sup>13</sup> for the series of cyclic metaphosphates eluted from the same substrate. Evidence that the individual chain lengths noted on the vertical axis of Figure 1 are indeed correct comes from several sources. First, the corresponding polyphosphates from the crystallization of the acridinium salts were shown to fall in the same positions in thin-layer chromatographic analysis. Secondly, samples of tri- and tetrapolyphosphate run through the 240-cm column elute at the right place, and their NMR patterns corresponded to those given in the literature. Thirdly, the NMR spectra of the eluted samples exhibited the expected ratio of total end,  $e$ , to total middle,  $m$ , phosphate groups,<sup>20</sup> with no detectable orthophosphate,  $n$ , or branch,  $b$ , groups (i.e.,  $n < 0.2\%$  and  $b < 1.0\%$  of total P) and no resonances assignable to known cyclic metaphosphates.<sup>13</sup> Furthermore, the applicability of the mathematical analysis<sup>19</sup> of the NMR spectra discussed in this paper is also support for the singularity of each sample.

The spectra of the penta- and hexopolyphosphates, shown in Figure 2, exemplify the quality of fits obtained between the experimental (NMR) and theoretical (computer-simulated)  $^{31}\text{P}$  spectra from each member of the series of polyphosphate compounds of this study. The pentaphosphate spectrum has the most complicated appearance of any of those obtained from the family of condensed phosphates; however, the number of transitions is not excessively high because of the limited number of interacting nuclei, and the spectrum can be computed with a high degree of certainty. For the chain phosphates, the number of resolvable transitions decreases as the molecular size increases from that of pentaphosphate because of the crowding of individual transitions, so that the spectrum appears more simple. The theoretical complexity, however,

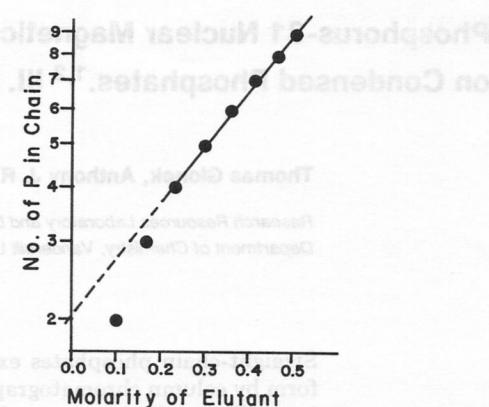


Figure 1. The elution pattern of the chain polyphosphates from the 240-cm column of diethylaminoethyl cellulose, using an 18-l. linear salt gradient of triethylammonium bicarbonate.

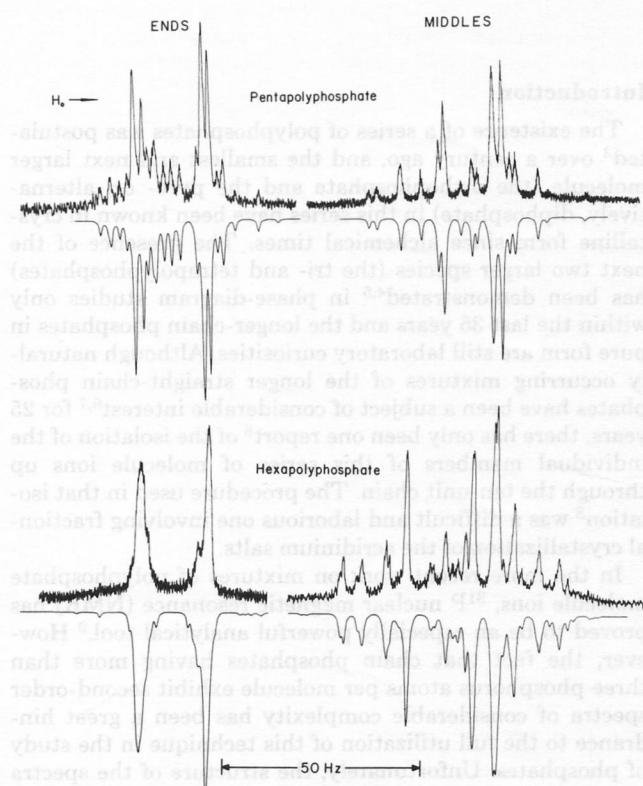


Figure 2. Experimental and simulated (inverted)  $^{31}\text{P}$  NMR spectra of sodium penta- and hexaphosphate, each at pH 10.2. The signal width at half-height in the calculated spectra was 1.2 Hz.

