Gallium-71 and Phosphorus-31 Nuclear Magnetic Resonance Studies of the Interactions of Gallium with Phosphoric Acid in Aqueous Solution[1a]

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Abstract: Because inorganic phosphate has been shown to inhibit gallium uptake by L1210 leukemic cells, the interactions of gallium with phosphoric acid was investigated using both ^{71}Ga and ^{31}P FT-NMR spectroscopy. The ^{71}Ga NMR results are consistent with the formation of several types of complexes with different stoichiometries. The ^{31}P resonances of the phosphate species present were used to characterize the complexes more fully. At 5–7 °C, the ^{31}P NMR spectrum of various gallium phosphate solutions can contain as many as five distinct peaks at 0, ~2.5, 4.0, 5.1, 8.5, and 11 ppm from external phosphoric acid reference. The peak at 0 ppm can be assigned to the uncomplexed phosphate species which are in rapid chemical exchange with one another on the ^{31}P chemical shift time scale. The peaks at 4.0 and 5.1 ppm have been identified as the $GaH_3PO_4^{3+}$ and $GaH_2PO_4^{2+}$ complexes, respectively. We suggest that the broad peak observed at 2.5 ppm arises from the formation of gallium phosphate polymers and that the remaining resonance at 11 ppm can be assigned to a gallium complex of the dimeric ion of phosphoric acid, $H_5P_2O_8^-$.

The clinical use of ^{67}Ga as a tumor-scanning agent[2] has stimulated investigations into both the mechanism of the cellular incorporation of gallium[3] as well as the serum transport of this nuclide.[4] In our laboratories, such investigations have proceeded along two lines: in vitro studies of the uptake of gallium by normal and malignant cells[5-9] and NMR studies on the aqueous chemistry of gallium[10-12] In these studies, we have made use of multinuclear NMR techniques to monitor the interactions of gallium with low molecular weight ligands[10-12] which had been shown to inhibit ^{67}Ga uptake in our model cell system. The relative ability of these agents to inhibit the cellular incorporation of gallium paralleled their relative affinity for gallium. We have also found that gallium can form polymers with some of these ligands under certain conditions. Similar observations have also been made by other investigators.[13]

As part of this overall study, we investigated the interaction of gallium with phosphate using ^{71}Ga NMR.[12] The results of these experiments indicate that complex formation is complicated. Therefore only a limited amount of information concerning this process was available from the ^{71}Ga results. In order to gain additional insight into this problem, we have approached the characterization of gallium phosphate complexes by monitoring both their ^{71}Ga and ^{31}P NMR signals. A similar approach has been employed by Akitt[14] in the study of aluminum phosphate complexes. Apart from any implications that our findings may have on the cellular incorporation or serum transport mechanism of gallium, the approach illustrated in this study may prove useful in the characterization of the phosphate complexes of other metals whose resonances can be routinely detected by multinuclear NMR techniques.[15]

Experimental Section

A. **Sample Preparation.** $Ga(NO_3)_3 \cdot 9H_2O$ (Alfa Inorganics, Beverly, Mass.) was used to make up the required concentrations of gallium solution in D_2O (Merck Sharp and Dohme, Montreal, Canada). The phosphoric acid concentrations were made up from 85% orthophosphoric acid (Baker Analyzed Reagent, Phillipsburg, N.J.). In this study, two types of ^{31}P NMR experiments were performed. In one, the H_3PO_4 concentration was kept constant at 1.72 M while the gallium concentration was varied by adding the appropriate volume of a solution containing 3.00 M gallium and 1.72 M H_3PO_4 to a solution which is 1.72 M in H_3PO_4. In the others the gallium concentration was kept constant at 1.0 M while the H_3PO_4 concentration was varied by adding the appropriate volumes of a solution containing 1.0 M gallium and 1.72 M H_3PO_4 to a solution containing 1.0 M gallium.

