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Study of the Tautomeric Equilibrium of Pyridoxine in 1,4-Dioxane/Water Mixtures by ^{13}C Nuclear Magnetic Resonance. Thermodynamic Characterization and Solvent Effects

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Received April 2, 2004

A significant temperature dependence has been found for the ^{13}C NMR chemical shifts of pyridoxine in 10%, 20%, 30%, 40%, 50%, and 60% v/v 1,4-dioxane/water mixtures (pH = 7.0). The nuclei most sensitive to the temperature effect were C-3 and C-6 in all of the mixtures. This dependence has been explained on the basis of a thermally induced tautomeric equilibrium shift between the neutral and the dipolar forms of the pyridoxine molecule. The thermodynamic characterization of this tautomeric equilibrium, which interconverts quickly on the NMR time scale, has been achieved by considering the observed average ^{13}C NMR chemical shifts at different temperatures through fitting the experimental data to a theoretical curve. The fitting accuracy is greatly improved on using linear correlations between the average chemical shifts obtained from different nuclei at the same temperature. The methodology outlined above allows the $\Delta H°$ value to be calculated for the tautomeric process and the chemical shifts of the pure extreme forms, i.e., neutral and dipolar, to be deduced. These values have been used to calculate the thermodynamic parameters of the tautomerization equilibrium in each dioxane/water mixture. The effect of solvent on the tautomeric equilibrium and the averaged chemical shift has been explained in terms of a multiparameter equation developed by Kamlet and Taft. The overall solvent effect is the sum of two different effects: the dipolarity and polarizability of the solvent and the ability of the solvent to act as a hydrogen-bond donor toward a solute.

Introduction

The study of proton tautomerization processes is a field of great interest due to the importance of tautomerism in many areas of chemistry and biology.[1−4] Despite the clear importance of quantitative thermodynamic data in this area, there is little information in the literature about these processes in solution and data for mixed solvent systems are practically nonexistent.[1,5,6]

The techniques used for the measurement of tautomeric populations are mainly spectroscopic,[7−9] and of particular interest is UV−visible spectroscopy,[10,11] for which it is necessary to obtain the spectra of the pure forms.

The temperature dependence of NMR signals for compounds that undergo rapid chemical exchange can be used as a sensitive probe to study the equilibrium in question. Experimental chemical shifts $\langle\delta\rangle$ obtained from the spectra of isomers that interconvert rapidly on the NMR time scale are the weighed average of the contributing tautomeric forms. To estimate the tautomeric populations of the isomers, it is necessary to determine the chemical shifts for each pure tautomeric form. The study of this type of equilibrium suffers from particular difficulties in that it is usually impossible to measure directly the signals corresponding to the pure isomers. Several authors have determined the chemical shifts of the pure isomers from model compounds such as methyl derivatives, where tautomerism has been avoided by replacing protons with substituent groups.[8] However, much more reliable values for the extreme tautomeric forms can be obtained from NMR data by a fitting process.

In previous papers[7,12] we described a new method for the thermodynamic characterization of tautomeric equi-

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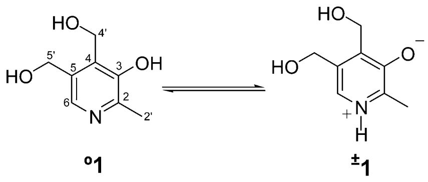
10.1021/jo0494533 CCC: $27.50

SCHEME 1. Tautomeric Equilibrium of Pyridoxine



$$K(T) = \frac{[^{\pm}1]}{[^{0}1]} = \frac{x_B}{x_A}$$

libria from multinuclear magnetic resonance techniques. This method allowed us to calculate tautomeric equilibrium constants at different temperatures, as well as the corresponding free energy, enthalpy, and entropy data for the tautomerization process from variable temperature ^{13}C NMR and ^{15}N NMR signals.