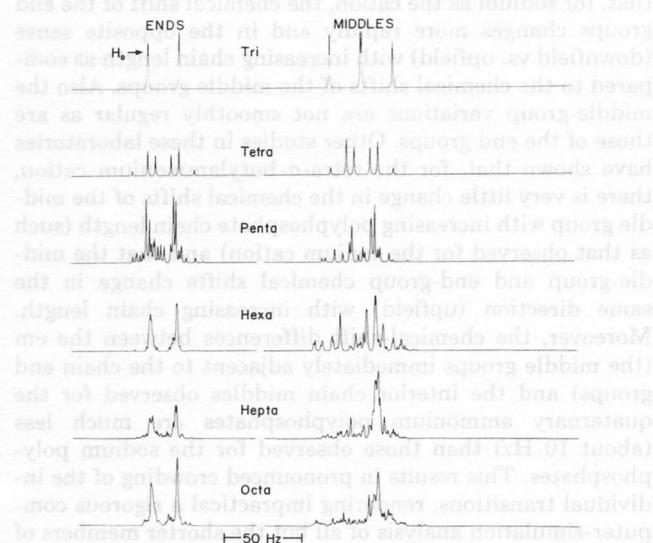
must increase with increasing molecular size. Thus, for phosphates larger than the pentaphosphate, the errors inherent in the calculated values of the chemical shifts and coupling constants increase with the size of the phosphate so that calculations on the nona- and higher polyphosphates, should they be performed, would be of limited accuracy.

Fortunately, as the molecular size increases, the  $^{31}\text{P}$  spectrum simplifies. The nona- and decaphosphate essentially exhibit a "simple" doublet in the end-group spectral region and a single broad envelope in the middle-group region. For chain lengths greater than ca. 150 phosphorus atoms, the end group (when detectable) is a sharp doublet while the middle-group envelope appears as a sharp singlet. For a carefully prepared solution of Kurrol's

**TABLE I:**  $^{31}\text{P}$  NMR Parameters for Solutions (0.1 M in P) of the Pure Sodium Polyphosphates at pH 10.2

$n$ in $\text{Na}_{n+2}\text{P}_n\text{O}_{3n+1}$	Chemical shifts, <sup>a</sup> Hz					Coupling constants, <sup>b</sup> Hz			
	Middle groups					$J_{1,2}$	$J_{2,3}$	$J_{3,4}$	$J_{4,5}$
	End e	em	emm	emmm	emmm				
2	264.1								
3	251.4	743.0				20.6			
4	228.1	748.0				20.2	15.0		
5	207.6	753.7	766.5			19.2	15.5		
6	198.2	732.6	758.6			18.1	16.0	16.0	
7	197.4	757.1	774.6	775.0		17.7	16.2	16.3	
8	197.2	756.2	778.4	778.8		17.6	16.3	16.3	16.3
9	197.2	767.5 <sup>c</sup>	778.4 <sup>c</sup>	778.8		17.6 <sup>c</sup>			
$\infty$	197.2 <sup>d</sup>	768.0 <sup>e</sup>		778.8 <sup>d,e</sup>		17.4 <sup>d</sup>			

<sup>a</sup> The chemical shifts measured from the hydrogen lock are referenced to "external" 85%  $\text{H}_3\text{PO}_4$ , with positive shifts being upfield and 1 ppm = 36.4 Hz. The symbol e refers to the phosphate end group and, for example, emm refers to the phosphate middle group which is based on the third phosphorus from the end of the chain. <sup>b</sup>  $J_{i,(i+1)}$  is the coupling constant between the pair of neighboring phosphorus atoms in position  $i$  and  $(i + 1)$  where  $i$  is the number of the phosphorus atom when counting from the end of the chain. <sup>c</sup> Estimated by extrapolation. <sup>d</sup> Directly measured on several long-chain polyphosphate compositions. <sup>e</sup> This middle-group chemical shift corresponds to phosphorus atoms so far from the ends of the chains as to be unaffected by them.



**Figure 3.** Simulated  $^{31}\text{P}$  NMR spectra of the sodium tri- through octapolyphosphates. The amplitude of each multiplet was chosen to correspond to that of the experimental spectrum to which it was fitted and, therefore, the relative areas of the end and middle groups are not comparable. The signal width at half-height used in the simulation was 0.5 Hz. The spectra correspond to those of the sodium polyphosphates at pH 10.2 (solutions 0.1 M in phosphorus and 0.2 M in EDTA).

salt, exhibiting a chain length of ca. 10,000 phosphorus atoms, the middle-group line width was measured to be less than 0.2 Hz, the resolution limit of the spectrometer at that time.