B. **NMR Spectra.** Time average Fourier transform ^{71}Ga (27.45 MHz) and ^{31}P (36.43 MHz) NMR spectra (frequency sweep mode) were recorded on a Bruker HX-90 (18-in. magnet) spectrometer, equipped with a NIC-1085 computer (Nicolet Instrument Corp., Madison, Wisc.). The ^{71}Ga spectra were recorded at ambient probe temperature (28 °C). The ^{31}P spectra were taken at low temperature (5–7 °C) using the Bruker variable temperature unit. Chemical shifts for the ^{71}Ga resonances were referred to the ^{71}Ga resonance of external $GaCl_4^-$. Chemical shifts for ^{31}P NMR were referenced to H_3PO_4 (85%). Sample tubes were 10 mm for ^{71}Ga and ^{31}P experiments. A 4-mm coaxial capillary tube was used for external reference. Integrated spectral intensities were measured by weighing peaks of the spectra.

Results and Discussion

I. **^{71}Ga NMR.** Gallium-71 is a spin $3/2$ nucleus (39.8% natural abundance) with a quadrupole moment of $0.11-0.15e \times 10^{-24}$ cm^2. The ^{71}Ga results which we have previously reported[10,12] were confined to the detection of the resonances of octahedral $Ga(H_2O)_6^{3+}$ (247.7 ppm upfield from external $GaCl_4^-$ reference), which is the predominant species in acidic solution,[10-12] and tetrahedral $Ga(OH)_4^-$ (25.5 ppm downfield from external $GaCl_4^-$ reference), which is the predominant species of gallium in basic solution. We have previously noted that, as H_3PO_4 is added to a solution of 1.0 M $Ga(NO_3)_3$, the intensity of the $Ga(H_2O)_6$ peak decreases, indicating that the gallium is in slow exchange between its free and complexed states.[12] In the present study we also observed the appearance of a broad peak or set of peaks (line width of ca. 1.0–1.5 kHz) centered at 273.2 ppm upfield from $GaCl_4^-$ reference. The intensities of the observed ^{71}Ga resonances (relative to external $GaCl_4^-$ reference) are shown in Figure 1 as a function of the phosphate to gallium ratio. Note that the integrated intensity of the broad peak or set of peaks reaches a maximum value corresponding to the approximately 30% of the total gallium species even when all the gallium is complexed. Therefore, we can conclude that these resonances arise from only part of the gallium complexes formed. The resonances of the other gallium phosphate complexes are probably too broad to observe. In order to characterize further the various species of gallium phosphate present, we found it necessary to monitor complex formation using ^{31}P NMR.

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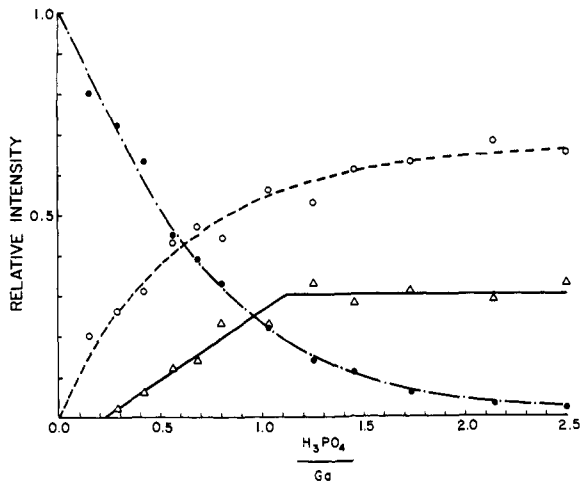

Figure 1. The relative intensities of the peaks present in the ^{71}Ga NMR spectrum as a function of H_3PO_4/Ga ratio, where open circles represent the $Ga(H_2O)_6^{3+}$ peak, triangles the broad gallium phosphate peak, and filled circles unresolved phosphate peak (calculated by subtracting the intensities of free gallium peak and broad gallium complex peak from 1 M total gallium concentration in the solution).