In the work described here we increased the scope of this methodology by addressing the study of tautomeric processes in solvent mixtures. This new method was applied to the study of the tautomeric equilibrium $^{0}1 \rightleftharpoons {}^{\pm}1$ of pyridoxine (Scheme 1) in a number of 1,4-dioxane/water mixtures; $^{0}1$ and $^{\pm}1$ represent the neutral and the dipolar forms of the molecule, respectively. The ^{13}C NMR spectra were obtained in 1,4-dioxane/water mixtures [10%, 20%, 30%, 40%, 50%, and 60% (v/v) in dioxane] and at temperatures ranging from 20 to 80 °C. The resulting information allowed the thermodynamic parameters of the tautomeric equilibrium to be obtained.

Experimental Section

Commercial samples of pyridoxine were used without further purification. 1,4-Dioxane was distilled from sodium prior to use. ^{13}C NMR spectra were acquired at 100 MHz in a 400 MHz spectrometer under standard proton decoupling techniques. ^{13}C NMR variable temperature experiments in the range 20−80 °C were performed in 5 mm tubes with 0.08 M solutions of pyridoxine in 10%, 20%, 30%, 40%, 50%, and 60% v/v 1,4-dioxane/water mixtures. The pH measurements in 1,4-dioxane/water mixtures have been carried out according to the procedure previously described in the literature.[13−15] The pH of each sample was adjusted to 7.0 by careful addition of tiny amounts of solid sodium hydroxide and sodium dihydrogen orthophosphate to the initial aqueous solution. A coaxial insert with a solution of hexamethyldisiloxane (HMDSO) in DMSO-d_6 was used as external reference ($\delta = 1.93$ ppm) and as the field lock. The FID was acquired with 64 K points and transformed with zero filling, leading to a resolution of 0.35 Hz/point. A delay of 900 s was used to stabilize each temperature. A recovery delay of 1.0 s was used between pulses and the acquisition time for each pulse was 1.2 s. Each FID was recorded with a variable number of transients, typically between 1200 and 2500, until an acceptable signal-to-noise ratio was obtained.

Theoretical Basis

If the relaxation time for an equilibrium $A \rightleftharpoons B$ is small compared with the NMR time scale, the average chemical shift of a particular nucleus $\langle\delta\rangle$, is given by

$$\langle\delta\rangle = x_A\delta_A + x_B\delta_B \tag{1}$$

where x_A, x_B, δ_A, and δ_B are the molar fractions and the chemical shifts of the pure A and B forms, respectively. When the NMR spectra are obtained at different temperatures, a change in the average chemical shift will be observed because a shift in the equilibrium is induced. It is worth noting that the chemical shifts of both the pure forms and the NMR reference can also be affected by the temperature variation. In general, this change in chemical shifts is very small[12] when compared with the chemical shift variation induced by modifications in the equilibrium composition. In the following theoretical treatment this temperature effect on the chemical shifts has been neglected and therefore δ_A and δ_B have been considered constant with the temperature. This simplification will be briefly discussed later on.

The tautomerization equilibrium constant is defined as

$$K(T) = \frac{x_B}{x_A} = \frac{\langle\delta\rangle - \delta_A}{\delta_B - \langle\delta\rangle} \tag{2}$$

The following expression can be obtained from eq 2:[12]

$$\langle\delta\rangle = \frac{\delta_A\Delta_B + \delta_B\Delta_A E}{\Delta_B + \Delta_A E} \tag{3}$$

where

$$E = \exp\left[\frac{\Delta H°\Delta T}{RTT_r}\right]; \quad \Delta_B = \delta_B - \langle\delta_r\rangle; \quad \Delta_A = \langle\delta_r\rangle - \delta_A; \quad \Delta T = T - T_r$$

in which T_r and $\langle\delta_r\rangle$ are the values corresponding to a single experimental datum used as a reference.

ΔH° is the standard enthalpy change for the process, which is considered to be constant with temperature. Thus, the standard enthalpy change and the chemical shift of the pure forms can be estimated by a nonlinear fitting procedure using eq 3, where the values for $\langle\delta\rangle$ and T are the experimental results. Each series of experimental data obtained from any nucleus sensitive to the prototropic exchange must give the same thermodynamic parameters within experimental error.

