Application of INDOR Method to a Relatively Slow Exchange System. OH Protons of the 1,3-Dihydroxyacetone Dimer in Dimethyl-d_6 Sulfoxide

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The nuclear magnetic multiple-resonance method developed by Forsén and Hoffman for the study of exchange rates has been applied to the two different types of OH protons of the 1,3-dihydroxyacetone dimer in dimethyl-d_6 sulfoxide, *i.e.*, OH (α) and CH_2OH (β). Here, "decay" and "recovery" curves were obtained using the so-called "INDOR" method, instead of a rapid repetitive scan method. Since the INDOR method continuously monitors a peak height, decay and recovery curves as fast as 1 s can be drawn by storing them in a computer after a single scan. The concentration dependence was examined as a two-site exchange system by neglecting the small amount of water (γ) contained in the dimethyl sulfoxide solvent. Both the lifetime, τ, and the longitudinal relaxation time, T_1, obtained from this assumption correlate well with the concentration variation of the 1,3-dihydroxyacetone dimer. The difference in the types of OH protons on the concentration dependence is clearly seen for the latter parameter, T_1. From the triple-resonance experiments including γ, it was found that the β proton is more "labile" than the α.

One of the most powerful uses of nuclear magnetic resonance spectroscopy in physical chemistry is its application to rate processes, such as chemical exchange reactions, restricted internal rotations and ring inversions. Two types of experimental and analysis methods have been developed so far for studies using a continuous wave (cw) NMR spectrometer, one is the line-shape analysis method originated by Gutowsky, McCall, and Slichter[1,2] and further extended and widely applied by many investigators[3] and the other is the double- or multiple-resonance method presented by Forsén and Hoffman,[4,5] which will hereafter be referred to as the FH method. This latter method is somewhat specific, since it can be applied for relatively slow-rate processes and in a narrower range than the former, *i.e.*, in the 0.01—1 s^{-1} range of a first-order rate constant. Therefore, examples of investigations using the FH method have been relatively few up to now.[6] These limitations arise for theoretical and technical reasons, *i.e.*, the lifetime of an exchanging nucleus must be comparable with its spin-lattice relaxation time, T_1, and the "decay" of the signal intensity upon sudden irradiation to another signal must be accurately followed. This latter gives the upper limit of the rate process which can be followed. Forsén and Hoffman have employed the rapid repetitive scan method in a state of no spin and misadjustment of the field homogeneity which prevents signal distortion due to wiggling and maintains slow passage conditions. Anet and Bourn have devised their equipment to continuously receive the intensity of a sharp signal with the sample spining and with the help of a field-frequency lock system.[7] This method can handle somewhat more rapid processes and closer signals of exchanging nucleus than the rapid repetitive scan method.

In the present work, an attempt was made to deal with relatively fast rate processes for the FH method, *i.e.*, processes with lifetimes less than about one second. This fills the gap between the fast limit of the FH method and the slow limit of the line-shape analysis method and extends the rate constant range over which the FH method can be applied. For this purpose, continuous observation of the signal height, a method similar to that of Anet and Bourn was adopted although the necessary decay curves were obtained differently. The so-called "INDOR" technique, first described and applied by Baker,[8] was employed in this work. In order to follow and record fast changes in the signal height, CAT (time-averaging computer) was used. For a preliminary examination, a dimethyl sulfoxide solution of a 1,3-dihydroxyacetone dimer was chosen as an example and it was examined to what extent this INDOR technique could follow a fast rate process within the framework of the FH method.

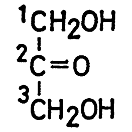
Experimental

Materials. The highest purity 1,3-dihydroxyacetone dimer (hereafter abbreviated as DAD) was obtained from the Tokyo Kasei Kogyo Co., Ltd, and dimethyl-d_6 sulfoxide (DMSO-d_6) was obtained from the Stohler Isotope Chemicals (SIC) Co., Ltd. These were used without further purification including in the deoxygenation and drying procedures, since from the preliminary experiment the magnitude of the lifetimes, τ, and spin-lattice relaxation times, T_1, of the OH protons of DAD were appropriately suited to this purpose. The balancing of the magnitudes of these two parameters is a very important point of the FH method.[4,5]