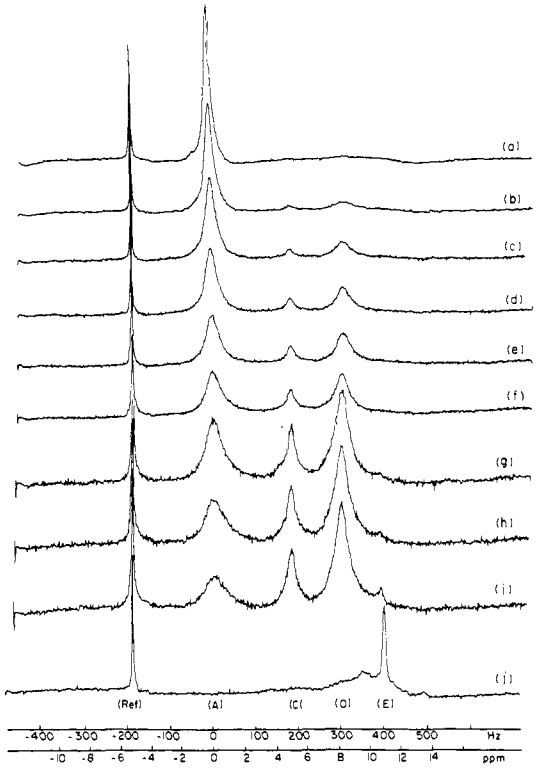

Figure 2. Low temperature (5-7 °C) phosphorus-31 spectra of 1.7 M phosphoric acid in the presence of gallium nitrate at varying Ga/H_3PO_4 ratios: (a) 0.10, (b) 0.16, (c) 0.23, (d) 0.29, (e) 0.35, (f) 0.40 (g) 0.50, (h) 0.58, (i) 0.72, (j) 1.74. (128 total scans per spectrum). Vertical scale in spectra g to j is twice as large as in spectra a to f.

II. **^{31}P NMR.** At room temperature, the ^{31}P spectrum of a solution containing 1.00 M gallium nitrate and 1.00 M phosphoric acid contains a single broad peak at ca. 7 ppm upfield from external phosphoric acid. When this solution is cooled to 5-7 °C, several peaks can be resolved in this spectrum, indicating that, at room temperature, chemical exchange occurs between the various phosphorus containing species at rates intermediate on the ^{31}P chemical shift scale. The low temperature ^{31}P spectra of a solution containing 1.70 M H_3PO_4 in the presence of various concentrations of $Ga(NO_3)_3$ are shown in Figure 2. The ^{31}P spectra of a solution containing 1.0 M $Ga(NO_3)_3$ in the presence of various concentrations of H_3PO_4 are shown in Figure 3. In these spectra there are as many as five peaks present at 0.0, 4.0, 5.1, 8.5, and 10.5 ppm respectively. These have been labeled peaks A through E, respectively, in Figure 3. A secondary external reference, K_3PO_4, was employed in order to avoid overlap with the five peaks.