The experimental average chemical shift data for two sensitive nuclei, obtained at the same temperature, follow eq 4:[12]

$$\langle\delta\rangle_1 = \frac{\delta_{A1}\delta_{B2} - \delta_{A2}\delta_{B1}}{\delta_{B2} - \delta_{A2}} + \frac{\delta_{B1} - \delta_{A1}}{\delta_{B2} - \delta_{A2}}\langle\delta\rangle_2 \tag{4}$$

where subscripts 1 and 2 refer to data from the two different nuclei. Equation 4 indicates that a plot of $\langle\delta\rangle_1$ versus $\langle\delta\rangle_2$ (for data obtained at the same temperature) is a straight line. Indeed, very good linearity and a high level of accuracy were obtained for the different signals (see below).

From the experimental slope (s) and intercept ($o.o$) from eq 4 it is possible to demonstrate that each signal for nucleus 1 (for example, δ_{A1}) is related in a linear manner with the corresponding signal for nucleus 2 (δ_{A2}):

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$$\delta_{A1} = o.o + s\cdot\delta_{A2} \quad (5)$$

The same situation holds for δ_{B1} and δ_{B2}.

A simultaneous multiple function curve fitting of several signals can be performed using these linear correlations as restricting conditions. The functions share both the temperature and the standard enthalpy of the process. The use of this methodology enables the experimental data points corresponding to all the different nuclei to be used together in the same fitting process. The calculation gives the enthalpy of the process ($\Delta H°$) and the chemical shifts of the pure forms for a given nucleus (δ_A and δ_B). In addition, the chemical shift of the signal used as the reference (δ_r) can also be obtained from the fitting in order to avoid the introduction of experimental error in the calculation. The same parameters for the other nuclei can be determined from the previously obtained parameters and the linear correlations. It is worth pointing out that a good qualitative assessment of the results is provided by comparing the calculated and experimental values for the reference chemical shift.

The advantages of the multiple function curve fitting with the linear correlations allow an accurate way of solving the problem using only ^{13}C NMR data.

Another possibility for data analysis is based on the derivation of eq 2, from which it is possible to obtain

$$\left(\frac{d\langle\delta\rangle}{dT}\right) = \frac{\Delta H°}{RT^2}\left[\frac{(\langle\delta\rangle - \delta_A)(\delta_B - \langle\delta\rangle)}{\delta_B - \delta_A}\right] \quad (6)$$

Equation 6 allows the fitting of the experimental results without the use of a single datum as a reference. Nevertheless, the use of this equation is not necessarily advantageous because the derivatives need to be calculated.

Solvent Effects. There is significant interest in the study of physicochemical phenomena in mixed solvent systems and in the interpretation of such phenomena with regard to the preferential solvation of solutes by one of the components in the solvent mixture.[16,17] Preferential solvation in a binary solvent mixture is a composite effect determined by both solvent−solvent and solvent−solute interactions. Such interactions have been found to be important in solution chemistry to explain spectroscopic, equilibrium, and kinetic data.[18] One example of a successful quantitative treatment of solvent effects using a multiparameter equation was described by Kamlet and Taft,[19−21] and this approach is also known as the linear solvation energy relationship (LSER).

The equation developed by Kamlet and Taft explains the variation of the properties of any solute with solvent composition in terms of a linear combination of the microscopic parameters of the solvent π^*, α, and β. The solvatochromic parameter π^* measures a blend of dipolarity and polarizability of the solvent. Parameter α is a quantitative, empirical measurement of the ability of a bulk solvent to act as a hydrogen-bond donor (HBD) toward a solute. Finally, β represents the ability of the solvent to act as a hydrogen-bond acceptor (HBA) or electron-pair donor (EPD) toward a solute, i.e., to form a solute-to-solvent hydrogen bond or a solvent-to-solute coordinate bond, respectively. The solvatochromic comparison method, introduced by Kamlet and Taft[19−21] and further developed by Abboud and Abraham,[21] makes use of eq 7:

$$Y(s) = Y(o) + a\alpha + b\beta + s\pi^* \quad (7)$$

where Y(s) and Y(o) represent the solute property in question in a given solvent or in a hypothetical solvent for which $\alpha = \beta = \pi^* = 0$, and a, b, and s are the respective susceptibilities to change in the α, β and π^* values of the solute property in question.

