N.M.R. Study of the Hydration of Pyruvic Acid

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The ratio of the concentration of pyruvic acid to that of its hydrate (2,2-dihydroxypropanoic acid) has been determined from the ratio of the intensities of their methyl resonances at 60 Mc/sec. The kinetics of the hydration of pyruvic acid have been studied; the hydrogen-ion-dependent rate constant of the hydration is 5.6 l. mole⁻¹ sec⁻¹, and that of the dehydration 8.7 l. mole⁻¹ sec⁻¹. The results generally confirm the conclusions drawn from a limited study of these phenomena at 28 Mc/sec.

Bellamy and Williams 1 showed, by employing infra-red spectra, that the structures of lithium pyruvate monohydrate and lithium hydroxy pyruvate monohydrate were consistent with the diol form. Jencks and Carriulo 2 showed the presence of the carbonyl group from the infra-red spectrum of an aqueous solution of sodium pyruvate in heavy water. From Raman spectra, Long and George 3 showed the absence of the carbonyl group in solid lithium pyruvate monohydrate and then its presence in an aqueous solution of the salt in heavy water. Becker and Strehlow 4 employed polarography in the study of the hydrate. Becker, 5 from intensity measurements of the n.m.r. spectrum of pyruvic acid, found that less than 2% of the acid was in the enol form. He also obtained the dissociation constants of pyruvic acid and its hydrate, 2,2-dihydroxypropanoic acid:

$$\text{CH}_3\cdot \text{CO}\cdot \text{COOH} + \text{H}_2\text{O} \rightleftharpoons \text{CH}_3\cdot \text{C(OH)}_2\cdot \text{COOH}$$

(1)

This hydration is similar to that of the acetaldehyde carbonyl group in forming a gem-diol.

The kinetics of the hydration of pyruvic acid have been studied by a relaxation method 6 and by n.m.r. 7, 8, 9. Strehlow 6 employed conductivity measurements to observe the readjustment of the hydration equilibrium due to a pressure jump of 50 atm; the hydration was acid-based catalyzed.

The previous n.m.r. study of the kinetics of the hydration 7, 8 was made at 28 Mc/sec. There was overlapping of the signals, hence, errors were present in the measurement of the intensities and of the line-widths. However, at the frequency of 60 Mc/sec, the signals are all well separated. The previous study employed the broadening of only the methyl resonance of pyruvic acid, and did not use any data from the broadening of the methyl resonance of 2,2-dihydroxypropanoic acid. Therefore, only the hydrogen-ion-dependent rate constant of the hydration was determined.

The purpose of the present study was to verify whether the explanation of the observed broadening of the methyl resonance of pyruvic acid and of its hydrate was correct. At the same time, the new results yield more accurate values of the ratio of the concentration of pyruvic acid to that of 2,2-dihydroxypropanoic acid and also of this hydrogen-ion-dependent rate constant of both the hydration and dehydration processes at 24°C.
The commercially available R.S.2. nuclear magnetic resonance spectrometer manufactured by A.E.I. was employed using a 60 Mc/sec probe. The magnet and probe were thermally insulated at 24 ± 1°C. The samples were contained in egg-shell glass tubes of 5 mm ext. diam. in order to increase the filling factor and hence the signal-to-noise ratio, and they were allowed several minutes in the probe to attain the ambient temperature. The chemical shifts were measured by the side-band technique with the aid of a Muirhead audio oscillator. The spectra were recorded on 10 in. chart paper so that accurate measurements of the half-width could be made.

The pyruvic acid was distilled under reduced pressure to a colourless liquid and its purity tested by obtaining the lowest freezing point and also by observing the n.m.r. spectrum. The acid catalyst employed was prepared from A.R. hydrochloric acid, and the solutions were then standardized with A.R. sodium tetraborate decahydrate, using methyl red as indicator.

RESULTS AND DISCUSSION

The proton magnetic resonance spectrum of pyruvic acid consists of two signals at 10.7 and 2.6 p.p.m. with respect to an internal reference of tetramethylsilane. The intensities of these signals were in the expected ratio of 1:3 respectively. The carboxyl proton resonance may be assigned to the signal observed at the lower magnetic field, 10.7 p.p.m., and the resonance of the methyl protons, to the signal at the higher field, 2.6 p.p.m. The separation of the signals was critically dependent on the purity of the pyruvic acid, the greater the purity, the greater the separation. This dependence may be due to intermolecular hydrogen bonding.

