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### Phosphorus-31 Chemical Shift Variations with Counteranion and Ionic Strength for the Various Ethyl Phosphates

A. J. R. Costello, T. Glonek, and J. R. Van Wazer\*

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Observations in our laboratories have shown  $^{31}\text{P}$  nuclear magnetic resonance (NMR) chemical shifts of a number of biological phosphorus compounds (studied by the Fourier-transform method in aqueous and nonaqueous solvents, as well as in intact living cells) to be differently affected by various simple, singly charged cations.<sup>1-6</sup> In order to provide a foundation for the understanding of such studies, we have carried out  $^{31}\text{P}$  shift determinations on some simple orthophosphate compounds (and a phosphonate), as reported herein.

### Experimental Section

Field frequency stability of the Bruker HFX-5 spectrometer<sup>2,4</sup> ( $^{31}\text{P}$  field 36.43 MHz) was provided by the protons of tetramethylsilane contained in a capillary coaxially mounted in the 13-mm spinning sample tube. As is customary,<sup>7</sup> the NMR chemical shifts are reported relative<sup>8</sup> to "external" 85%  $\text{H}_3\text{PO}_4$ ,<sup>9</sup> with positive shifts being upfield. The salts of the phosphorus oxy acids were prepared in the chosen solvent by previously published procedures.<sup>10</sup> All of the reported data correspond to the phosphorus concentrations being held at 0.010 M and the pH at 7.00 at a temperature of  $25.0 \pm 0.2^\circ\text{C}$ . The pH was adjusted without use of a buffer so that foreign ions were not introduced.

### Results and Discussion

The variations with added tetramethylammonium chloride or sodium chloride of the  $^{31}\text{P}$  chemical shifts at pH 7.00 of dilute, aqueous solutions of triethyl orthophosphate,  $(\text{C}_2\text{H}_5\text{O})_3\text{PO}$ , the diethyl orthophosphate anion,  $(\text{C}_2\text{H}_5\text{O})_2\text{PO}_2^-$ , the monoethyl orthophosphate anion,  $(\text{C}_2\text{H}_5\text{O})\text{PO}_3\text{H}_{1.3}^{0.7-}$ ,

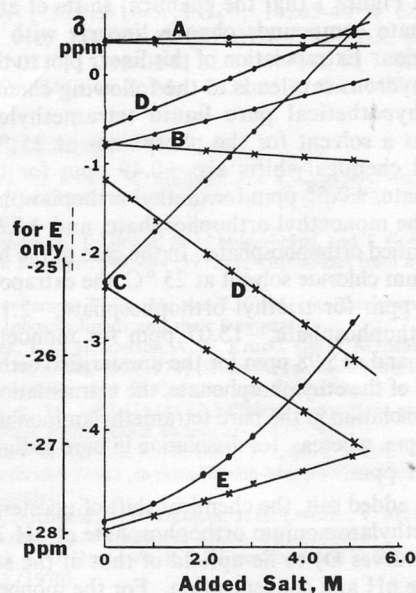


Figure 1.  $^{31}\text{P}$  chemical shifts,  $\delta$ , of 0.010 M aqueous solutions at pH 7.00 as a function of added salt concentration for (A) triethyl orthophosphate, (B) the diethyl orthophosphate anion, (C) the monoethyl orthophosphate anion, (D) the inorganic orthophosphate anion, and (E) the ethylphosphonate anion. The upper curves of each set (with data points denoted by black circles) correspond to the added salt being tetramethylammonium chloride with the counterion to the phosphorus acids being the tetramethylammonium ion. The lower lines of each set (with data points denoted by crosses) correspond to the added salt being sodium chloride with the counterion to the phosphorus acids being the sodium ion.

and the unesterified orthophosphate anion,  $\text{PO}_4\text{H}_{1.6}^{1.4-}$ , are shown in Figure 1, along with the same information for the ethylphosphonate ion,  $\text{C}_2\text{H}_5\text{PO}_3\text{H}_{1.5}^{0.5-}$ . If tetramethylammonium bromide or sodium bromide are employed in place of the respective chloride, exactly the same curves as shown in Figure 1 are obtained. Although less work was done with the perchlorate or sulfate salts, it also appeared that the results were essentially the same. In other words, the role of the anion in causing these chemical shift changes is unimportant. At