Figure 3 shows the calculated NMR spectra obtained for the tri- through the octapolyphosphate. For illustrative purposes, these spectra are presented instead of the experimental NMR spectra because spectral noise in the experimental records often hides the fine points of multiplet structure. The signal width at half-height was chosen to be 0.5 Hz which corresponds to the best resolution that was obtained for this series of phosphates. The best-fit parameters that were used in simulating the spectra are given in Table I.

The spectra for the pyrophosphate (a singlet in the end-group spectral region) and tripolyphosphate (an end-group doublet and middle-group triplet) have been reported many times elsewhere.<sup>9</sup> Note that the center resonance of the tripoly middle-group triplet is composed of two closely spaced transitions; under optimum scan conditions, this multiplicity can be detected in experimental spectra. Also note that, consistent with the behavior of pseudo first-order NMR spectra, the intensities of the inner signals of each multiplet are greater than that of their outer counterparts. This phenomenon, which is also apparent in the spectrum of the tetrapolyphosphate, is observed experimentally.

The apparent complexity of the end-group multiplet goes from a simple doublet for the tripolyphosphate to a complicated second-order pattern for the tetrapolyphosphate. Although the end-group multiplet structure of the pentapolyphosphate is still quite complicated, the overall envelop is that of a doublet. On proceeding to the longer-chain polyphosphates, the simple-doublet appearance of the end-group multiplet becomes more pronounced.

A similar change in superficial appearance is observed for the middle-group region of the spectrum where the apparent triplet of the tripolyphosphate turns into more complicated patterns for the tetra- through hexapolyphosphates, with the overall shape of the envelop becoming simpler with increase in chain length until finally, for very long chains, a single resonance signal exhibiting a very narrow line width<sup>2</sup> at half-height is observed. Because of its unique symmetry as an aa'xx' molecule, the pattern in the end-group region of the tetrapolyphosphate ion is the mirror image of that in the middle-group region.

The theoretical spectra, which were plotted in Figure 3 assuming a spectral resolution of 0.5 Hz, give no indication of the great complexity of the transition patterns from which they result. For the linear array of spins involved in going from the di- through the octapolyphosphate, the number of transitions are 4, 8, 24, 66, 157, 432, and ca. 2000, respectively, for  $\text{Na}_4\text{P}_2\text{O}_7$  through  $\text{Na}_{10}\text{P}_8\text{O}_{25}$ .

It should be emphasized here that the spectra shown in Figure 3 and the corresponding NMR parameters of Table I refer solely to the polyphosphate anions in aqueous solu-

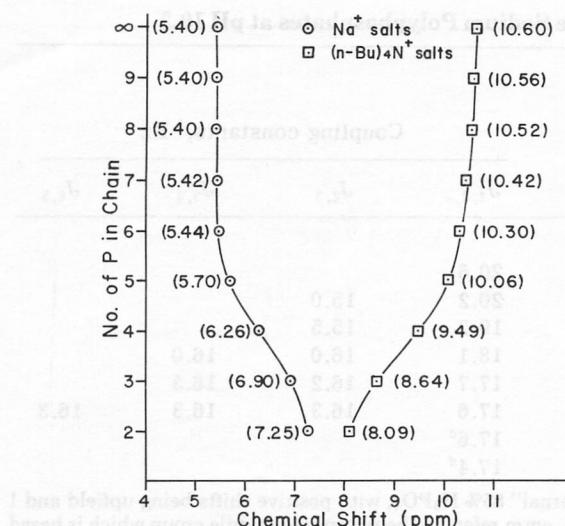


Figure 4. Chemical shifts of the end groups of the sodium and tetra-*n*-butylammonium polyphosphates at pH 10.2 (i.e., no associated protons, except for pyrophosphate). The numbers in parentheses give the precise values of the chemical shifts in ppm and  $\infty$  symbolizes a polyphosphate glass of  $\bar{n} = 100.8$ .

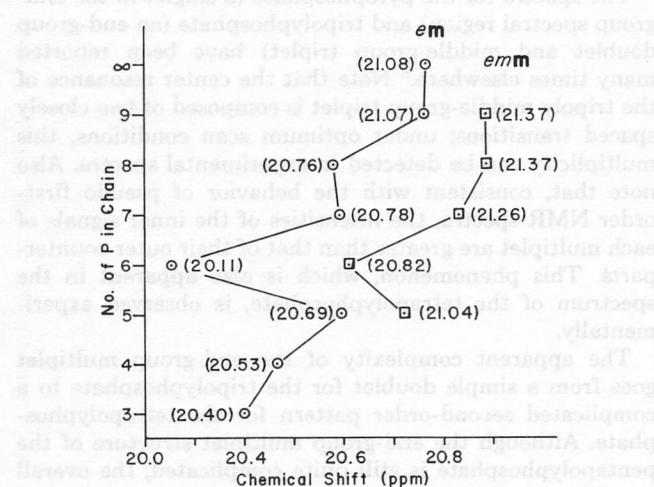


Figure 5. Chemical shifts of the middle groups of the sodium polyphosphates at pH 10.2. The numbers in parentheses give the precise values of the chemical shifts in ppm and  $\infty$  symbolizes a polyphosphate glass of  $\bar{n} = 100.8$ . Only the shifts of the second and third phosphorus atoms from the ends of the chains are plotted (em and emm, respectively).