The proton magnetic resonance spectrum of pyruvic acid in deuterium oxide consists of three signals, the chemical shifts of which are 5.47, 2.61 and 1.73 p.p.m. Due to the occurrence of fast proton exchange, the signal at 5.47 p.p.m. may be

Fig. 1.—Graph of the ratio $K_{eq}$ of the concentration of 2,2-dihydroxypropanoic acid to that of pyruvic acid against the cube of the water concentration in moles $L^{-1}$.
assigned to the resonances of the carboxyl, hydroxyl and water protons. The resonance at 2.61 p.p.m. may be assigned to the methyl protons of pyruvic acid, as previously. The additional resonance at 1.73 p.p.m. may be accounted for if it is assumed that a hydrate of pyruvic acid, 2,2-dihydroxypropanoic acid, has been formed. The signal could then be assigned to the methyl proton resonance of the hydrate because (i) this resonance would be expected to occur at a higher magnetic field than the methyl resonance of pyruvic acid since the loss of the diamagnetic anisotropy of the carbonyl group in the hydrate would increase the screening of the methyl protons. (ii) The separation of the methyl proton resonance of acetaldehyde from that of its hydrate is 0.91 p.p.m. the hydrate resonance occurring at the higher magnetic field. Similarly, the separation of this additional high-field resonance from that of the methyl proton resonance of pyruvic acid is 0.88 p.p.m. These values are in good agreement. (iii) The intensity of this resonance was dependent on the relative concentration of pyruvic acid to water, the intensity decreasing with increase of pyruvic acid concentration.

By measuring the intensities of the methyl proton resonance of the hydrate and of pyruvic acid, the ratio $K_{eq}$, the concentration of the hydrate to that of pyruvic acid, was determined at various concentrations (fig. 1):

$$K_{eq} = \frac{\text{intensity of the hydrate methyl resonance}}{\text{intensity of the pyruvic acid methyl resonance}}.$$  

The results indicate a definite third-power dependence of the ratio $K_{eq}$ on the water concentration. The probable explanation for this involves hydrogen-bonding. The value of $K_{eq}$ for a solution of 4.2 mole l$^{-1}$ of pyruvic acid was 0.68 at 24°C.

The hydration-dehydration exchange of pyruvic acid is acid-catalyzed. As the exchange rate was increased, due to the addition of hydrochloric acid, the methyl proton resonances of pyruvic acid and its hydrate were observed to broaden. With further increase in hydrochloric acid concentration, the methyl resonances coalesced and finally a single, narrow resonance was observed (fig. 2). The concentrations of all the pyruvic acid solutions were 4.2 mole l$^{-1}$, because of signal-to-noise considerations. Before and after each sample, the spectrum of a solution of pyruvic acid in water was taken.
The half-widths (the widths of the resonances at half their maximum) of the methyl proton resonances of pyruvic acid and its hydrate were measured at various hydrochloric acid concentrations. Hence, the mean lifetimes of pyruvic acid and its hydrate form were determined.

**Figure 3.** Graph of reciprocal of mean lifetime of pyruvic acid against HCl concentration.

**Figure 4.** Graph of reciprocal of mean lifetime of hydrate form of pyruvic acid against HCl concentration.
hydrate were obtained from eqn. (3):

\[
\frac{1}{T'_{2A}} = \frac{1}{T_{2A}} + \frac{1}{\tau_A},
\]

(3)

where \(2/T'_{2A}\) is the half-width of the exchange-broadened signal A and \(T_{2A}\) is the transverse relaxation time of the nuclei in their environment. The mean lifetime \(\tau_A\) must be large compared with the reciprocal of the separation of the exchange-broadened signals. The reciprocals of the mean lifetimes of both pyruvic acid and its hydrate were plotted against the hydrochloric acid concentration (fig. 3 and 4 respectively). The straight line graphs obtained indicate a rate law of the type:

\[
\text{specific rate} = k_0 + k_{H^+}[H^+],
\]

(4)

where \(k_0\) is the spontaneous rate constant and \(k_{H^+}\) is the hydrogen-ion-dependent rate constant.

The spontaneous rate constants of the hydration and dehydration reactions cannot be determined by n.m.r. due to the nature of the experiment. The kinetics appear to follow simple hydrogen-ion catalysis, thus confirming the previous results of n.m.r. and relaxation methods. This is in agreement with the general acid-base catalysis observed in the hydration of carbonyl-containing compounds.

For the hydration, the hydrogen-ion-dependent rate constant was 5·61. mole\(^{-1}\) sec\(^{-1}\), from the slope of the straight line obtained in fig. 3, which is of similar magnitude to that found by Strehlow for dilute solutions. This agreement between the results of relaxation and n.m.r. methods indicates the validity of both experimental techniques.

For the dehydration, the slope of the straight line of fig. 4 gave the hydrogen-ion-independent-rate constant as 8·71. mole\(^{-1}\) sec\(^{-1}\). The ratio of the hydrogen-ion-dependent rate constant of the hydration to that of the dehydration is 0·65. This is in good agreement with the value 0·68 of \(K_\text{eq}\) at the concentration of pyruvic acid of 4·2 mole l.\(^{-1}\). This agreement again indicates the validity of the n.m.r. technique.

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