\* To whom correspondence should be addressed at Vanderbilt University.

the chosen pH of 7.00, it should be noted that the diethyl orthophosphate anion is completely dissociated ( $pK_1 = 1.39$ ), whereas the monoethyl orthophosphate ( $pK_2 = 6.62$ ) and the unesterified orthophosphate ( $pK_2 = 7.10$ ) anions exhibit approximately 1.3 and 1.6 associated hydrogen atoms, respectively. Likewise, the ethylphosphonate anion has about 1.5 associated hydrogens.

Aside from the different chemical shifts in pure water (a phenomenon which has been previously explained<sup>7</sup>), the most noteworthy feature of Figure 1 is found in the fact that addition of tetramethylammonium chloride to the solution causes an increase in the chemical shifts, whereas addition of sodium chloride causes a decrease or, in the case of the ethylphosphonate, a lesser increase in the chemical shift. Furthermore, it should be noted in the chosen phosphate group of compounds, that the change of the  $^{31}\text{P}$  chemical shift with added salt concentration is very small for the triester and increases with increasing degree of ionization. Thus, the difference in chemical shift due to substitution of sodium for tetramethylammonium ion is found to be 0.02 ppm/mol of added salt for the uncharged triethyl orthophosphate, 0.17 for the single-charged diethyl orthophosphate, and 0.75 and 0.94 for the unesterified orthophosphate and for the monoethyl orthophosphate, each of which exhibit an average molecular charge of ca. 1.5 electrons.

Note in Figure 1 that the chemical shifts of all of the orthophosphate compounds change linearly with added salt concentration. Extrapolation of this linear plot to the molarity of the anhydrous salt leads to the following chemical shifts. For the hypothetical pure liquid tetramethylammonium chloride as a solvent for the phosphates at 25 °C, the extrapolated chemical shifts are +0.49 ppm for triethyl orthophosphate, +0.55 ppm for diethyl orthophosphate, +3.40 ppm for the monoethyl orthophosphate, and +1.63 ppm for the unesterified orthophosphate. In the case of the hypothetical liquid sodium chloride solvent at 25 °C, the extrapolated shifts are -0.03 ppm for triethyl orthophosphate, -2.14 ppm for diethyl orthophosphate, -15.07 ppm for monoethyl orthophosphate, and -15.98 ppm for the unesterified orthophosphate. In the case of the ethylphosphonate, the extrapolation chemical shift for dissolution in the pure tetramethylammonium chloride is -14.88 ppm, whereas, for dissolution in pure sodium chloride, it is -22.01 ppm.

With no added salt, the chemical shift of unesterified 0.010 M tetramethylammonium orthophosphate at pH 7 is seen in Figure 1 (curves D) to lie upfield of that of the sodium salt at the same pH and concentration. For the monoester, these two counterions lead to a lesser difference in chemical shift; and, for the diester, this shift difference is negligible.

There are two general approaches which may be used for explaining solvent-induced chemical shift changes. One of them, which is generally called "long-range" nuclear shielding, considers<sup>11</sup> that distant atoms or groups exhibiting spin behavior act together to modify the applied magnetic field as seen by the nucleus under observation. The other<sup>7,12</sup> considers the effect of the "unbalanced p and d electrons"<sup>13</sup> of the nucleus under observation brought about by changes induced in its electronic interaction with neighboring atoms (the electronic structures of which in turn are affected by local changes in their environment). Although either of these two theoretical approaches may be used independently to explain either an increase or decrease in chemical shift due to solvent changes (such as the incorporation of a salt into a solvent), they are basically different (long-range vs. short-range shielding) and real situations may involve both of them.

