Massachusetts Institute of Technology
Department of Chemistry
5.33 Advanced Chemical Instrumentation
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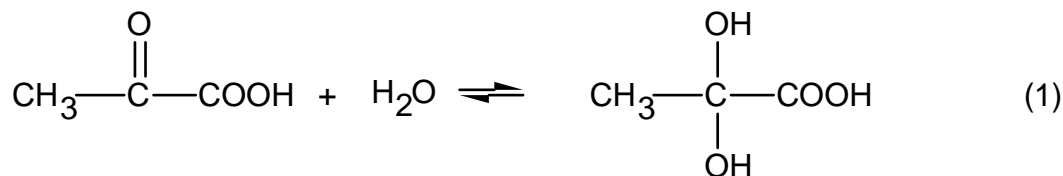## EXPERIMENT #2A: MAGNETIC RESONANCE SPECTROSCOPY

I.   Introduction

Magnetic resonance spectroscopy is one of the most widely used techniques in chemistry. Both nuclear-spin (NMR) spectroscopy and electron-spin resonance (ESR) spectroscopy provide important information about chemical systems. Applications of NMR to the analysis of chemical systems, which you have already encountered in organic chemistry and laboratory subjects, include use of the spectrum as a qualitative means of identifying particular molecules and quantitative examination of intramolecular interactions which determine chemical shift and coupling in proton spectra. The positions of bands reflect intramolecular chemical shifts and spin-spin splitting interactions and also give data on the formation of molecular complexes (which might be viewed as extended molecular species having their own intramolecular interactions).

In this experiment, you will explore two tools that are widely used in the determination of reaction mechanisms, be they organic, biochemical, organometallic, or inorganic. These are: the use of variable temperature NMR (VT-NMR) and line-shape analysis. Line shape analysis includes two components:

> (1) the relative areas of the bands give the relative numbers (concentrations) of different types of nuclei present;
> (2) the width of bands in systems where exchange is occurring gives information on the mean lifetimes of nuclei in various environments.

The model system you will be studying is the interaction of pyruvic acid with water, by itself as well as under acidic conditions. The reaction is represented by the equilibrium:

$$CH_3-\overset{\overset{\textstyle O}{\|}}{C}-COOH \ + \ H_2O \ \rightleftharpoons \ CH_3-\overset{\overset{\textstyle OH}{|}}{\underset{\underset{\textstyle OH}{|}}{C}}-COOH \qquad (1)$$

You are going to obtain two sets of data on this system. The set measured at room temperature will allow you to draw conclusions about the dependence of the reaction mechanism on the acidity of the medium. The VT set will allow you to draw conclusions

about the temperature dependence of the equilibrium and rate constants. In your report, you should discuss what your data tell you about the reaction mechanism, and what it does not.

II.   References

A.E. Derome, *Modern NMR Techniques for Chemistry Research*, Pergamon Press, New York, 1987.
E.F.H. Brittain, W.O. George, and C.H.J. Wells, *Introduction to Molecular Spectroscopy, Theory and Experiment,* Academic Press, New York, 1970.
J. Sandstrom, *Dynamic NMR Spectroscopy*, Academic Press, New York, 1982.
J. Kaplan and G. Fraenkel, *NMR of Chemically Exchanging Systems*, Academic Press, New York, 1980.
H. Friebolin, *Basic One- and Two-Dimensional NMR Spectroscopy*, 2nd Ed. (VCH Publishers, 1993).

Also see Experiment 21, pp. 256-264, in C. W. Garland, J. W. Nibler and D. P. Shoemaker *Experiments in Physical Chemistry*, 7th Edition, McGraw-Hill Publishing Co., New York, 2003.

Related discussion may be found in:

J. R. Dyer, *Applications of Absorption Spectroscopy of Organic Compounds,* Prentice-Hall, Englewood Cliffs, N.J., 1965, Chapter 4, pp. 58-132.
D. J. Pasto and C. R. Johnson, *Organic Structure Determination*, Prentice-Hall, Englewood Cliffs, N.J., 1979, Chapter 6, pp. 180-264.