NMR Measurements. All measurements were performed at 26 °C with a Varian HA-100 D spectrometer operating in the frequency sweep mode at 100 MHz and with a 20-dB RF-field attenuation. Tetramethylsilane was used as an internal lock signal. The leakage from the transmitter coil to the receiver coil was minimized as much as possible with probe tuning paddles to avoid receiver overload when perturbing H_2 and H_3 fields were switched on. The "INDOR" experiments were performed using a Hewlett-Packard Model 4204 A oscillator as an additional oscillator for observing the H_1 field. Attention was paid to the amplitude of the H_1 field so as not to reduce the observing signal height by saturation. For triple-resonance experiments, a Wavetek Model 111 oscillator was employed. The perturbing field strengths of H_2 and H_3 used were determined to sufficiently saturate the signals but not to cause receiver overload. A Varian C-1024 time-averaging computer (CAT) operating in the internal trigger mode was used to follow and to store the fast changes in the signal intensity and then to read out the data on the recorder of the HA-100 D spectrometer. The sweep time of the C-1024 was arbitrarily set in the range from 2.5 to 10 s depending on the duration of the decay and recovery curves. A pen response time of 5 Hz was used for filtering.

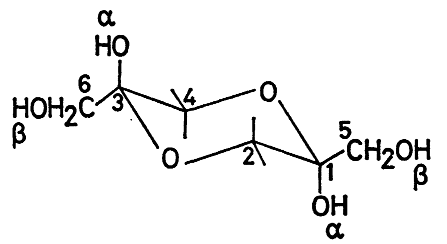
Results and Discussion

1,3-Dihydroxyacetone (I) is known to be present as a dimer having a 1,4-dioxane structure (II) in the crystalline state.



$$\begin{array}{l} {}^{1}CH_2OH \\ {}^{2}C{=}O \\ {}^{3}CH_2OH \end{array}$$

I



II

This form is maintained as the main species in a dimethyl sulfoxide solution, at least for a few hours after dissolution.[9] Figure 1 shows a typical proton magnetic resonance spectrum of DAD in a DMSO-d_6 solution (2.0 M) together with assignments using the notation and numbering shown in II. The weak signal at about 3.65 ppm is due to water protons and is also designated as γ. As is seen in Fig. 1, since the two types of OH protons, α and β, are well separated from each other and also from the other signals, DAD can be irradiated safely by the strong rf fields inherent to the FH method. The concentration dependence of the features of the α and β signals is shown in Fig. 2, in which the α and β signals for 2.0, 1.0, 0.5, and 0.25 M solutions are presented. When the concentration was lowered, both the α and β signals became sharp. This was particularly clear for the β signals. This sharpening implies a slowdown in the exchange rate, although an increase in the spin-spin relaxation time, T_2, may also contribute to this sharpening. Accordingly, the sharpening of the α and β signals cannot be explained simply. It was difficult to monitor the γ signal for 2.0 M by the INDOR technique due to its weakness. When the solutions were diluted from 2.0 M, the γ signal shifted to higher field with concomitant narrowing of the line width and at 0.25 M it overlapped the low-field signal of the $C_{2,4}H_a$ doublet. For these reasons, the exchange reactions of the α and β protons were first investigated using the INDOR technique over the 2—0.25 M range neglecting the presence of the γ proton. The elimination of the γ proton from the exchange pathway in the experiment gives an apparent lifetime, τ, and a spin-lattice relaxation time, T_1, which may be useful as relative parameters to observe trends. Subsequently, a three-site exchange system, which includes γ as an exchangeable site, was investigated for 1.0 and 0.5 M and compared with the results of the two-site exchange approximation.