The study of the effect of solvent on the tautomerization equilibrium constants using the Kamlet and Taft multiparametric method can be used to obtain correlations between the equilibrium constant and the solvatochromic parameters. The use of linear solvation energy relationships to predict solvent effects in chemical equilibria is well documented.[17,22−25] In this way, extensive data on related keto/enol equilibria and Kamlet−Taft correlations on chemical shifts are given in the literature.[26−29]

Results and Discussion

The ^{13}C NMR spectra of pyridoxine in 1,4-dioxane/water mixtures (10%, 20%, 30%, 40%, 50%, and 60% v/v in dioxane, pH = 7) were recorded at temperatures ranging from 293 to 358 K. However, at temperatures higher than 343 K the evaporation of solvent from solutions with a high 1,4-dioxane content became significant. This fact precluded the use of a wider range of temperatures. Experimental data were therefore obtained for all the mixtures between 293 and 338 K. Under these conditions the pyridoxine is present in equilibrium between the neutral (^{0}1) and the zwitterionic form ($^{\pm}$1) (see Scheme 1).[30]

The ^{13}C NMR spectra show nine well-defined signals, and several of these are very sensitive to temperature variation. The average chemical shifts measured for the most sensitive nuclei at different temperatures are given in Table 1. To keep the amount of data to a minimum, they are presented as a mathematical second-order function. The most marked temperature effects are observed for the signal assigned to C-6, followed by the signals corresponding to C-3, C-2, and C-4. C-5 and the exocyclic nuclei (C-2′, C-4′, and C-5′) show rather small

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TABLE 1. Temperature Dependence of ^{13}C NMR (C-3, C-2, C-4, and C-6) Average Chemical Shifts ($\langle\delta\rangle$, ppm) for the Equilibrium $^{0}\mathbf{1} \rightleftharpoons {}^{\pm}\mathbf{1}$ of Pyridoxine in Some 1,4-Dioxane/Water Mixtures (pH = 7)[a]

	c	*d*	$e \times 10^4$
10%			
C-3	157.637	−0.0676	1.72
C-2	143.790	0.0249	−0.34
C-4	136.662	−0.0376	1.39
C-6	126.580	0.1096	−2.67
20%			
C-3	157.111	−0.0670	2.25
C-2	143.928	0.0245	−0.43
C-4	136.324	−0.0365	1.56
C-6	127.343	0.1107	−4.07
30%			
C-3	155.975	−0.0683	3.30
C-2	144.221	0.0257	−0.77
C-4	135.602	−0.0381	2.19
C-6	128.997	0.1093	−4.64
40%			
C-3	154.199	−0.0508	2.63
C-2	144.697	0.0202	−0.34
C-4	134.416	−0.0287	1.80
C-6	131.553	0.0838	−0.35
50%			
C-3	152.763	−0.0331	1.85
C-2	145.113	0.0174	−0.48
C-4	133.355	−0.0189	1.46
C-6	133.718	0.0601	−2.60
60%			
C-3	151.740	−0.0182	0.82
C-2	145.404	0.0146	−0.22
C-4	132.475	−0.0077	0.75
C-6	135.218	0.0443	−1.68
p error	±0.005	±0.0005	$\pm 10^{-5}$

[a] Reference: HMDSO, $\delta = 1.93$ ppm. Parameters of the fitting to $\langle\delta\rangle = c + d(T - 298) + e(T - 298)^2$. The indicated percentage of 1,4-dioxane is given by volume (v/v).