In the case of the orthophosphate compounds for which data are reported in Figure 1, there is some information which may be used to distinguish if one of these two alternatives pre-

dominates. If long-range shielding were dominant, we would expect that the various phosphate compounds would show about the same slopes with increasing concentration for all of the tetramethylammonium chloride cases or, alternatively, for all of the sodium chloride cases; but this is not so. Furthermore, one would not expect the effect to be anion independent. Indeed, the data strongly indicate that the observed chemical shift changes must be attributable to short-range interactions between the cations and the orthophosphate anion under NMR observation. This kind of situation is probably best handled by a treatment such as that which has been previously applied<sup>14</sup> to the interpretation of the change in  $^{31}\text{P}$  chemical shift of some oxy acids of phosphorus with degree of neutralization.

Approximate quantum chemical calculations<sup>12</sup> have shown that the change in the chemical shift,  $\Delta\delta$ , for these compounds may be treated by the relationship<sup>14</sup>

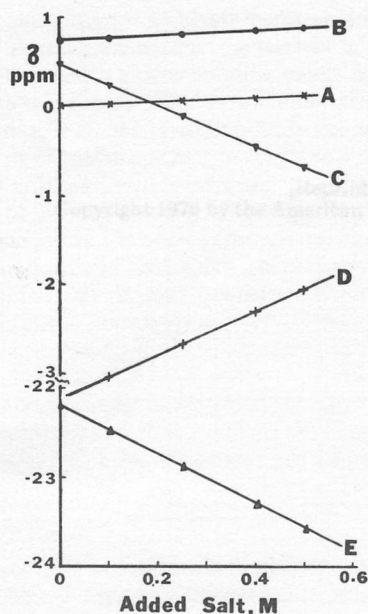
$$\Delta\delta = 180\Delta\chi_0 - 147\Delta n_\pi - A\Delta\theta \quad (1)$$

where  $\Delta\chi_0$  is the change in the effective electronegativity of the  $\text{PO}_4$  oxygens caused by the change in added salt concentration,  $\Delta n_\pi$  is the concomitant change in the phosphorus  $d_\pi$ -orbital occupation due to variation in the  $\pi$  character of the P-O bonds thus induced, and  $\Delta\theta$  is any change in the OPO bond angle caused by the addition of salt to the solution. Since  $A$  is a small number lying in the range of 0-0.3, the expected small change in bond angles can surely be neglected. From this equation, it is clear that an increase in  $^{31}\text{P}$  chemical shift upon addition of the salt corresponds to more  $\sigma$ -electron withdrawal by the oxygens from the phosphorus and/or less  $p_\pi \rightarrow d_\pi$  donating by the oxygens to the phosphorus atom. Therefore, we conclude from eq 1 that the presence of the excess sodium ion causes these oxygens to give electrons to the phosphorus. Now, if we consider only the association of a sodium with a  $\text{PO}_4$  oxygen (i.e., formation of a weak Na-O chemical bond), we might think that the resulting change in chemical shift ought to be upfield not downfield as observed.

Since the tetramethylammonium ion is usually considered to be wholly noncomplexing, bonding between it and a  $\text{PO}_4$  group should not be considered. In its role as a positive-charge carrier, this ion in close approach to a  $\text{PO}_4$  ion will draw electron density on a  $\text{PO}_4$  oxygen toward it and hence will tend to withdraw electrons from the P-O bond, resulting in the observed upfield change in chemical shift with increasing salt concentrations. We explain the opposite effect of the sodium ion as a replacement (by the "associated" sodium) of the hydrogen bonds which tie the oxygens of the  $\text{PO}_4$  group to the water matrix. In other words, we conclude from these tenuous arguments that the upfield change in chemical shift upon adding excess tetramethylammonium ion is predominantly due to the positive charge of this ion, whereas the opposite effect of the sodium ion results from the penetration of the phosphate hydration sphere by the alkali ion.

The  $^{31}\text{P}$  chemical shift behavior of the same group of phosphate compounds and the same phosphonate upon adding tetra-*n*-butylammonium chloride to their anhydrous solutions in tetramethylurea is reported in Figure 2. The apparent pH to which the data in this figure correspond is again 7.0. This pH adjustment was achieved by neutralization of the phosphorus compound in aqueous solution followed by a careful process of dehydration and resolution into the anhydrous solvent, according to the method<sup>10</sup> we have developed. In interpreting the data of Figure 2, it is important to remember that tetramethylurea acts as one of the few good solvents for inorganic orthophosphates, presumably because of an affinity for these acid anions due to its weakly basic character. Furthermore, ion-pair formation should be much more likely in this medium than in water.