III.   Experimental

This experiment will be done by groups of three students. Indicate clearly on the spectra and in your notebooks who did what. Each student should obtain the data from the spectra for each part and enter it in her/his own notebook. The further analysis, calculations, and discussion indicated below in Sections IV and V should be done independently. Results and conclusions may be compared.

A.   <u>Sample Preparation</u>

The pyruvic acid has been distilled[1] by your TAs to remove water, decomposition products, and other impurities (see Fig. 1). The distilled sample is stored in a refrigerator to keep it away from moisture, light, and oxygen[2].

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[1] Boiling points from literature (temperature in °C/pressure in Torr): 165/760 ; 106-108/126; 73/28; 68/17; 60/12.
[2] You may want to give some thought to the effect of temperature, light, moisture, and oxygen on the compound, and therefore on your experimental results.

Figure 1. Vacuum distillation apparatus for pyruvic acid. See your TAs for details.

Just prior to carrying out the experiment, prepare solution (A) and 3 of the (1-9) solutions as directed by your TA. You will collect data for a third of the solutions and share it with the other groups in your rotation:

(A) 0.010 mL pyruvic acid + 0.60 mL $CDCl_3$

(1) 0.010 mL pyruvic acid + 0.60 mL D_2O
(2) 0.010 mL pyruvic acid + 0.575 mL D_2O + 0.025 mL HCl
(3) 0.010 mL pyruvic acid + 0.550 mL D_2O + 0.050 mL HCl
(4) 0.010 mL pyruvic acid + 0.525 mL D_2O + 0.075 mL HCl
(5) 0.010 mL pyruvic acid + 0.500 mL D_2O + 0.100 mL HCl
(6) 0.010 mL pyruvic acid + 0.475 mL D_2O + 0.125 mL HCl
(7) 0.010 mL pyruvic acid + 0.450 mL D_2O + 0.150 mL HCl
(8) 0.010 mL pyruvic acid + 0.425 mL D_2O + 0.175 mL HCl
(9) 0.010 mL pyruvic acid + 0.400 mL D_2O + 0.200 mL HCl

Use concentrated HCl solution (approx. 11.7 M).

B.   **Measurement of the Spectra**

Specific instructions for operation of the NMR instrument will be given by your TA during your scheduled time on the NMR spectrometer.

C.   **1/T$_2$ determination**

Record the spectrum of pyruvic acid dissolved in CDCl$_3$ (solution A). Measure the linewidth of the pyruvic acid methyl proton peak.

D.   **H$^+$ and Temperature Dependence**

Record the spectrum of pyruvic acid (solutions 1-9) at 20, 27, 35, 42, 50, and 57 $^\circ$C. Integrate the pyruvic acid and hydrate methyl resonances and calculate their width at half maximum using the computer.

Every time you put a new NMR tube in the probe, you should allow it to equilibrate thermally by spinning it inside the probe for about 10 minutes before collecting data[3]. The thermometer inside the probe may not be well calibrated.

When varying the temperature, it is important to change the temperature by no more than 20 °C at a time between each equilibration in order to prevent thermal shock to the probe. Ideally, you would take data at 5 degree intervals between room temperature and 70 °C. However, since it may take 10 minutes or so for the tube to come into thermal equilibrium with the probe, you may not have enough time to carry out the entire series. In this case, you will have to make a choice between using larger temperature intervals or smaller intervals over a restricted temperature range. It is also a good idea to use nitrogen gas at elevated temperatures in order to prevent oxidation of the shim coils.

When you are finished with the experiment, please leave the spectrometer at room temperature so the next group can use it. You have to allow approximately 10 minutes for every 20 degrees that the probe needs to be cooled down.

IV.   Data Analysis

A.   **Theory of the NMR Spectrum**

You should be aware that you are using a pulsed FT-NMR spectrometer, not a continuous-wave (CW) one as you may have done in earlier laboratories. The pulsed NMR spectrometer transmits a pulse over the entire spectral width at once. The free induction decay (FID) of the signal emitted by the excited protons is observed and converted to a frequency-domain spectrum with the use of Fourier Transforms[4]. **The FID is the rate of decay of the**

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[3] To avoid having to do this for the nine room-temperature runs, check the temperature reading of the spectrometer and use that as the temperature of the experiment. You can assume that the inside and the outside of the probe are at the same temperature.
[4] For a discussion of Fourier Transforms, see Friebolin (*op. cit.*), Chapter 1, and Appendix A of this Manual.

excited nuclear spins. The value of dynamic NMR lies in that it allows the study of reactions with half-lives close to the half-life for the relaxation of a magnetically active nucleus in the system.