tion at pH 10.2 in the presence of only sodium as the cation. Miscellaneous experiments with highly purified ortho-, pyro-, and trimetaphosphates have led us to believe that the presence of the EDTA in the solutions studied here has essentially no effect on the phosphate  $^{31}\text{P}$  NMR parameters other than that of rendering a high degree of precision to the measurements; the reported chemical shifts are not moved by more than 0.1–0.2 Hz. The dramatic effect of changing the counterion while holding the pH constant is shown in Figure 4 in which the findings for the end groups of the sodium salts of the di- through the octopolyphosphate may be compared with those for the respective tetra-*n*-butylammonium salts. Analogous data have been presented for the cyclic metaphosphates.<sup>13</sup> Note in Figure 4 that the difference in chemical shift upon changing the cation becomes greater with increasing chain length and finally achieves constancy for the larger chain lengths.

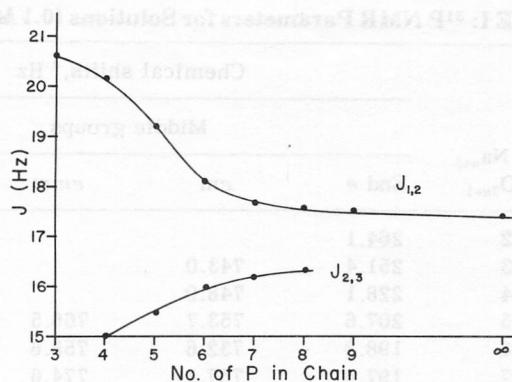


Figure 6. Variation of the coupling constants  $J_{1,2}$  and  $J_{2,3}$  with phosphate chain length, where phosphorus atom 1 is in an end group.

The change in the middle-group chemical shifts with increasing chain length is shown for the sodium polyphosphates in Figure 5. From comparison of the lower-field curve of Figure 4 with the curves of Figure 5, it can be seen that, for sodium as the cation, the chemical shift of the end groups changes more rapidly and in the opposite sense (downfield vs. upfield) with increasing chain length as compared to the chemical shifts of the middle groups. Also the middle-group variations are not smoothly regular as are those of the end groups. Other studies in these laboratories have shown that, for the tetra-*n*-butylammonium cation, there is very little change in the chemical shifts of the middle group with increasing polyphosphate chain length (such as that observed for the sodium cation) and that the middle-group and end-group chemical shifts change in the same direction (upfield) with increasing chain length. Moreover, the chemical-shift differences between the em (the middle groups immediately adjacent to the chain end groups) and the interior chain middles observed for the quaternary ammonium polyphosphates are much less (about 10 Hz) than those observed for the sodium polyphosphates. This results in pronounced crowding of the individual transitions, rendering impractical a rigorous computer-simulation analysis of all but the shorter members of the family.

## Discussion

As shown in Figures 4 and 6, there is a smooth variation with increasing polyphosphate chain length of the end-group  $^{31}\text{P}$  chemical shift as well as a smooth variation of the P–O–P coupling constants between the end and its neighboring middle group ( $J_{1,2}$ ) and between this middle group and its neighboring middle group ( $J_{2,3}$ ). However, in Figure 5, the middle-group chemical shifts vary irregularly with increasing chain length, with the most pronounced deviation from a smooth change occurring in the case of the hexopolyphosphate ion. Note in Figure 5 that the chemical shifts of the middle-group phosphorus atoms immediately adjacent to the end groups are upfield for the chains having an odd number of phosphorus atoms, as compared to the shifts of the neighboring even-numbered chains. Since none of this irregular behavior is found when the sodium counterions are replaced by noncomplexing tetraalkylammonium cations, it appears that these observations are related to the overall conformational ordering of the inherently flexible polyphosphate chains in relationship to the sodium ions with which they are complexed and/or associ-

ated. We are quite certain that the irregularity of the plots given in Figure 5 is a physically real phenomenon since these sets of points have been checked by using entirely different phosphate preparations and carrying through the full experimental and theoretical analysis. Furthermore, attempts at using points from various smooth curves drawn through the data from Figure 5 have consistently led to calculated spectra which simply did not fit the experimental observations. Extension of this study to pH 7 (where all end groups are partially protonated) led to the same situation in which the middle-group chemical shifts gave rise to virtually the same irregularities while the other NMR parameters again exhibited smooth variations with increasing chain length.

