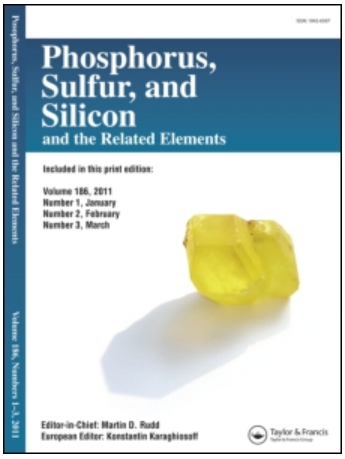

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Deuterium Discrimination in P--H/P--D Exchange of Phosphorous Acid

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DEUTERIUM DISCRIMINATION IN P–H/P–D EXCHANGE OF PHOSPHOROUS ACID

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Deuterium discrimination in P–H/P–D exchange is first reported. Quantitative ^{1}H and ^{31}P NMR analyses of the phosphorous acid in H_2O/D_2O solutions indicate that there is a strong discrimination against deuterium bonding with phosphorus. The $([PD]/[PH])_{nmr}$ ratio, where $[PD]_{nmr}$ and $[PH]_{nmr}$ are the NMR determined concentration of phosphorus attached to deuterium and proton, respectively, is only 67% of the stoichiometrical D/H ratio in the studied solutions. The very strong isotope discrimination should originate from the isotope effect in tautomerization involving an intermediate having the structure of $P(OH)_3$ where the three OH's are equivalent.

Keywords: Deuterium discrimination; isotope exchange; phosphorous acids

INTRODUCTION

Isotope discrimination is a common phenomenon in natural biochemical reactions. C-13 discrimination in photosynthesis, which was first discovered early in 1960s,[1] is a typical example of isotope discrimination, where lighter carbon dioxide $^{12}CO_2$ is more actively involved than the heavier $^{13}CO_2$. In the study of C-13 discrimination in plant physiology, a parameter $\Delta_{C\text{-}13}$ has been defined,[1–3] which describes the ability

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of carbon isotope discrimination against C-13.

$$\delta_{\text{C-13}} = \frac{([^{13}\text{C}]/[^{12}\text{C}])_{\text{sample}} - ([^{13}\text{C}]/[^{12}\text{C}])_{\text{atmosphere}}}{([^{13}\text{C}]/[^{12}\text{C}])_{\text{atmosphere}}} \quad (1)$$

Currently, the parameter $\Delta_{\text{C-13}}$ has become an important indication for many biological processes.[2–4] In addition to C-13 discrimination, O-18 and S-34 discrimination has also been reported in photosynthesis.[5,6] In this article we report the hydrogen isotope discrimination against H-2 found in D/H exchange of phosphorous acid by NMR spectroscopy. While $\delta_{\text{C-13}}$ is usually in the range between -0.03 to -0.05, we found $\delta_{\text{H-2}}$, or δ_{D}, which is defined in this article as a quantitative specification for H-2 discrimination in D/H exchange of phosphorous acid, is found to be as large as -0.32, 10 times as big as $\Delta_{\text{C-13}}$.

H/D exchange has been intensively and extensively investigated in the past decades. H^N/D^N exchange, with its tremendous importance in structure and dynamics studies of biomolecules, has been the dominant topic in discussion of H/D exchange;[7–10] here we use X^Y to denote X atom attached to Y atom. Compared to H^N/D^N exchange, H^P/D^P exchange is not so common, but the H^P/D^P exchange may provide unique information about phosphorus chemistry. Phosphorous acid is an important inorganic acid in biology and chemistry. It is suggested that phosphorous acid is involved in the first development of life.[11] Phosphorous acid has also been used for DNA modification.[12] In electrochemistry or synthetic chemistry, it has been proved to be very useful as a reductive agent.[13–17] Many chemical products synthesized from phosphorous acid and its derivatives show biological, medical, and corrosion-inhibited activities.[18–20]

RESULTS AND DISCUSSION

Phosphorous acid (H_3PO_3) is a fairly strong inorganic acid. It has been well known that one of the three hydrogens is attached to phosphorus. This hydrogen, however, is labile. It can exchange with water but the exchange is extremely slow with a rate constant in the magnitude order of h^{-1}. When the phosphorous acid is dissolved in D_2O, three ^1H NMR peaks and six ^{31}P NMR peaks are observed. In the ^1H spectrum, there is a doublet due to the ^1H—^{31}P one-bond coupling ($J = 1400$ Hz) and a strong solvent peak. In the ^{31}P spectrum, there is a doublet due to the one-bond ^1H—^{31}P coupling, a triplet due to the one-bond ^2H—^{31}P coupling and a weak singlet that is assigned to H_3PO_4 as being an impurity in H_3PO_3 due to the oxidation by the atmospheric oxygen. The

triplet indicates that in the solution, P–H/P–D exchange has occurred

$$H^P + D^O \rightleftharpoons D^P + H^O \quad (2)$$

But the exchange is so slow that the rate constants cannot be determined by two-dimensional exchange and saturation transfer experiments. The rate constant has been estimated to be 1.1×10^{-5} $s^{-1}M^{-1}$ by monitoring the decay of the ^{1}H-P signal within one-month period.