The spectrum of pure pyruvic acid consists of two resonances at 9.3 ppm and 2.6 ppm which are due to the carboxyl proton and the methyl protons, respectively. The separation of the two is dependent upon the purity of the sample; the greater the purity, the greater the separation.

The spectrum of an aqueous solution of pyruvic acid consists of three bands. The bands at 2.6 ppm and 1.75 ppm represent the resonances of the methyl protons of pyruvic acid and 2,2-dihydroxypropanoic acid, respectively, while the third band which represents the resonances of the carboxyl, hydroxyl, and water protons appears at variable positions in the spectrum dependent upon the composition of the mixture. This latter band is a singlet since the proton exchange rate between the three different environments is fast compared with the time of transition between different magnetic environments.

The quantities you will be measuring in the experiment is the integrated area under peaks and the linewidth at half the peak height (FWHM or $\Delta v_{1/2}$), as show below:
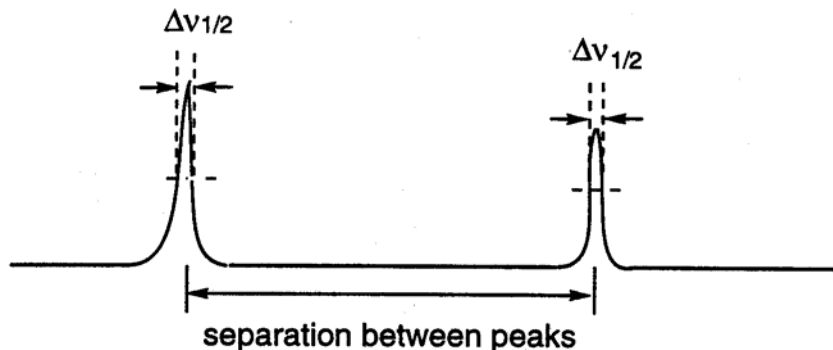
The full width of the exchange broadened bands at half the band height is inversely proportional to the net relaxation time (T'_2) for the proton, so that

$$\Delta v_{1/2} = \text{FWHM (in Hertz)} = \frac{1}{\pi T'_2} \qquad (2)$$

with

$$\frac{1}{T'_2} = \frac{1}{T_2} + \frac{1}{\tau} \qquad (3)$$

where T_2' is the relaxation time of the fully exchanged protons and T_2 is the relaxation time in the absence of exchange; thus τ is the mean lifetime of the proton.

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B. Reaction Kinetics Overview

In principle, the reaction between pyruvic acid and water could follow either an uncatalyzed or an acid (H^+)-catalyzed path, as shown below. Are there any other mechanisms possible?
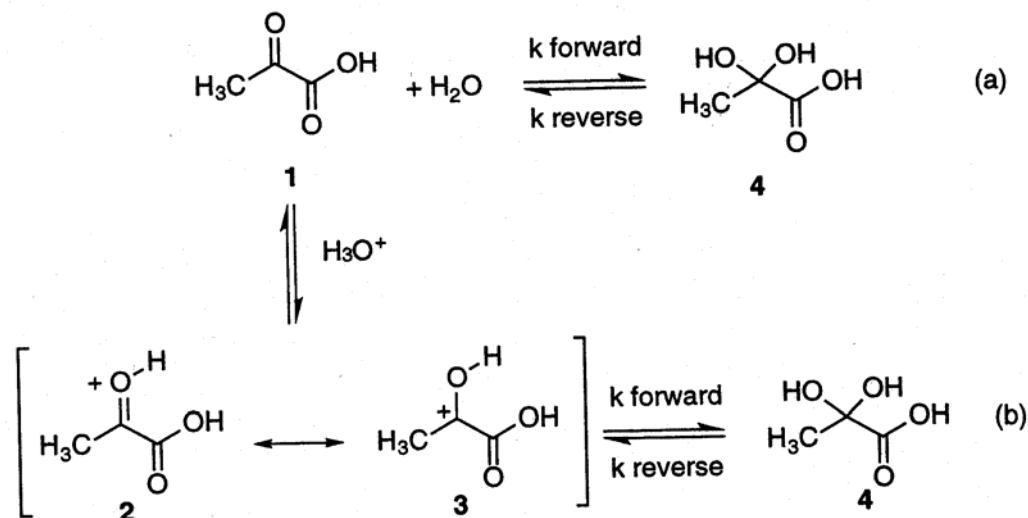
As either the temperature or the H^+ concentration is increased, the rate of exchange between the pyruvic acid form and the 2,2-dihyroxypropanoic acid form is increased. As this happens, the bands due to the methyl protons in these forms will broaden.