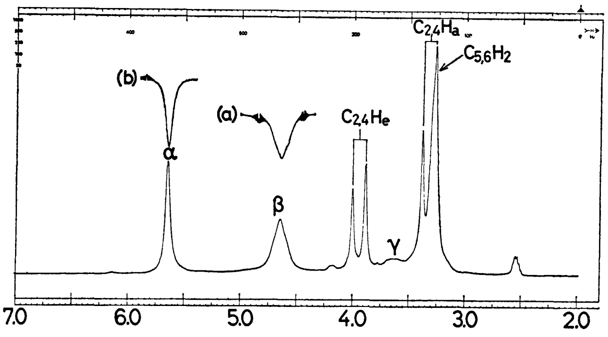


Fig. 1. The proton magnetic resonance spectrum of DAD observed in 2.0 M solution in DMSO-d_6, together with the assignments and INDOR spectra for α and β resonances. (a) INDOR spectrum for α(β), (b) INDOR spectrum for β(α).

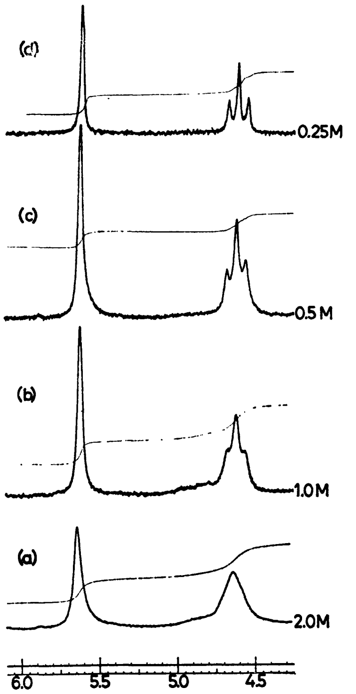


Fig. 2. The α and β resonances of DAD, showing the effect of concentration.

(a) 2.0 M, (b) 1.0 M, (c) 0.5 M, and (d) 0.25 M in DMSO-d_6.

Two-site Exchange System between α and β Protons. Figures 1 (a) and (b) show examples of INDOR spectra in cases where the α and β signals were monitored and the β and α signals were perturbed, respectively. Only one negative INDOR signal is obtainable in each case, since the spin transferred by chemical exchange from the saturating site simply reduces the monitoring signal intensity. Here, an abbreviation for the formal description of the multiple resonance experiment, is used with the nucleus under study outside the parenthesis and the nucleus being irradiated inside the parenthesis, *e.g.*, $\alpha(\beta)$ for case (a) and $\beta(\alpha)$ for case (b). If a recorder pen is set at the frequency of the bottom position of the INDOR spectrum and then if the irradiation field is switched on, the decay curve of the monitoring signal can be obtained by sweeping the CAT at a constant speed. Similarly, a recovery curve can be obtained when the radiation is switched off after the transient decay reaches a steady state. Figures 3 and 4 show the decay and recovery curves obtained at 2.0 and 0.25 M

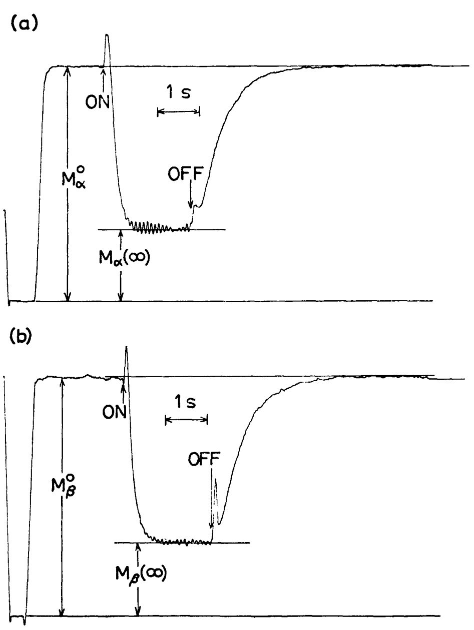


Fig. 3. The decay and recovery of the magnetization of DAD, 2.0 M in DMSO-d_6 solution for (a) $\alpha(\beta)$ and (b) $\beta(\alpha)$ arrangements. "ON" and "OFF" indicate the start and the removal of the irradiation, respectively.