When the NMR spectrometer is well adjusted, the line width at half-height at pH 10.2 for the pyrophosphate ion is about 4 Hz; for the tripolyphosphate, about 1 Hz; and for the tetra- through the decapolypophosphate, about 0.5 Hz. As previously noted, phosphate chains exhibiting more than ca. 150 phosphorus atoms exhibits a line width approaching that of cyclic trimetaphosphate, a value which is essentially at the resolution limit of our spectrometer (ca. 0.2 Hz) at the time of these measurements. We conclude from these findings that the middle groups making up the long-chain phosphates are all in the same chemical environment so that in aqueous solution the macromolecular polyphosphate chains exhibit a regular configuration. In a previous publication, we have postulated that, for sodium as the countercation in aqueous solution, this configuration is a helix having three phosphorus atoms per turn.<sup>13</sup>

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## References and Notes

- (1) T. Glonek, R. A. Kleps, E. J. Griffith, and T. C. Myers, *Phosphorus*, in press.
- (2) T. Glonek, R. A. Kleps, E. J. Griffith, and T. C. Myers, *Phosphorus*, in press.
- (3) T. Fleitmann and W. Henneberg, *Annalen*, **45**, 304, 387 (1845).
- (4) E. P. Partridge, V. Hicks, and G. V. Smith, *J. Am. Chem. Soc.*, **63**, 454 (1941).
- (5) R. K. Osterheld and R. P. Langguth, *J. Phys. Chem.*, **59**, 76 (1955); R. P. Langguth, R. K. Osterheld, and E. F. Karl-Kroupa, *ibid.*, **60**, 1335 (1956); also see R. P. Langguth, M.S. Thesis, Cornell University, Ithaca, N.Y., 1952; and M. Amadori, *Atti. R. Ist. Veneto Sci. Lett. Arti*, **76**, 419 (1916).
- (6) J. R. Van Wazer and K. A. Holst, *J. Am. Chem. Soc.*, **72**, 639 (1950).
- (7) J. R. Van Wazer, *J. Am. Chem. Soc.*, **72**, 644 (1950).
- (8) E. J. Griffith and R. L. Buxton, *J. Am. Chem. Soc.*, **89**, 2884 (1967).
- (9) M. M. Crutchfield, C. H. Dungan, J. H. Letcher, V. Mark, and J. R. Van Wazer, "P<sup>31</sup> Nuclear Magnetic Resonance", in M. Grayson and E. J. Griffith, Eds., "Topics in Phosphorus Chemistry", Vol. 5, Wiley-Interscience, New York, N.Y., 1967.
- (10) J. R. Van Wazer, *J. Am. Chem. Soc.*, **72**, 647 (1950).
- (11) J. R. Van Wazer, E. J. Griffith, and J. F. McCullough, *Anal. Chem.*, **26**, 1755 (1954).
- (12) E. J. Griffith and C. F. Callis, *J. Am. Chem. Soc.*, **81**, 833 (1959).
- (13) T. Glonek, J. R. Van Wazer, M. Mudgett, and T. C. Myers, *Inorg. Chem.*, **11**, 567 (1972).
- (14) P. S. Chen, T. Y. Toribara, and H. Warner, *Anal. Chem.*, **28**, 1756 (1956).
- (15) T. Glonek, J. R. Van Wazer, and T. C. Myers, *Bioinorg. Chem.*, **1**, 1 (1971).
- (16) J. M. Tanzer, M. I. Krichevsky, and B. Chassy, *J. Chromatogr.*, **38**, 526 (1968).
- (17) T. O. Henderson, T. Glonek, R. L. Hilderbrand, and T. C. Myers, *Arch. Biochem. Biophys.*, **149**, 484 (1972).
- (18) T. Glonek, T. O. Henderson, R. L. Hilderbrand, and T. C. Myers, *Science*, **169**, 192 (1970).
- (19) J. D. Swalen, Programs 33, 35, and 36 in the Quantum-Chemistry Program-Exchange Catalog, Chemistry Department, Indiana University, Bloomington, Ind., 1973.
- (20) D. W. Matula, L. C. D. Groenweghe, and J. R. Van Wazer, *J. Chem. Phys.*, **41**, 3105 (1964).