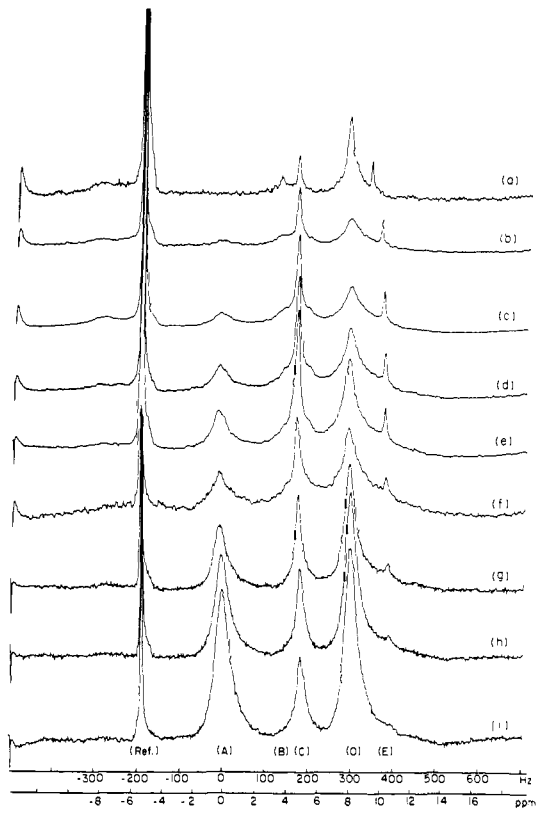

Figure 3. Low temperature (5-7 °C) phosphorus-31 spectra of 1 M $Ga(NO_3)_3$ with varying amounts of H_3PO_4 in aqueous solution. The H_3PO_4/Ga ratios are: (a) 0.15 (2 K), (b) 0.29 (2 K), (c) 0.42 (2 K), (d) 0.56 (1 K), (e) 0.68 (1 K), (f) 0.80 (512), (g) 1.03 (512), (h) 1.25 (512), (i) 1.45 (512). The number of scans of each spectrum is shown in parentheses. The vertical scale of spectra f to i is half the size of that for spectra a to e.

When a solution containing 1.70 M H_3PO_4 is cooled to 5-7 °C, a single ^{31}P resonance is observed at 0 ppm, indicating that at this temperature the various phosphoric acid and phosphate species are present in rapid chemical exchange on the ^{31}P NMR time scale. For the purposes of this discussion we will refer to these species as the free or uncomplexed phosphoric acid present in solution. In Figures 2 and 3 we note that peak A has the same chemical shift as free H_3PO_4 and its intensity decreases monotonically as gallium is added (Figure 2). For these reasons, this peak can be assigned to the free phosphate species present in solution. On the basis of the data shown in Figures 2 and 3 alone, it is difficult to make any further assignments of the ^{31}P resonances.

Assignment of Peaks C and D. In acidic solution, (pH $\leq$1.0), H_3PO_4 and $H_2PO_4^-$ should be the predominant species of phosphoric acid present. The other ionized forms of phosphoric acid HPO_4^{2-} (pK_2 = 7.2) and PO_4^{3-} (pK_3 = 12.32) are present in such small concentrations (less than 10^{-7} and 10^{-12} M, respectively) that they can be excluded from consideration in assigning the ^{31}P peaks observed. Thus, the most probable complexes associated with peaks C and D are the gallium complexes of H_3PO_4 and $H_2PO_4^-$, respectively.

In designing experiments which might enable us to assign peaks C and D to specific kinds of gallium phosphate complexes, we considered the effects of changing the hydrogen ion concentration on the relative concentrations of the phosphoric acid species present in solution. If

$$H_3PO_4 \rightleftharpoons H^+ + H_2PO_4^-$$

is the predominant dissociation of phosphoric acid in acidic solution, then the addition of H^+ will decrease the relative concentration of $H_2PO_4^-$ by protonation (i.e., forcing the equilibrium toward H_3PO_4). The effects of adding concentrated HCl to a solution containing 1.0 M $Ga(NO_3)_3$ and 1.5 M H_3PO_4 are shown in Figure 4a. It is clear that of the two peaks, the intensity of peak D decreases with increasing HCl concentration concomitant with an increase in the intensity of the free phosphate peak (A). We can therefore assign peak D to a gallium complex of $H_2PO_4^-$.

We have also considered the effects of decreasing the H^+ concentration in the phosphoric acid solution. These decreases should bring about increases in the relative concentration of $H_2PO_4^-$ concomitant with decreases in the relative concentration of H_3PO_4. The decreases in H^+ concentration were brought about by additions of NaH_2PO_4 to the solution.

The effects of such additions of NaH_2PO_4 to a solution containing 1.0 M $Ga(NO_3)_3$ and 1.0 M H_3PO_4 are shown in Figure 4b. These additions bring about two changes in the solution; the H^+ concentration is lowered while the total phosphate concentration is raised. From Figure 4b it is clear that the additions of NaH_2PO_4 bring about decreases in the intensity of peak C and increases in the intensities of peaks A and D. After the addition of 2 mol of NaH_2PO_4 (bringing the total phosphate concentration to 3.0 M), there are 0.96 mol of D present, 0 mol of C, and 2.00 mol of A present. From Figure 1, it is evident that at this phosphate concentration, virtually all of the gallium (1.0 M) should be bound. Since peak D corresponds to the only gallium phosphate complex present and since we know that this complex contains 1 mol of gallium per mole of phosphate we can assign peak D to the $Ga(H_2PO_4)^{2+}$ species. We therefore can exclude the possibility that species of the type $Ga(H_2PO_4)_n^+$ where $n > 1$ contribute to peak D.