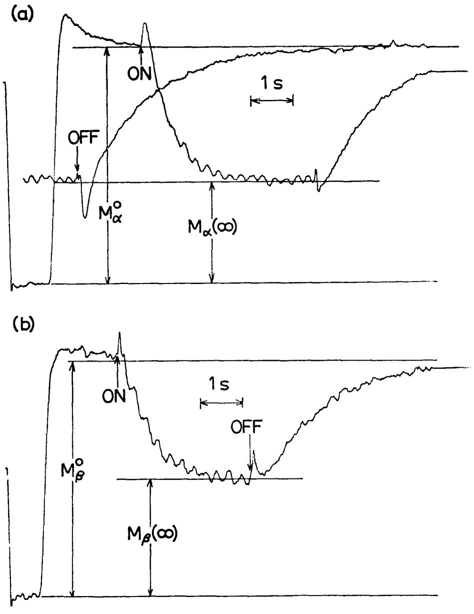


Fig. 4. As for legend to Fig. 3, except that 0.25 M in DMSO-d_6 solution was used.

for both $\alpha(\beta)$ and $\beta(\alpha)$ experimental arrangements. In analyzing the decay curves, the following three points were taken into account:

(a) There is a time lag until complete saturation after the irradiation power is switched on.

(b) A time lag due to pen response may be present. For example, in this experiment, a response frequency of 5 Hz was used, which causes a time lag of at most 0.2 s for the decay curve as a whole.

(c) "Shock noise" appears when the irradiation rf power is switched on or off, as Figs. 3 and 4 show. This noise also causes a lag in the recovery curve.

These three points introduce errors in the analysis of the decay curves. However, in practice, the scale of the decay curves is less than 10 cm, so that the time lag due to (b) may be less than 0.1 s. This time lag may have some effect in the case of the decay of a 2.0 M solution, but may not be as large for the case of the slow decay of a 0.25 M solution. The time lag due to (a) is inevitable, in principle, but due to (c), the decay curves were analyzed after the decay had passed through its initial value, *i.e.*, $M_\alpha{}^0$ or $M_\beta{}^0$. This delay may compensate the time lags due to (a) and (b) to approximately the same extent. Figure 5 shows the logarithmic plots ($\ln[M_i(t)-M_i(\infty)]$ *versus* t) of the magnetization decay for 2.0, 1.0, 0.5 and 0.25 M solutions. The slopes of the lines give $\tau_{1\alpha}=0.14$, 0.21, 0.38, and 0.55 s and $\tau_{1\beta}=$ 0.15, 0.31, 0.47, and 0.62 s from 2.0 to 0.25 M, respectively. From these τ_1, τ, and T_1 for each concentration can be calculated using Eqs. 2 and 10 of Ref. 4 and are plotted in Figs. 6(a) and (b), respectively. The results of two series of experimental trials are shown. In

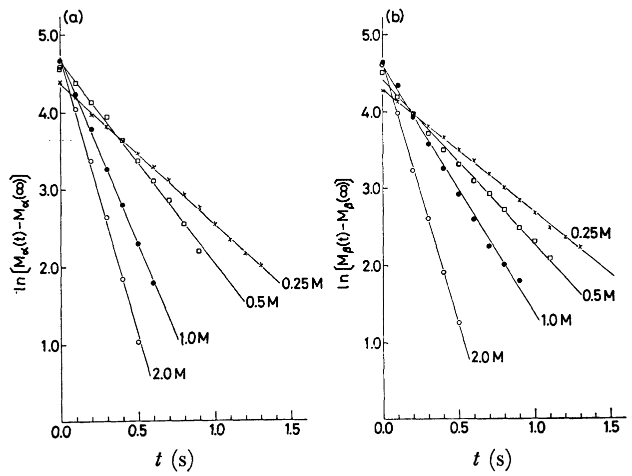


Fig. 5. Logarithmic plots (base e) of the decay of magnetization for (a) $\alpha(\beta)$ and (b) $\beta(\alpha)$ arrangements at various concentrations. The experimental points are taken from a smooth curve drawn through the decay curve "by-eye".

