# JOURNAL OF THE AMERICAN CHEMICAL SOCIETY

(Registered in U. S. Patent Office)



VOLUME 81 JANUARY 12, 1960 NUMBER 24


## PHYSICAL AND INORGANIC CHEMISTRY


[CONTRIBUTION FROM MONSANTO CHEMICAL COMPANY, RESEARCH DEPARTMENT, INORGANIC CHEMICALS DIVISION]


# Structure and Properties of the Condensed Phosphates. XVI. Pyrophosphate Formation in Concentrated Orthophosphate Solutions and its Biological Implications


BY CHARLES D. SCHMULBACH, JOHN R. VAN WAZER[1] AND RIYAD R. IRANI



RECEIVED JANUARY 5, 1959



At 100°, 0.16 g. of pyrophosphate calculated as $Na_2H_2P_2O_7$ is found in equilibrium in 100 g. of an orthophosphate solution originally containing 60.0 g. of $NaH_2PO_4$ per 100 g. of soln. Enthalpy of the reaction whereby the pyrophosphate hydrolyzes is −4.7 kcal., and the so-called "phosphate bond energy" (free energy of hydrolysis at 25°) is −3.2 kcal. An explanation, not based on "high-energy bonds," is given for the role of phosphates in biology.


The term "high-energy phosphate" is commonly used in biochemistry, where it is applied to such compounds as the pyro- and tripolyphosphates and their esters. "High-energy" refers to the free energy of hydrolysis and means that the hydrolytic equilibrium is shifted strongly toward the orthophosphate hydrolysis products. In this study, the orthophosphate–pyrophosphate equilibrium in a concentrated aqueous solution of monosodium orthophosphate was determined by a highly sensitive paper chromatographic procedure.

## Experimental Section

**Chemicals.**—Merck reagent-grade monosodium orthophosphate monohydrate was used without further purification. By drying to constant weight at 104°, this salt was found to contain 1.28 $H_2O$ of crystallization. The *p*H of a 1% aqueous solution was 4.49. This salt contained only a very small amount of pyrophosphate, 0.05% $Na_2H_2P_2O_7$ by weight, as measured by the paper chromatographic procedure described below.

The disodium phosphate used in this study was a multiply recrystallized dihydrate which was shown to be free of pyrophosphate according to the paper chromatographic method employed. The $Na_4P_2O_7 \cdot 10H_2O$ was prepared by recrystallization from Monsanto commercial tetrasodium pyrophosphate.

**Procedure.**—A calculated amount of the $NaH_2PO_4 \cdot 1.28H_2O$ was weighed into a 250-ml. beaker on a pan balance with an accuracy of ±0.1%. Water was weighed into a 500-ml. three-necked, round-bottomed flask on the same balance. This flask was then fitted with a 24 in. water-cooled condenser, a motor-driven glass stirrer and a mercury thermometer, plus a 5-cc. hypodermic syringe, the needle of which was extended to the bottom of the flask by a glass capillary. A slurry was formed by slowly adding through the condenser the $NaH_2PO_4 \cdot 1.28H_2O$ to the well-stirred water at room temperature. This slurry was then rapidly heated to the desired reaction temperature with a Meeker burner, with less than 5 minutes being required to reach the highest temperature used, 110°. At this point, the $Na_4P_2O_7$ crystals were added in those cases where the equilibrium was approached from the high pyro side. The reaction vessel then was transferred immediately to a constant temperature bath filled with light mineral oil.

At periodic intervals, 0.8 to 1.0-ml. aliquots of the solution were removed with the hypodermic syringe. The aliquots were transferred immediately to weighed 50-ml. volumetric flasks which were immersed in an ice-bath. The system was considered "frozen" under these conditions. Water was weighed into the flasks to give a 1.67 wt. % solution, and the solution was promptly employed in the chromatographic procedure.

**Paper Chromatography.**—The one-directional band chromatographic procedure described in a previous publication[2] from this Laboratory was employed in this study. However, the spots were heavily overloaded by using the relatively concentrated solution noted above. In addition, to get an even lower limit of detection and better accuracy, 30 droplets of 5 μl. of the 1.67% sample solution were applied along the starting line. Droplets of an orthophosphate–pyrophosphate reference solution were put at either side of the band corresponding to the sample solution. The chromatographic paper was four inches high and nine inches wide. The quantitative determination was carried out as previously described,[2] with the results being expressed as wt. % $Na_2H_2P_2O_7$ in the anhydrous $NaH_2PO_4$.