If the assumptions made about the most probable species of gallium complexes present are correct, peak C can be assigned to a gallium complex of H_3PO_4. Since the additions of NaH_2PO_4 are expected to decrease the relative concentration of H_3PO_4 in solution, the observations made in Figure 4b are consistent with the above assignment. In Figure 4a, the intensity of peak C decreases to a lesser extent than the intensity of peak D. This observation is also consistent with our assignment. The stoichiometry of this complex can be determined by relating the data shown in Figure 1 with the spectra given in Figure 3. In Figure 3c only peaks A, C, and D are present, each with relative intensities of 0.39, 0.11, and 0.50, respectively. Since the total phosphoric acid concentration is known to be 1.45 M, the molar concentrations of the three species giving rise to these three resonances are 0.57, 0.16, and 0.72 M, respectively. From Figure 1, we can estimate that at this concentration of phosphoric acid (0.45 M), the concentration of bound gallium is 0.88 M. Since the concentration of $Ga(H_2PO_4)^{2+}$ is 0.72 M, the concentration of gallium that is complexed by the other species is 0.16 M. Note that there is only one other type of gallium complex present in Figure 3i, namely, peak C. Since the complex associated with peak C contains 0.16 M of gallium per 0.16 M of phosphate, it can be assigned to the $Ga(H_3PO_4)^{3+}$ species.

Assignment of Peak B. From Figure 3 it is clear that this peak is considerably broader than the other resonances. In addition this peak is present only when there is a large excess of gallium in solution. This observation taken together with

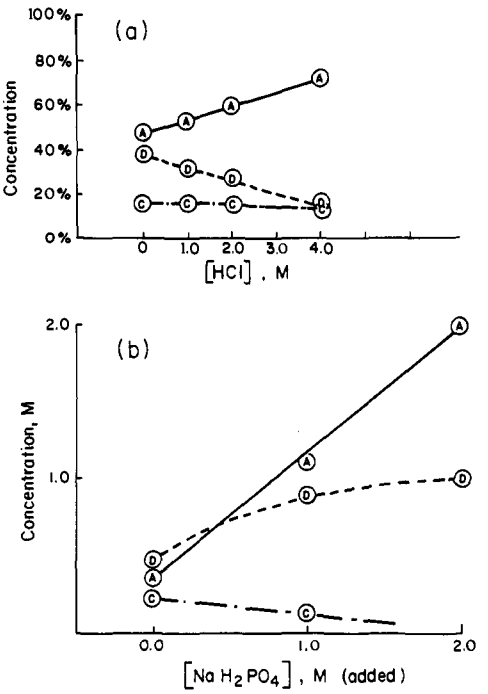

Figure 4. (a) The relative concentrations of species A, C, and D present in a solution containing 1.0 M $Ga(NO_3)_3$, 1.5 M H_3PO_4, and the concentrations of HCl indicated. (b) The concentrations of species A, C, and D present in a solution containing 1.0 M $Ga(NO_3)_3$, 1.0 M H_3PO_4, and the concentrations of NaH_2PO_4 indicated.

evidence found in other systems, that gallium forms polymer when the gallium/ligand concentration ratio is high, suggests that peak B can be assigned to a polymeric species of gallium phosphate. Similar conclusions have been made concerning the interactions of gallium with perchlorate, citrate, and hydroxide ions.[11,13]

Assignment of Peak E. Elmore et al.[16] have presented evidence for the presence of a stronger acid than H_3PO_4 in solution. This species was identified as the $H_6P_2O_8$ dimer and its associated triple ion $H_5P_2O_8^-$. These authors have calculated the concentrations of the various species present in acidic phosphoric acid solutions. At the concentration of H_3PO_4 (1.72 M) for which the ^{31}P spectra are shown in Figure 3, the two dimeric species $H_6P_2O_8$ and $H_5P_2O_8^-$ should make up ~4 and ~15%, respectively, of the total concentration of species present. On the basis of electrostatic considerations, we suggest that gallium will form complexes most readily with the ionic species $H_5P_2O_8^-$. If this assignment is correct, this species must also have the strongest affinity for gallium of all the species present since it is the most intense peak in the ^{31}P spectrum when excess gallium is present.