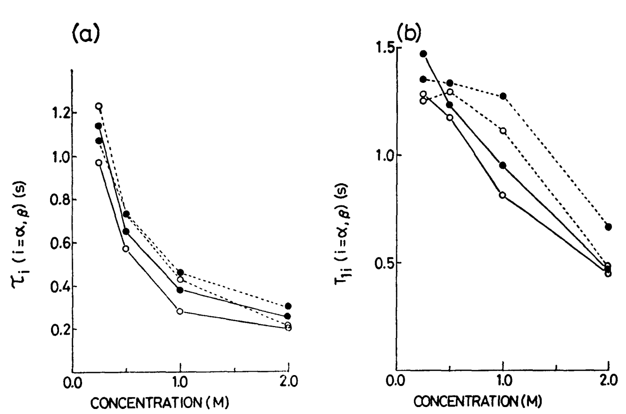


Fig. 6. The effect of concentration on (a) τ_i and (b) T_{1i} ($i=\alpha,\beta$). ○: Experiment 1, ●: Experiment 2. ——: α, ------: β.

Experiment 1, sample solutions of 1.0, 0.5, and 0.25 M were prepared by successive dilution from a 2.0 M solution, whereas in Experiment 2, each sample solution was prepared by weighing the corresponding amount of DAD individually. These two series were made to see the extent of scatter of τ and T_1 by the sampling method. However, as shown in Figs. 6(a) and (b), both Experiments 1 and 2 give similar trends. This indicates that τ and T_1 are not appreciably influenced by a small difference in the water content arising from the difference in sampling methods.

As shown in Fig. 6(a), when the concentration is lowered, both τ_α and τ_β increase steeply. This trend clearly shows the slowing down of the exchange rate between the α and β protons which was implied by sharpening of the signals upon dilution, as seen in Fig. 2. The steep increase in τ indicates that the exchange reaction may be controlled by the collision probability which depends on the concentration. On the other hand, with regard to T_1, $T_{1\alpha}$, and $T_{1\beta}$ show different tendencies with $T_{1\alpha}$ increasing monotonically and $T_{1\beta}$ reaching a maximum at about 1.0 M. This difference may be caused by the difference in the magnetic environments of the α and β protons, namely, the β proton has neighboring methylene protons while the α proton does not.

For both Experiments 1 and 2, τ_β was slightly larger than τ_α. This is reasonable since $M_\beta{}^0$ is 1.1—1.2 times larger than $M_\alpha{}^0$. Another important point for checking the reliability of τ and T_1 is to observe the degree of fitness between the recovery curve calculated using these parameters and the experimental recovery points. Since the recovery process is slower than the decay process and also is free from apprehension for receiver overload, a more precise curve than the decay curve is obtainable. Figure 7 shows the calculated recovery curves and the experimental points, with $\ln[M_i{}^0 - M_i(t)]$ plotted *versus t*. The necessary parameters for drawing the theoretically-determined full curves were calculated from Eqs. 15, 19, and 20 of Ref. 4 and are shown in Table 1. The scales of C_1 and C_2 are adjusted to the present experimental size in terms of $M_i{}^0$. All the theoretically-predicted curves are nearly straight on the whole and, since λ_1 is much larger than λ_2, the exponential part of λ_1 decays quickly to zero. In order to obtain the best fit, the experimental points were read off by delaying the null-point time by about 0.1—0.2 s from the actual moment that the irradiating field was removed. This type of time lag has also been reported by Forsén and Hoffman.[4] The resulting fit between the theoretically-predicted curves and the experimental points is satisfactory for all cases. These results show clearly that the two-site exchange approximation is justifiable for determining the relative tendencies and that the INDOR technique used here can follow the fast rate processes which is impossible by the rapid repetitive-scan method used up to now.