The accuracy of the method was found to be ±0.03% of the total anhydrous phosphate as $Na_2H_2P_2O_7$. This was established by boiling the 20% solution of $NaH_2PO_4$ for ten hours to remove all pyrophosphate, and then adding known amounts of pyrophosphate to it. The lower limit of detection was variable, ranging from 0.02 to 0.05% $Na_2H_2P_2O_7$.

## Results and Conclusions

As shown in Fig. 1, the equilibria were approached from both sides. This is proof that the

(1) To whom communications concerning this article should be directed.

(2) E. Karl-Kroupa, *Anal. Chem.*, **28**, 1091 (1956).

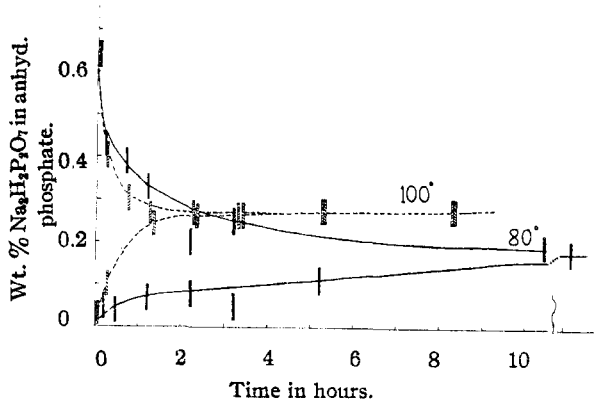


Fig. 1.—Change of pyrophosphate content in aqueous solutions containing 60 wt. % of $NaH_2PO_4$.

final values truly represent equilibrium conditions. Since there was no trace of condensed species other than the pyrophosphate, equilibria with the more condensed phosphates must necessarily be ignored. Our activity coefficients were so chosen that the corrected equilibrium constants $K_{true}$ correspond to the ionic equation

$$H_2P_2O_7^- + H_2O \rightleftarrows 2H_2PO_4^- \qquad (1)$$

Equilibrium constants based only on concentrations $K_{apparent}$ were calculated according to the equation

$$K_{true} = \frac{\gamma_o^2\,[\text{orthophosphate}]^2}{\gamma_p\,[\text{pyrophosphate}]\cdot\gamma_w\,[\text{water}]} = K_{appar}\left(\frac{\gamma_o^2}{\gamma_p\gamma_w}\right) \qquad (2)$$

where $\gamma_p$ and $\gamma_o$ are the mean ionic activity coefficients of the $Na_2H_2P_2O_7$ and $NaH_2PO_4$, and $\gamma_w$ is the activity coefficient of the water. The values of $K_{appar}$ were found to be $2.18 \times 10^2$ at 80°, $1.52 \times 10^2$ at 100°, and $1.30 \times 10^2$ at 110°. The free energy of hydrolysis was computed in two different ways. In the first one, the activity coefficients given in equation 2 were calculated on a molality basis for a temperature of 100°. Thus, $\gamma_o$ at any molality greater than unity was related by the Gibbs–Duhem equation to its value at a molality of 1 by use of vapor pressure data.[3] The value at 1 molal and 100° was computed from the extended Debye–Hückel theory,[4] using an ionic radius of 1.84 Å. This led to a value of $\gamma_o$ of 0.159 at a molality $m$ of 12.5. The activity coefficient of the water $\gamma_w$ was obtained directly from the vapor pressure data and found to be 0.74 at 100° and the same orthophosphate molality. An approximate value of $\gamma_p$ was estimated from $\gamma_o$ to be 0.23 on the basis of the Debye–Hückel extended law,[4] assuming that the radius of the pyrophosphate is 1.5 times that of the orthophosphate. This gives a value of $K_{true} = 0.22 \times 10^2$ at 100°. Use of the extended Debye–Hückel equation at 80 and 110°, employing the "ionic radius" value used at 100°, gave $K_{true} = 0.37 \times 10^2$ at 80°, and $0.18 \times 10^2$ at 110°. By plotting the logarithms of $K_{true}$ against reciprocal absolute temperature, the enthalpy of hydration $\Delta H°$ was found to be −4.7 kcal. This allowed a calculation of $K_{true} = 2.2 \times 10^2$ at 25°. From this constant, the free energy of hydration $\Delta F°$ was calculated to be −3.2 kcal. at 25°. At the same temperature, the entropy of hydrolysis $\Delta S°$ thus equals −5.0 e.u.