In summary, we wish to point out that this work began with the observation of five distinct resonances in the ^{31}P spectrum of a solution containing both gallium ions and phosphoric acid. These peaks arise from the free phosphate species and four different kinds of gallium phosphate complexes. The identification of each of these species was achieved by monitoring the NMR resonances of both the metal and ligand nuclei. Similar experiments may prove useful in characterizing the phosphate complexes of other metals whose resonances are detectable by NMR spectroscopy.

References and Notes

(1) (a) This investigation was supported by Public Health Service Grant CA-13148 from the National Cancer Institute and Grant DT-51 from the American Cancer Society. This work was presented as part of the doctoral dissertation of C.H.F. Chang to the Department of Physics, University of Alabama in Birmingham. (b) University of Alabama in Birmingham, Bir-

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Chemistry of Thiono- and Selenonophosphoranes. A Mechanistic Study of Chlorination Reactions of Phosphorus Thionoesters >P(S)OR: Reactive Intermediates and Stereochemistry[1]

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Abstract: Low-temperature ^{31}P NMR spectroscopy and stereochemical data obtained from compounds having optically active phosphorus and carbon centers have been applied to elucidate the mechanism of the reaction between phosphorus thionoesters >P(S)OR (**1**) and chlorine or sulfuryl chloride. Phosphonium intermediates were unambiguously detected and depending on the substituent at the phosphorus atom may or may not undergo nucleophilic displacement of ligands. Chloridates >P(O)Cl (**4**), elemental sulfur, and alkyl halides are formed in the first case and oxophosphoranesulfenyl chlorides >P(O)SCl (**3**) and alkyl halides in the latter. The structures of the phosphonium intermediates were confirmed through independent synthesis by Arbuzov-type reactions.

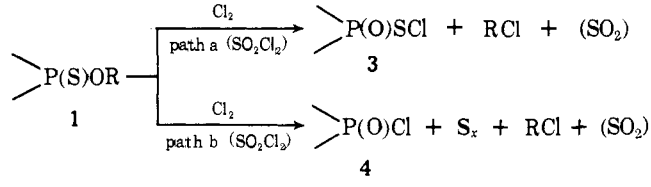
Two series of esters of phosphorus monothio acids are known: thionates **1** and thiolates **2.** Among the chemical transformations of esters **1** and **2** reactions with halogens and sulfuryl chloride are of special interest. Saville[2] and Stirling[3] were the first to describe the reaction of **2** with halogens. It has been demonstrated in this laboratory that this reaction is of general importance and can be widely employed for synthetic and stereochemical purposes.[4]

$$>P(O)SR \xrightarrow{X_2} >P(O)X + XSR$$

2

The reaction of trialkylphosphorothionates $(RO)_3P{=}S$ with chlorinating agents such as elemental chlorine or sulfuryl chloride has been reported by Michalski and Skowrońska and explored as a general route to compounds containing the >P(O)SCl (**3**) functional group.[5] We have examined the influence of the environment at the phosphorus atom on the reaction of thionoesters **1** with elemental chlorine and sulfuryl chloride. We have found that depending on the structure of **1** reaction with a chlorinating agent can proceed either predominantly via path a with the formation of oxophosphoranesulfenyl chloride (**3**) and alkyl chloride or via path b with the formation of chloridate **4,** elemental sulfur, and alkyl chloride. Acyclic phosphorothionates and six-membered cyclic thionates are chlorinated exclusively with release of alkyl chlorides and formation of oxophosphoranesulfenyl chlorides **3.** In contrast, acyclic phosphono- and phosphinothionates, as well as some other types of thionates **1,** release alkyl chloride and elemental sulfur with the formation of the corresponding chloridates **4.** Cases in which both paths a and b are followed were also encountered. We report a detailed study of these reactions and propose a general mechanistic scheme based both on intermediates detected by low-temperature ^{31}P NMR spectroscopy and on stereochemical changes observed.

Scheme I



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