Just as the H^N/D^N exchange in biochemistry is accomplished with the participation of H_2O/D_2O, the H^P/D^P exchange also occurs in the presence of H_2O/D_2O, which are expressed in Eq. 2 in the form of OH and OD. If there were no deuterium discrimination in the exchange, the equilibrium constant for the above equation would be unity, and the integration ratio of the doublet to the triplet should equal to the total stoichiometric [H]/[D] ratio. However, there is a remarkable difference between the ^{31}P NMR determined $[P^H]/[P^D]$ ratio and the stoichiometry. The $[P^H]/[P^D]$ ratio is approximately 50% larger than the total [H]/[D] ratio in the solution (see Table I).

The evidence of the strong deuterium discrimination can also be obtained from the ^{1}H NMR spectra where the integration of H^P and H^O signals can be precisely obtained. For convenience, we define [O] as the concentration of the oxygen site at which proton and deuteron can reside. Theoretically, the probability of finding a hydrogen (or a deuteron) attached to oxygen is proportional to [O] with $[O] = 2[W] + 2[P3] + 3[P5]$, where [W] denotes the concentration of

TABLE I NMR Determined $[P^H]/[P^D]$ (by ^{13}P NMR), $[H^P]/[H^O]$ (by ^{1}H NMR), Exchange Equilibrium Constant, and Deuterium Discrimination Parameter for 13 Phosphorous Acid Solutions. As a Comparison, the Stoichiometric [H]/[D] and [P3]/[O] of the Solutions are Also Shown

No.	[H]/[D]	$[P^H]/[P^D]$	[P3]/[O]	$[H^P]/[H^O]$	δ_D	K_{frac}
1	0.139	0.202	0.0392	0.0581	−0.31	0.68
2	0.141	0.206	0.0397	0.0588	−0.31	0.67
3	0.126	0.183	0.0355	0.0525	−0.31	0.68
4	0.142	0.215	0.0399	0.0571	−0.34	0.65
5	0.172	0.257	0.0437	0.0611	−0.33	0.66
6	0.169	0.249	0.0391	0.0554	−0.32	0.67
7	0.183	0.279	0.0346	0.0480	−0.34	0.65
8	0.333	0.488	0.0390	0.0520	−0.32	0.67
9	0.216	0.313	0.0454	0.0655	−0.31	0.68
10	0.481	0.714	0.0373	0.0489	−0.33	0.66
11	3.360	4.920	0.0409	0.0450	−0.32	0.67
12	14.47	21.51	0.0355	0.0366	−0.33	0.66
13	28.19	41.66	0.0315	0.0319	−0.32	0.67

water which is the sum of [D_2O], [HDO] and [H_2O], [P3] denotes the concentration of phosphorous acid which is the sum of [H_3PO_3], [H_2DPO_3], [HD_2PO_3] and [D_3PO_3], [P5] denotes the concentration of phosphoric acid which is the sum of [H_3PO_4], [H_2DPO_4], [HD_2PO_4], and [D_3PO_4]. The probability of finding a hydrogen (or a deuteron) attached to phosphorus is proportional to [P3]. Since for a given sample [W], [P3], and [P5] are known, the total ratio [P3]/[O] is also known, which, compared to the experimental determined [H^P]/[H^O], is always smaller, as can be seen from Table I. The larger [H^P]/[H^O] as compared to the stoichiometric [P3]/[O] should be an indication that during the H^P/D^P exchange, discrimination against deuterium happened.

In order to quantitatively describe the deuterium discrimination, it is better to define a parameter, δ_D, in accordance with $\delta_{C\text{-}13}$ in the discussion of ^{13}C discrimination in plant physiology (see Eq. 1). The deuterium discrimination parameter can be defined by

$$\delta_D = \frac{([D^P]/[H^P])_{nmr} - ([D]/[H])_{stoich}}{([D]/[H])_{stoich}} \tag{3}$$