The reciprocal of the mean lifetime τ is equal to the specific rate of the reaction in a particular environment. The specific rate in turn is equal to $k_0 + k_{H^+} [H^+]$, where k_0 is the rate constant of the uncatalyzed reaction. The rate of the acid-catalyzed reaction is proportional to the concentration of protonated pyruvic acid, which is in turn proportional to the $[H^+]$ concentration. Thus, the acid-catalyzed rate is $k_{H^+} [H^+]$, and Eq. 3 can be written in the form of eq. 4.

$$\frac{1}{T_2'} = \text{constant} + k_{H^+}\left[H^+\right] = \left[\frac{1}{T_2} + k_0\right] + k_{H^+}\left[H^+\right] \tag{4}$$

C. Determination of Rate and Equilibrium Constants:

$1/T_2$:   If one assumes that there is no water in the pyruvic acid and chloroform solution, a rough measurement of $1/T_2$ can be obtained from the NMR spectrum of solution (1) (part IIIC). Depending on the quality of your data, a better way of calculating $1/T_2$ would be to fit your data given the following relationships between $1/T_2$, k_0, and k_{H^+}.

k$_{H+}$:   Based on (4), a plot of $1/T_2$' versus [H$^+$] at constant temperature should yield a straight line with a slope equal to the value for the acid-catalyzed rate constant. By measuring the bandwidth at half-height of methyl resonances in the spectra of pyruvic acid and 2,2-dihydroxypropanoic acid at different acid concentrations, the acid-catalyzed rate constants for the forward and back reactions may be determined.

k$_0$:   There are two ways to find k$_0$. The intercept of the plot of $1/T_2$' vs k$_{H+}$ is equal to $1/T_2+k_0$ (see (4)). Similarly, because there is no HCl added, the rate observed for solution (1) is the uncatalyzed reaction rate.

K$_{eq}$:   *K$_{eq}$* can be calculated using two methods based on integrated areas and linewidth measurements:

(1) By measuring the integrated areas of the methyl resonances, the equilibrium constant for the system can be evaluated at each temperature for each solution (1-9).

(2) From the Principle of Detailed Balance, the forward and reverse rate constants for any reaction pathway are related to give *K$_{eq}$*:

$$K_{eq} = \frac{k^{forward}}{k^{reverse}} \tag{5}$$

D.   Determination of E$_{act}$ and ΔH°

The Arrhenius theory of reaction rates predicts a temperature dependence of the rate constant given by

$$k = A \exp\left(-\frac{E_{act}}{RT}\right) \tag{6}$$

where E$_{act}$ is the activation energy for the reaction, interpretable as the energy required to reach a "transition state" or "activated complex" intermediate between reactants and products, and A is a unit-bearing pre-exponential factor. Eq. (6) leads to the so-called Arrhenius plot,

$$\frac{d \ln(k)}{d \frac{1}{T}} = -\frac{E_{act}}{R} \tag{7}$$

Thus, a plot of ln(k) vs. reciprocal absolute temperature should be a straight line of negative slope, with the magnitude of the slope equal to the activation energy in units of the gas constant R.