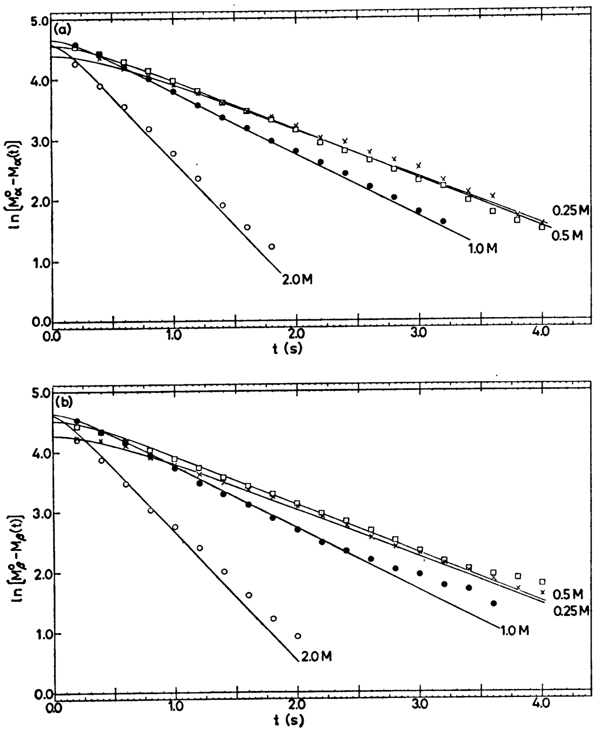


Fig. 7. Logarithmic plots (base e) of the recovery of magnetization for (a) α and (b) β nuclei at various concentrations. The full curves represent the theoretically predicted recoveries. The experimental points are taken from a smooth curve drawn through the recovery curve and are shown by the following symbols for each concentration: ○, 2.0 M; ●, 1.0 M; □, 0.5 M; ×, 0.25 M.

Table 1. Calculated parameters used to draw the recovery curves in Fig. 7

Concentration	type of proton	C_1(mm)	C_2(mm)	λ_1(s^{-1})	λ_2(s^{-1})
2.0 M	α	21.8	−119.8	11.8	2.1
	β	21.9	−120.3		
1.0 M	α	18.9	−124.4	7.0	1.0
	β	18.4	−120.9		
0.5 M	α	24.7	−120.2	3.9	0.8
	β	23.4	−113.9		
0.25 M	α	34.7	−115.7	2.6	0.8
	β	30.7	−102.2		

Three-site Exchange System for α, β, and γ Protons. In the case of a three-site exchange system, including γ which originates from the water protons, exactly the same procedures as those for the two-site exchange system can be utilized, except that a triple resonance is considered. The triple resonance is rather difficult to carry out, since it causes more beating than does double resonance. However, fortunately, this was not such a serious problem in the present experiments. Figure 8 shows, in level-diagram form, the concentration dependence of the steady-state ratios necessary for calculating τ_i and λ_{ij}. Ratios for a 0.25-M solution are included for reference, although the measurement of the γ signal at this concentration is somewhat questionable because of overlapping with other signals. The parameter λ_{ij} is the probability per unit time for a nucleus at site i to be transferred to site j and is generally related to the life time τ_i by

$$1/\tau_i = \sum_{j \neq i} \lambda_{ij}. \qquad (1)$$

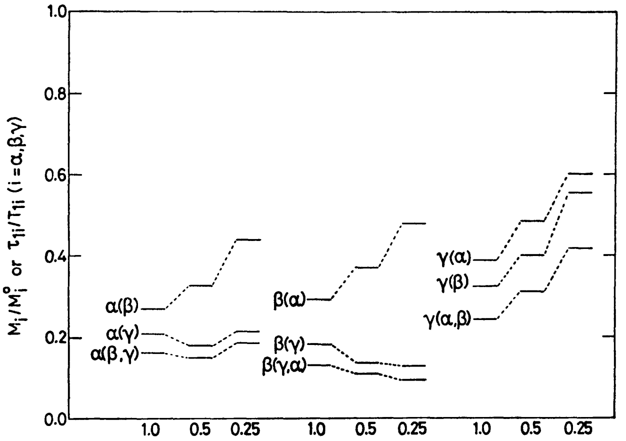


Fig. 8. The schematic illustration of the intensity ratios M_i/M_i^0 and τ_{1i}/T_{1i} for various experimental arrangements. Experiment 1 series of samples were used. Double resonance experiments (α(β) *etc.*) give ratios M_i/M_i^0 and triple resonance experiments (α(β,γ) *etc.*) give ratios τ_{1i}/T_{1i}.