In the alternate method of calculation, a value of $K_{appar}$ at 25° and of the respective enthalpy (an approximate value of $\Delta H°$) was found by plotting the logarithms of the three experimental values of $K_{appar}$ *versus* reciprocal absolute temperature. In order to obtain $\Delta F°$, a value of $\gamma_o$ of *ca.* 0.25 at 25° was obtained by extrapolating measured[5] activity coefficients from $m = 6.0$ to $m = 12.5$. Likewise, $\gamma_w$ was found to equal *ca.* 0.72 at 25° from extrapolating the osmotic coefficients of the $NaH_2PO_4$ solutions.[5] The value of $\gamma_p$ was roughly estimated to be 0.36 at 25° by the same procedure used at 100°. This approximation leads to these several values for the hydrolysis process: $\Delta F° \approx -3.1$ kcal., $\Delta H° \approx -4.6$ kcal., and $\Delta S° \approx -5.0$ e.u. It should be noted that the major error in both calculations lies in the assumptions employed in estimating the value of $\gamma_p$ from that of $\gamma_o$.

Since the sodium complexes[6] are weak, the $\Delta H°$ value for the reaction of eq. 1 will reasonably represent the over-all enthalpy which would be found calorimetrically by hydrolysis of a moderately dilute solution of disodium pyrophosphate, $Na_2H_2P_2O_7$. Thus, the enthalpy of hydration obtained in this work ($\Delta H° = -4.7$ kcal.) lies between the more accurate over-all values of −4.42 kcal. obtained by Giran[7] in a strongly acid solution and −5.81 kcal. by Ging and Sturtevant[8] at *p*H 7.3. The fact that our value, which corresponds to compositions having a *p*H value of 4.5 when the water concentration is high, lies between the other two values indicates that the over-all enthalpy of hydration may increase with increasing *p*H due to changes in the amounts of the different ionic species.

The equilibrium between pyro- and orthophosphate also was measured in a more alkaline solution than corresponds to monosodium orthophosphate. Thus, at 100°, $K_{appar}$ was found to equal $2.8 \times 10^2$ for a solution containing 55% of anhydrous solid orthophosphates and having a mole ratio of $NaH_2PO_4/Na_2HPO_4 = 2.37$. Likewise, paper chromatographic measurements[9] on the distribution of molecular species in concentrated phosphoric acids showed that the equilibrium constants[10] $K_{appar}$ for the hydrolysis of pyrophosphoric acid to orthophosphoric acid is approximately $3 \times 10^2$ for a temperature between 25 and 100°. The fact that the various values of $K_{appar}$ are of the same order of magnitude shows that there is not a large shift in the orthophosphate–pyrophosphate equilibrium with changes in the particular ionic species involved.

(3) "International Critical Tables," Vol. III, McGraw-Hill Book Co., New York, N. Y., 1928, p. 297.

(4) H. S. Harned and B. B. Owen, "The Physical Chemistry of Electrolyte Solutions," 3rd Ed., Reinhold Publ. Corp., New York, N. Y., 1958; see eq. 9-5-3 on p. 415.

(5) R. A. Robinson and R. H. Stokes, "Electrolyte Solutions," Academic Press, Inc., New York, N. Y., 1955, pp. 478, 469.

(6) J. R. Van Wazer and C. F. Callis, *Chem. Revs.*, **58**, 1011 (1958).

(7) H. Giran, *Compt. rend.*, **135**, 961 (1902).

(8) N. S. Ging and J. M. Sturtevant, THIS JOURNAL, **76**, 2087 (1954).

(9) A. L. Huhti and P. A. Gartaganis, *Can. J. Chem.*, **34**, 785 (1956).

(10) J. R. Van Wazer, "Phosphorus and Its Compounds," Interscience Publishers, New York, N. Y., Vol. I, 1958, p. 490.