**Figure 2.**  $^{31}\text{P}$  chemical shifts,  $\delta$ , of 0.010 M solutions in tetramethylurea as a function of the concentration of added tetrabutylammonium chloride for (A) triethyl orthophosphate and the tetrabutylammonium salts of (B) the diethyl orthophosphate anion, (C) the monoethyl orthophosphate anion, (D) the orthophosphate anion and (E) the ethylphosphonate anion. The solutions were all adjusted so as to exhibit a pH of 7 when the tetramethylurea was replaced by water.

Our interpretation of the data of Figure 2 is based on the same ideas used in explaining Figure 1. We conclude that there is little effect of the added salt on the chemical shift of either the triethyl or the diethyl orthophosphate since neither of these have associated hydrogen atoms through which the solvent can interact with the  $\text{PO}_4$  groups. The downward change of the chemical shift with added salt for the monoester is then attributed to the added cations disrupting the interaction of the solvent with the proton associated with the  $\text{PO}_4$  groups (presumably through hydrogen bonding). Apparently the upfield change in shift observed for the unesterified orthophosphate upon the salt addition is due to retention of the interaction with the solvent through the very weakly acidic proton plus an increase in magnetic shielding brought about by close approach of the positive ions.

It should be noted in Figure 1 that the chemical shifts corresponding to no additional salt are very close for the sodium and tetramethylammonium cations of all of the phosphate structures except the unesterified orthophosphate, for which it can be seen that the chemical shift of the sodium orthophosphate at pH 7.00 is appreciably lower than that of

the tetramethylammonium salt at the same pH. This finding led us to investigate the effect of different countercations on the orthophosphate chemical shift corresponding to pH 7 in aqueous solutions containing 0.010 mol of P/l., with no added chloride or other salt. The results for the various ammonium and alkali metal ions are as follows:  $(\text{CH}_3)_4\text{N}^+$ ,  $-0.61$  ppm;  $\text{NH}_4^+$ ,  $-0.82$  ppm;  $\text{Li}^+$ ,  $-0.71$  ppm;  $\text{Na}^+$ ,  $-1.21$  ppm;  $\text{K}^+$ ,  $-0.62$  ppm;  $\text{Rb}^+$ ,  $-1.66$  ppm;  $\text{Cs}^+$ ,  $-0.77$  ppm. Values were also obtained for two of the alkaline earth metal ions, which were present on a 1:1 molar basis with respect to the phosphorus, with additional tetramethylammonium ion to bring the precursor aqueous solution<sup>10</sup> to pH 7.00. The results are as follows:  $\text{Mg}^{2+}$ ,  $-1.65$  ppm;  $\text{Ca}^{2+}$ ,  $-0.65$  ppm. Unfortunately the strontium and barium phosphates are not sufficiently soluble to have been included in this study. An interesting feature of these findings is the alternation between high and low values of the chemical shift upon going from the lighter to the heavier alkali metal ions.

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**Registry No.**  $(\text{C}_2\text{H}_5\text{O})_3\text{PO}$ , 78-40-0;  $(\text{C}_2\text{H}_5\text{O})_2\text{PO}_2^-$ , 48042-47-3;  $(\text{C}_2\text{H}_5\text{O})\text{PO}_3^{2-}$ , 57919-10-5;  $\text{PO}_4^{3-}$ , 14265-44-2;  $\text{C}_2\text{H}_5\text{PO}_3^{2-}$ , 16486-09-2;  $\text{Na}$ , 7440-23-5;  $(\text{CH}_3)_4\text{N}^+$ , 51-92-3;  $(t\text{-Bu})_4\text{N}^+$ , 10549-76-5;  $\text{NH}_4^+$ , 14798-03-9;  $\text{Li}$ , 7439-93-2;  $\text{K}$ , 7440-09-7;  $\text{Rb}$ , 7440-17-7;  $\text{Cs}$ , 7440-46-2;  $\text{Mg}$ , 7439-95-4;  $\text{Ca}$ , 7440-70-2;  $^{31}\text{P}$ , 7723-14-0.

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