TABLE 2. TIME CONSTANTS OBTAINED FROM THE DECAY CURVES TREATED AS THREE-SITE EXCHANGE SYSTEMS

Concentration	Type of proton i	τ_{1i}(s)	T_{1i}(s)	τ_i(s)	τ_i(s) from Eq. 1
1.0 M	α	0.13	0.85	0.16	0.18
	β	0.11	0.91	0.12	0.16
	γ	0.14	0.58	0.18	0.21
0.5 M	α	0.17	1.05	0.20	0.23
	β	0.14	1.32	0.16	0.18
	γ	0.26	0.84	0.39	0.41

Table 2 shows the time constants which were obtained from an analysis of the triple-resonance decay curves. In the triple-resonance experiment, when the γ site was included as the irradiation site, the transient decay rate increased above that of the double-resonance case. This effect results in a smaller value of τ_1 than that of the corresponding two-site exchange approximation. However, it is interesting to note that it only affects the magnitude of τ leaving that of T_1 almost unchanged.

TABLE 3. CALCULATED λ_{ij} IN s^{-1} FOR 1.0 M (LEFT) AND 0.5 M (RIGHT) SOLUTIONS

i	j		
	α	β	γ
α	—	2.5, 1.2	3.0, 3.0
β	1.9, 1.1	—	4.6, 4.3
γ	1.8, 0.9	3.0, 1.6	—

TABLE 4. THE RATIO OF THE MAGNETIZATION WHICH FLOWS IN A SITE TO THAT WHICH EFFUSES FROM THE SITE PER UNIT TIME

Type of proton	Concentration	
	1.0 M	0.5 M
α	0.8	1.0
β	0.9	0.9
γ	1.2	1.1

Table 3 shows the values of λ_{ij} calculated from Eqs. 7 and 8 of Ref. 5, using the necessary values given in Table 2 and Fig. 8. The following ratios were also utilized: M_α^0: M_β^0: M_γ^0=1: 1.1: 1.4 for 1.0-M and 1: 1.2: 3.0 for 0.5 M-solutions. These were obtained by integrating the corresponding band of the single-resonance spectrum. Before using the values of λ_{ij} to attempt to obtain significant information, the reliability of these values must be checked. Since λ_{ij} depends on the difference between two asymptotic intensity ratios of similar magnitude, they may include large errors.[5] One way of checking the reliability of λ_{ij} is to test the agreement between the τ_i calculated from λ_{ij} using Eq. 1 and those calculated from the decay curves. In the last column of Table 2, the values of the τ_i calculated from λ_{ij} are given. As can be seen from this table, the agreement is satisfactory for all cases. The another important check is to test whether or not these λ_{ij} satisfy the balance equations of Ref. 5, namely, the magnetization which flows into a site must be equal to that which effuse from the site per unit time. This is shown in Table 4 as the ratio of the former to the latter for each site. All the calculated ratios are nearly equal to unity, so that the balance equations can be said to be well satisfied. Based on these checks, it can be said that the probability of proton transfer is in the following order of magnitude: $\beta\rightarrow\gamma>\alpha\rightarrow\gamma>\gamma\rightarrow\beta>\alpha\rightarrow\beta>\beta\rightarrow\alpha>\gamma\rightarrow\alpha$. This order indicates that:

(a) The probability of proton transfer is larger in the direction of the β and α protons to the γ proton than is the direct exchange between α and β protons. This implies that proton exchange between α and β sites is accelerated by the presence of water protons.

(b) The β proton is more "labile" than is the α proton. This is consistent with the well-known fact that primary alcohols are more acidic than tertiary alcohols and that the extent of esterification of the former is larger than that of the latter *etc.*,[10] although for DAD, the β nucleus is the hydrogen of the hydroxyl of a hemiacetal, which can be thought of as a fairly labile hydrogen.

Presently, the INDOR technique is a very popular method for analyzing spin-coupled spectra, with an appropriate attachment often being installed in commercially-available CW NMR spectrometers. This work was undertaken with the intention of applying this technique to the investigation of rate processes. It was shown that with the aid of CAT, the INDOR technique is more suitable and easier to use for following fast changes in magnetization than is the rapid repetitive-scan method. This will enable relatively fast rate processes to be investigated using the elegant multiple-resonance method presented by Forsén and Hoffman for the study of rate processes together with relaxation processes.

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