According to the limit of detection of the analytical procedure used and the measured equilibrium constant, pyrophosphate should not be detectable at equilibrium in monosodium orthophosphate solutions containing less than 30–35% salts. Thus in a 22% solution which had been boiled for several days, no pyrophosphate was found. By the way, it should be noted here that the formation[11] of condensed phosphates in relatively dilute aqueous solutions, as briefly reported by Sansoni, could not be reproduced in our laboratory.

From the equilibration curves such as are shown in Fig. 1, it was possible to estimate approximate rate constants and hence activation energies. For condensation (formation of pyrophosphate), the activation energy was *ca.* 30 kcal.; whereas, for hydrolysis, it was *ca.* 25 kcal. This latter value corresponds approximately to the activation energy measured[12] for hydrolysis of pyrophosphate in dilute solution. The activation energies are in agreement with the more accurately determined enthalpy, since 30–25 kcal. $\approx$ 4.7 kcal

## Discussion

The low values for $\Delta F^\circ$ and $\Delta H^\circ$ of hydrolysis found here for inorganic pyrophosphate are in accord with the low values for "high-energy phosphate bonds" now being accepted by biochemists.[13] These findings demonstrate that quantitative treatment of the concept of high-energy phosphates in terms of numerical values of $\Delta F^\circ$ of hydrolysis was an unfortunate choice. Thus, a value of $\Delta F^\circ$ of a million kcal. would, for all practical purposes, be identical to a value of ten in a reaction where the $\Delta F^\circ$ of the competing reaction is, say, two! Indeed, it appears that directly relating phosphates to energy in living systems was a facile expedient that has been confused rather than enlightened by the "high-energy phosphate bond" notation.

A living system can be defined as one in which energy transformations, as carried out through chemicalreactions, result in the growth and propagation of the system. By associating phosphates with energy, undue emphasis has been attached to phosphorus as the key to life. However, phosphorus is very important in vital processes and it is desirable to ask why. The answer to this question involves more than the hydrolysis equilibria (the "high-energy" concept). Our rationale of the importance of phosphates in biochemical transformations is briefly presented below:

As a Second-Row element, phosphorus has available d-orbitals which not only appear to be involved in $\pi$-bonding[14] for the $sp^3$ hybrids but which can and do enter into the $\sigma$-bond structure of activated complexes.[15] In carbon chemistry, the activated complexes of molecules based on $sp^3$ hybridization must assume a lower hybridization,[16] such as $sp^2$. With phosphorus, the activated complex often involves a higher coördination number than that corresponding to the ubiquitous hybrid, $sp^3$, so that the reactants are chemically bonded to each other, with the activated complex representing a chemical compound rather than a special orientation of unbonded molecules or molecular fragments. This is one reason for the presence of phosphorus in reactions of biological importance. This ability of the phosphorus atom to tie structures together through the use of d-orbitals explains why phosphorus-containing coenzymes appear in many enzymatically catalyzed reactions in which the phosphates themselves are not involved. The action of magnesium as a coenzyme in the reactions involving phosphorus probably represents another way of holding structures together through the use of d-orbitals of phosphorus in the $\pi$-bonds entering into the stabilization of a polyphosphato magnesium complex.[6]

Biological utilization of the phosphate family of compounds rather than other homologous series[15] based on phosphorus is due to the facts (1) that the vast majority of biochemical reactions (so far studied) take place in aqueous media or water interfaces (2) that the orthophosphates are thermodynamically stable[17] under the various conditions occurring during oxidation–reduction reactions involving carbon and oxygen.

The reason that condensed phosphates rather than the oxyacids of other Second-Row elements (*e.g.*, the silicates) are involved in basic biochemical reactions is the fact that the rate of hydrolysis and the related reactions of the phosphates are slow when enzymes are not present.[18] Although we have not seen data which are directly comparable, there is considerable chemical evidence to indicate that hydrolysis of sulfate or silicate condensed species or esters occurs much more rapidly than does the hydrolysis of equivalent phosphates under the same conditions. Speculation as to the basic cause of this difference in behavior so far has not been fruitful. Last and least of the reasons that phosphates play an important role in biology is the fact that the hydrolytic equilibria of various condensed species and esters are moderately but only moderately shifted toward hydrolysis. For the silicates, it would seem that the hydrolysis equilibria range around 10°; for the phosphates (as exemplified by the data of this paper), they range around $10^2$–$10^4$; whereas for the sulfates, it is guessed that the equilibria are very strongly shifted toward hydrolysis, with the average $K$ = *ca.* $10^8$–$10^{10}$. According to this picture a "moderate-energy bond" is better than a very high one, since in the latter case the amount of matter which can go through a sequence of competing reactions must necessarily be small.