The value of δ_D is listed in Table I. While the ^{13}C discrimination in photosynthesis is normally in the range from -0.03 to -0.05, the ^2H discrimination in H^P/D^P exchange is as big as -0.3, 10 times larger than $\delta_{C\text{-}13}$. Such a big discrimination should have its chemical origin. The H^P/D^P exchange involves a possible tautomerism of the phosphorous acid,[21] which is illustrated in Figure 1, where the phosphorous acid molecule is assumed to have the composition of H_2DPO_3. The B structure is an intermediate that has a very short lifetime and is not observed in NMR experiments. B can turn either to A or to C very rapidly, with a proton or a deuteron jumping from the oxygen site to the phosphorus site. Due to the isotope effect, the lighter H can jump more easily than the heavier D. The probability for the intermediate B to transfer to the stable structure C is therefore higher than that to form the stable

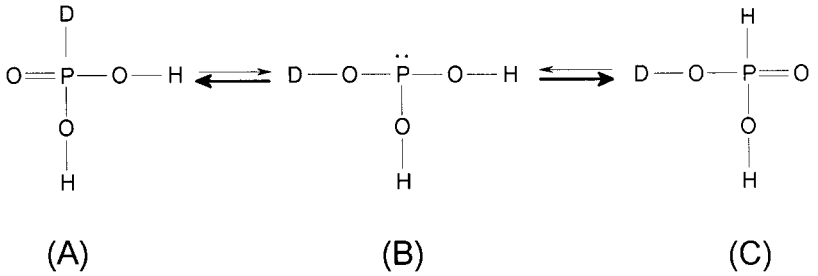

FIGURE 1 The tautomerism of phosphorus acid.

structure A. As a result, there is deuterium discrimination in the H^P/D^P exchange.

In addition to the discrimination parameter, the equilibrium constant for the exchange Eq. 2, usually called the fractionation factor, has also been used in discussion of isotope discrimination:

$$K_{\text{frac}} = \frac{[H^O][D^P]}{[D^O][H^P]} \tag{4}$$

From the ^{31}P spectra, $[P^D]/[P^H]$, which is equal to $[D^P]/[H^P]$, has been obtained, and from the ^1H spectra, $[H^O]/[H^P]$ has been obtained. Since the total [P3] is known and total [O] is known, $[D^O]$ is easily calculated. Hence K_{frac} can be determined experimentally, which are also presented in Table I. The average K_{frac} is 0.67, which deviates greatly from unity, indicating that the deuterium discrimination in H^P/D^P exchange is serious. Since D^O/H^O should be equal to the stoichometric [D]/[H] ratio in the solution, the fractionation factor is related to the discrimination parameter just by a simple equation:

$$K_{\text{frac}} = 1 + \delta_D \tag{5}$$

If we define two velocities, ν_H and ν_D, with which H and D jump between O and P, according to the mechanism shown in Figure 1 the fractionation factor should be equal to the ratio of ν_D to ν_H. The atom with larger mass should move slowly.[22] Suppose each atom has the same kinetic energy in the solution, that is, $(1/2)m_H{\nu_H}^2 = (1/2)m_D{\nu_D}^2$. Then K_{frac} can be estimated as the square root of the mass ratio (0.707), which is roughly in agreement with the experimental finding of 0.67.

EXPERIMENTAL

The chemical phosphorous acid (A.R.) was purchased from Beijing RedStar Factory of Chemicals. After it was thoroughly dried, it was then dissolved in D_2O to form a series (13 in total) of solutions where the stoichiometrical H/D ratio for the solutions was varied and strictly controlled by quantitative adding H_2O. The original ^1H content in D_2O was determined by NMR on a D_2O solution of CH_3COONa whose concentration was known. The NMR tubes were immediately sealed after the solutions were prepared. The samples were kept in the laboratory for one month so that the H/D exchange would reach equilibrium before NMR experiments were conducted.

All NMR experiments were performed on a Bruker ARX-500 spectrometer with an inverse broadband probe at a temperature of 298.0K. All ^1H NMR spectra were acquired by using a 30° pulse, 16 transients,

and 4 dummy scans, over a spectral width of 1500 Hz with 16384 data points. The relaxation delay was 20 s to ensure a reliable quantitative integration. For ^{31}P NMR (at 202.46 MHz), spectra were recorded by using a 90° pulse, over a spectral width of 2200 Hz with 16384 data points. Before acquisition, 4 dummy scans were used to get a stable transient. The scan number was 64, 128, and 256 according to the sample concentrations. The pulse repetition time was set to 5 T_1 (T_1 was measured by IR method). The phase and baseline of the signals were precisely adjusted before integration. In order to have high precision in integration, the integration range of each signal was carefully adjusted to be 10 times of the line width at the half height of the signal. For a Lorentzian line shape, the integration range so defined gives an integration value of 64.3% of the whole area (defined as unity by an integration from $-\infty$ to $+\infty$). By defining an integration range based on the line width, the error in integration can be dramatically reduced.

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