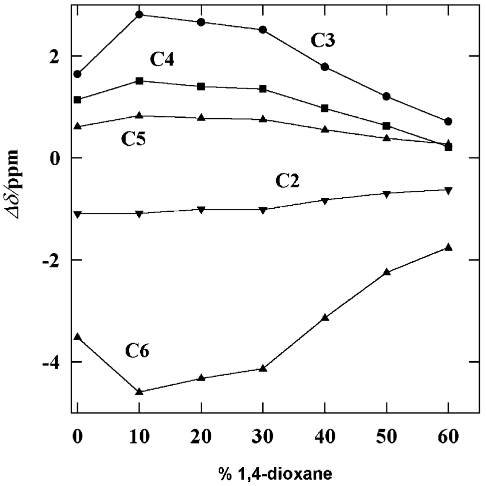

FIGURE 1. Temperature dependence of ^{13}C NMR average chemical shifts ($\Delta\delta = \langle\delta\rangle_{293} - \langle\delta\rangle_{338}$, ppm) for the equilibrium $^{0}\mathbf{1} \rightleftharpoons {}^{\pm}\mathbf{1}$ of pyridoxine (C-3, C-2, C-4, C-5, and C-6 nucleus) in the different 1,4-dioxane/water mixtures (v/v % in 1,4-dioxane). Data in pure water from ref 12.

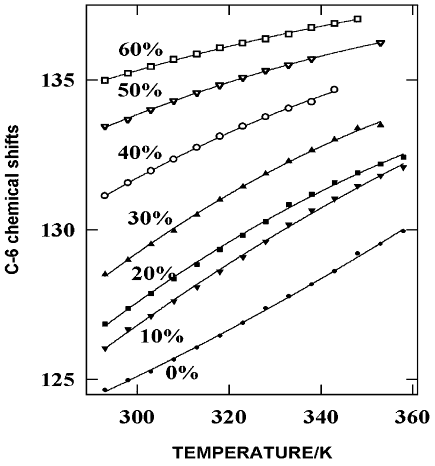

FIGURE 2. Experimental ^{13}C NMR average chemical shifts (ppm) of pyridoxine (C-6 nucleus) in the different 1,4-dioxane/water mixtures and at different temperatures. Data in pure water from ref 12.

variations in chemical shifts throughout the temperature range studied. This situation make these signals of little use in further calculations, and therefore they have not been included in Table 1. The reasons why a particular signal is affected to a greater or lesser extent by the temperature have been discussed in a previous paper.[12] Figure 1 shows a plot of $\langle\delta\rangle_{293} - \langle\delta\rangle_{338}$ for each carbon versus percent 1,4-dioxane (v/v) in the mixture. Although C-5 data are presented in Figure 1 for comparison, they will not be used further in calculations, as has been mentioned before. It can be seen that the behavior for the different nuclei is identical in all of the 1,4-dioxane/water mixtures. Thus, the shapes of these curves, as one would expect, are similar since they are all based on the same information. For instance, although $\langle\delta\rangle_{293} - \langle\delta\rangle_{338}$ is positive for C-3 and negative for C-6, both curves are nearly mirror images. The strongest effect resulting from the temperature variation, measured as the difference in chemical shifts, is observed for the 10% dioxane/water mixture. In this case, the chemical shift difference steadily decreases as the percentage of dioxane in the mixture increases.

The trend for the chemical shifts follows a similar pattern in 1,4-dioxane/water mixtures and in pure water.[12] Figure 2 shows a plot of the experimental chemical shifts for C-6 versus the temperature in water[12] and the different 1,4-dioxane/water mixtures. The "slope" of the curve decreases in proportion to the amount of 1,4-dioxane in the mixtures. This trend is similar to that seen in Figure 1.