(11) B. Sansoni, *Angew. Chem.*, **67**, 327 (1955).

(12) J. R. Van Wazer, E. J. Griffith and J. F. McCullough, THIS JOURNAL, **77**, 287 (1955).

(13) Compare P. Oesper, "Phosphorus Metabolism," Vol. I, (Ed. by W. D. McElroy and B. Glass), John Hopkins Press, Baltimore, 1951, p. 523, with T. H. Benzinger and R. Hems, *Proc. Natl. Acad. Sci.*, **42**, 896 (1956), the various papers by M. R. Morales, *et al.*, and the forthcoming chapters by B. J. Katchman in "Phosphorus and Its Compounds," Vol II, (Ed. by J. R. Van Wazer) Interscience Publishers, New York, N. Y., 1960.

(14) J. R. Van Wazer, THIS JOURNAL, **78**, 5709 (1956).

(15) See Chapt. 3 in ref. 10.

(16) In aliphatic carbon chemistry, the free radicals, carbonium ions and even the intermediate energy state in a Walden inversion (when the umbrella is half way between being inside out and outside out) are probably $sp^2$ hybrids.

(17) P. H. Emmett and J. F. Schultz, *Ind. Eng. Chem.*, **31**, 105 (1939); G. L. Frear, E. F. Ogg and L. H. Hull, *ibid.*, **36**, 927 (1944).

(18) For an earlier statement of this view, see F. Lipmann in the book of ref. 13, pp. 521–522.

In summary, it appears that the reaction schemes occurring in biology are dependent on employment of an element, for which d-orbitals are sufficiently available to play a role in the $\sigma$-bond structure of activated complexes but do not enter into the $\sigma$-bond structure of the common compounds. Primarily because of slow kinetics in simple aqueous solutions, but also because of a generally moderate tendency toward hydrolysis, phosphorus is the Second-Row element of outstanding biochemical activity.

**Acknowledgments.**—We wish to thank Dr. Editha Karl-Kroupa for her guidance in carrying out the chromatographic work, and Messrs. Lee Loveless and Thomas Kichline who gave us helpful advice.

ST. LOUIS, MO.

---

[CONTRIBUTION FROM THE MONSANTO CHEMICAL COMPANY, RESEARCH DEPARTMENT, INORGANIC CHEMICALS DIVISION]

# Principles of Phosphorus Chemistry. IV. The System of Fluorophosphoric Acids

BY DONALD P. AMES, SHIGERU OHASHI,[1] CLAYTON F. CALLIS AND JOHN R. VAN WAZER

RECEIVED JANUARY 2, 1959

In the single-phase liquid system $H_2O$–HF–$P_2O_5$, the nine structural entities herein listed were quantitatively determined from phosphorus and fluorine nuclear magnetic resonance spectra: mono-, di- and hexafluorophosphoric acids, orthophosphoric acid, end- and middle-phosphate structure units, free water, free hydrofluoric acid, and a new structure unit containing one fluorine per phosphorus. This new structure unit is believed to be a monofluorophosphate end group. Lines of equal concentration are shown for the nine structural entities in the single-phase region of a series of $H_2O$–HF–$P_2O_5$ triangular diagrams in which the two-phase area covers the region corresponding to more than *ca.* 55 mole % $P_2O_5$. Four equilibrium constants were calculated for the system.

The ease of transformation from one fluorophosphoric acid to another or into orthophosphoric acid in the presence of HF or $H_2O$ is well-known.[2] However, the equilibria involved have not been investigated quantitatively except for the reaction[3] between monofluorophosphoric acid and water to give orthophosphoric acid and hydrogen fluoride.