From Eq. (5), we must have:

$$\frac{d \ln K_{eq}}{d \frac{1}{T}} = \frac{d \ln k^{forward}}{d \frac{1}{T}} - \frac{d \ln k^{reverse}}{d \frac{1}{T}} \qquad (8)$$

$$= -\frac{1}{R}(E_{act}^{forward} - E_{act}^{reverse}) \qquad (9)$$

But we also know from the Gibbs-Helmholtz equation that:

$$\frac{d \ln K_{eq}}{d \frac{1}{T}} = -\frac{\Delta H^{o}}{R} \qquad (10)$$

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where ΔH° is the standard enthalpy change in the reaction; thus, we should have

$$E_{act}^{forward} - E_{act}^{reverse} = \Delta H^{o} \qquad (11)$$

E.   Determination of ΔG° and ΔS°

The Gibb's Free Energy of a reaction can be calculated at equilibrium based on the relationship

$$K_{eq} = exp\left(\frac{-\Delta G^o}{RT}\right) \tag{12}$$

Once the ΔG° and ΔH° have been determined, ΔS° can also be calculated from

$$\Delta G^o = \Delta H^o - T\Delta S^o \tag{13}$$

Which side of the reaction should be entropically favored? Which thermodynamic quantities should change with varying temperature? Varying acid concentration?
Substitution of Eq. (6) into Eq. (5) gives

$$K_{eq} = \frac{k_f}{k_r} = \left(\frac{A_f}{A_r}\right) exp\left(\frac{-\left(E_f^{act} - E_r^{act}\right)}{RT}\right) = \left(\frac{A_f}{A_r}\right) exp\left(\frac{-\Delta H^o}{RT}\right). \tag{14}$$

Note that Eq. (12) leads to an interesting interpretation of the pre-exponential factor in Eq. (6):

$$K_{eq} = exp\left(\frac{-\Delta G^o}{RT}\right) = exp\left(\frac{\Delta S^o}{R}\right) exp\left(\frac{-\Delta H^o}{RT}\right) \tag{15}$$

Comparing equations (14) and (15) shows that the ratio of the pre-exponential factors is directly related to the entropy of the reaction. One can even go so far as to define a forward and reverse "activation entropy" for the reaction analogous to the activation energies. This says that the reaction rate is favored by a large number of possible transition state configurations to cross the barrier.

V.   Notebook and Discussion

Record all procedures and observations carried out in preparing the solutions in your notebook. Incorporate all spectra as described in the "General Directions" handout for the course. Use a computer to analyze your data.

Discuss sources of error in the data. What is the largest source of error? Calculate standard errors on line fit parameters. Is the observed data scatter consistent with your error estimates? Discuss the results of your calculations and data analysis in terms of the theory on which they are based as presented in section IV above and in the references of section II. Also, discuss each of the points raised and answer the questions posed in the following for each of the sections of the experiment.

1. List the chemical shifts of the signals observed in the spectrum of pyruvic acid.
    Comment on the purity of the pyruvic acid.

2. Calculate the chemical shifts and assignments of signals observed in the spectrum of solution (1). Calculate the equilibrium constant for the pyruvic acid - 2,2-dihydroxypropanoic acid system in solution (1-9) using the integrated peak areas. Calculate ΔH° for each solution using the Gibbs-Helmholtz plot (Eq 10).

3. Measure the band widths at half-height of the methyl resonances in the spectra of solutions (1-9). Summarize the results in a table of the following form:

BAND WIDTHS ($1/T_2'$)

		Pyruvic Acid		Hydrate	
Solution #	HCl conc. (M)	Hz	Rad/s	Hz	Rad/s

4. Determine k_o using solution (1) and $1/T_2$. Determine k_{H+} and k_o using the slope and intercept of a linear fit of a plot of linewidths versus acid concentration at each temperature. Using k_{H+} and the two sets of k_o, plot the values for ln k for pyruvic acid and 2,2-dihydroxypropanoic acid in water against the reciprocal absolute temperature and thereby determine the activation energies for the uncatalyzed and catalyzed rate constants, $E_{act}^{forward}$ and $E_{act}^{reverse}$. Find $\Delta H°$ for the reaction according to Eq. (11), and compare the value you obtain with the values obtained from the peak area calculations. Comment on possible sources of error. Should ΔH° be the same or different for the acid-catalyzed and the uncatalyzed reactions? Does K_{eq} change as a function of $[H^+]$? Why?

5. Calculate and tabulate the $\Delta G°$ and $\Delta S°$ at varying temperatures and acid concentrations. Be prepared to discuss the thermodynamic results that one would logically expect and any discrepancies between these expected results and actual results.