A multiple function curve fitting of the most sensitive nuclei signals, i.e., C-3, C-2, C-4, and C-6, was carried out with eq 3 using 14 × 4 data points for each solvent mixture. The C-4 chemical shifts vary very little in the mixtures with 50% and 60% 1,4-dioxane (Figure 1). Therefore, these chemical shifts were not used in the fitting calculations. One significant advantage of this methodology is that it allows a large increase in the number of data to fit, and consequently the error of the fitted parameters is greatly reduced.

Equation 4 indicates that the plot of the chemical shift of a given signal versus the chemical shift of other nuclei must be linear for data obtained at the same temperature. When this correlation is calculated, excellent straight lines with very high correlation coefficients ($r > 0.999$) were obtained for each pair of nuclei in every 1,4-dioxane/water mixture. Table 2 shows the intercept and slopes

TABLE 2. Parameters (Intercept and Slope) and Standard Errors of Linear Relations between the Experimental Averaged Chemical Shifts of C-3, C-2, and C-4 versus C-6 (eq 4) in Different 1,4-Dioxane/Water Mixtures (v/v)

	intercept	slope
	10%	
C-3	235.11 ± 0.2	−0.612 ± 0.002
C-2	113.10 ± 0.2	0.242 ± 0.002
C-4	176.86 ± 0.4	−0.316 ± 0.003
	20%	
C-3	235.74 ± 0.5	−0.617 ± 0.004
C-2	112.17 ± 0.4	0.249 ± 0.003
C-4	176.75 ± 0.3	−0.318 ± 0.002
	30%	
C-3	233.67 ± 0.5	−0.602 ± 0.004
C-2	111.57 ± 0.3	0.253 ± 0.002
C-4	176.37 ± 0.5	−0.316 ± 0.004
	40%	
C-3	230.20 ± 0.9	−0.578 ± 0.008
C-2	109.29 ± 0.8	0.269 ± 0.002
C-4	175.05 ± 0.6	−0.309 ± 0.005
	50%	
C-3	221.11 ± 0.9	−0.511 ± 0.008
C-2	103.05 ± 0.7	0.315 ± 0.005
	60%	
C-3	205.38 ± 0.5	−0.397 ± 0.003
C-2	95.38 ± 0.7	0.370 ± 0.005

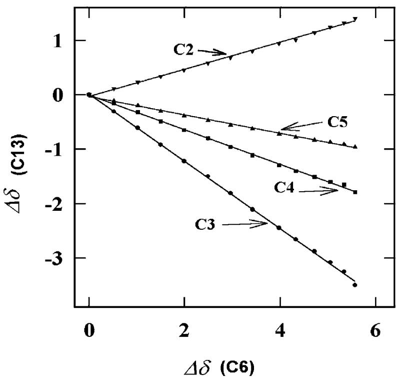

FIGURE 3. Linear plots of $\Delta\delta$(C13) versus $\Delta\delta$(C-6) in 20% 1,4-dioxane/water mixture. $\Delta\delta = \langle\delta\rangle_T - \langle\delta\rangle_{293}$.

TABLE 3. Parameters and Standard Errors Obtained from Multiple Function Curve Fitting of the C-3, C-2, C-4, and C-6 Signals to Equation 3 for Different 1,4-Dioxane/Water Mixtures[a]