This paper describes a study in which the relative proportions of the various molecular constituents in the system $H_2O$–HF–$P_2O_5$ at equilibrium were determined by nuclear magnetic resonance spectroscopy. This relatively new physical technique is particularly suited to the determination of equilibrium composition diagrams in liquid systems where the molecular species contain atoms exhibiting nuclear spin. The system involving fluorophosphoric acids is particularly adaptable to the n.m.r. technique, since naturally occurring hydrogen, fluorine and phosphorus have the optimum nuclear properties, *i.e.*, a predominant isotope with large magnetic moment and zero quadrupole moment.

## Experimental

**Samples Studied.**—Approximately 125 separate compositions, scattered throughout the homogeneous region shown in Figs. 2–10, were investigated in this study. The majority of these samples were made from eight concentrated preparations which were diluted with water. In a few cases, $P_2O_5$ and HF were added to the original preparation. The original preparations were obtained from Dr. Wayne E. White of the Ozark Mahoning Company, who simply combined phosphorus pentoxide and anhydrous HF to make all of them except the anhydrous difluorophosphoric acid, which was distilled. The original sample of difluorophosphoric acid must have contained some paramagnetic impurities, since the nuclear magnetic resonance peaks of this sample and of mixtures made by diluting it with water were exceptionally broad. A duplicate sample obtained from Dr. White exhibited sharp resonance peaks.

The various compositions studied were equilibrated at a temperature of 50° for more than 20 hr. in polyethylene bottles. Some of the tubes were stored thereafter for 48 hr. more at 50° with no change in their molecular composition. Further proof that equilibrium was reached was obtained by making samples having the same proximate analysis from different starting materials. In such cases, the same molecular composition was found.

**Nuclear Magnetic Resonance Measurements.**—All measurements were made with a Varian Model V-4300B nuclear induction magnetic resonance spectrometer utilizing a Varian magnet, Model V-4012-HR. The $P^{31}$ spectra were obtained with a 16.2 mc. radiofrequency oscillator using a field of 9395 gauss. The $F^{19}$ spectra were obtained with a 40.0 mc. radiofrequency oscillator using a field of 9985 gauss. The 40 mc. oscillator also was used to obtain hydrogen spectra at a field of 9395 gauss.

The samples were measured in 15 mm. o.d. polyethylene test-tubes containing 3 to 5 ml. of liquid. The tubes were sealed with a rubber stopper protected from the vapors with a layer of Saran Wrap®, and the stopper was affixed tightly with plastic pressure-sensitive tape. Referencing was carried out by a modification of the concentric-tube technique,[4] in which a small polyethylene tube filled with the reference material was inserted through a single-hole stopper into the sample under investigation. For the phosphorus spectra, 85% phosphoric acid was used as reference; whereas for the fluorine spectra 100% trifluoroacetic acid (Eastman reagent) was employed. The referencing was carried out by the side-band technique.[5] The side-band frequencies were counted with a Hewlett-Packard electronic counter, Model 524B. With either the fluorine or phosphorus spectra, no anomalies[6] were noted when the concentric-tube technique of referencing was employed. As expected, the hydrogen spectra were of little value in investigating the system studied, since the acidic hydrogens of the HF and the various phosphoric acids exchange with each other and with the water present so that only a single resonance peak is observed.

Both the phosphorus and fluorine spectra were obtained by utilizing the "intermediate-passage" absorption-mode

(1) On leave of absence from Kanazawa University, in Japan, during the 1957–1958 academic year.

(2) W. Lange, "Fluorine Chemistry," Vol. I, Edited by J. H. Simons, Academic Press, Inc., New York, N. Y., 1950, Chap. 3, pp. 125–188; John R. Van Wazer, "Phosphorus and Its Compounds. I. Chemistry," Interscience Publishers, Inc., New York, N. Y., 1958, Chap. 13, pp. 801–820.

(3) W. Lange, *Ber.*, **62B**, 1084 (1929); *Z. anorg. allgem. Chem.*, **214**, 44 (1933).

(4) J. R. Zimmerman and M. R. Foster, *J. Phys. Chem.*, **61**, 282 (1957).

(5) J. T. Arnold and M. E. Packard, *J. Chem. Phys.*, **19**, 1608 (1951).

(6) C. A. Reilly, H. M. McConnell and R. G. Meisenheimer, *Phys. Rev.*, **98**, 264 (1955).