	C-3*	C-2*	C-4*	C-6
		10%		
δ_A	152.23	145.92	133.85	135.39 ± 0.4
δ_B	159.74	142.94	137.73	123.12 ± 0.4
δ_r	157.63	143.78	136.64	126.58 ± 0.01
$\Delta H°$	−28.8 ± 2 kJ/mol; SEF = 0.033; R = 0.9999			
		20%		
δ_A	152.68	145.70	134.03	134.53 ± 0.2
δ_B	158.95	143.17	137.26	124.37 ± 0.2
δ_r	157.12	143.91	136.31	127.35 ± 0.01
$\Delta H°$	−33.2 ± 1 kJ/mol; SEF = 0.026; R = 1.00			
		30%		
δ_A	152.18	145.81	133.59	135.28 ± 0.2
δ_B	157.85	143.43	136.57	125.87 ± 0.3
δ_r	155.97	144.21	135.58	128.98 ± 0.01
$\Delta H°$	−34.7 ± 2 kJ/mol; SEF = 0.027; R = 1.00			
		40%		
δ_A	151.03	146.16	132.72	137.03 ± 0.7
δ_B	156.64	143.55	135.72	127.31 ± 0.9
δ_r	154.20	144.69	134.41	131.54 ± 0.01
$\Delta H°$	−25.4 ± 5 kJ/mol; SEF = 0.029; R = 0.9999			
		50%		
δ_A	150.49	146.49		138.14 ± 0.5
δ_B	154.71	143.90		129.89 ± 0.9
δ_r	152.75	145.10		133.71 ± 0.01
$\Delta H°$	−21.4 ± 4 kJ/mol; SEF = 0.019; R = 1.00			
		60%		
δ_A	149.93	147.08		139.79 ± 1
δ_B	153.49	143.76		130.81 ± 2
δ_r	151.74	145.39		135.22 ± 0.008
$\Delta H°$	−14.5 ± 5 kJ/mol; SEF = 0.017; R = 1.00			

[a] *Calculated values, see text. SEF = standard error of the fit.

of the straight lines resulting from the correlation of nuclei C-2, C-3, and C-4 with nucleus C-6 in all the studied solvent mixtures. As an additional graphical example, Figure 3 shows the plot of the chemical shifts for C-2, C-3, C-4, and C-5 versus the C-6 chemical shift in the 20% 1,4-dioxane/water mixture. The chemical shift values in Figure 3 are relative values with respect to the chemical shift at 293 K ($\Delta\delta = \langle\delta\rangle_T - \langle\delta\rangle_{293}$). It is therefore worth remembering that the intercepts and slopes given in Table 2 also relate the chemical shifts of the pure forms of a given carbon signal to the C-6 signal at a given temperature, as shown in eq 5.

Three parameters, δ_A, δ_B, and $\Delta H°$, are necessary to fit the experimental data for a given nucleus to eq 3, again using a single experimental datum as reference. The number of independent parameters in the multiple function curve fitting when considering four nuclei simultaneously (C-3, C-2, C-4, and C-6) is nine, eight for the chemical shifts of the pure forms and one for the enthalpy, since $\Delta H°$ must be the same in all cases. The use of the linear correlations mentioned above (eq 5) reduces the number of parameters markedly [for example, δ_A or δ_B (C-3) = (235.74 ± 0.5) − (0.617 ± 0.004)·(δ_A or δ_B)(C-6)] in the 20% 1,4-dioxane/water mixture (Table 2). Accordingly, six linear equations (two for each nucleus) were used as restricting conditions, and the total number of independent parameters to fit was reduced from nine to three. Furthermore, a single experimental datum at a given temperature (298 K, for instance) is required as the reference chemical shift in eq 3. Since this datum also satisfies the linear relation already presented, the C-6 chemical shift at 298 K, $\langle\delta_r\rangle_{298}$, was taken as the reference datum and was also used as a new adjustable parameter. In this way, the spread of the experimental error from the measurement of the reference chemical shift to the fitting process is avoided. In conclusion, the experimental chemical shifts for all four nuclei, C-2, C-3, C-4, and C-6, are used, and only four adjustable parameters are calculated in the fitting process; these are the C-6 chemical shifts of the limiting forms, the reference chemical shift, and the process enthalpy. From these values and the linear relations, the chemical shifts of C-3, C-2, and C-4 in their limiting forms and their chemical shifts at 298 K can be calculated.

The calculated values of δ_A, δ_B (the chemical shifts of the extreme forms), $<\delta_r>$ (chemical shift at 298 K), and $\Delta H°$ (the enthalpy of the tautomeric process) for all nuclei used in the fitting process are presented in Table 3. In addition, the standard errors for the C-3, C-2, C-4, and

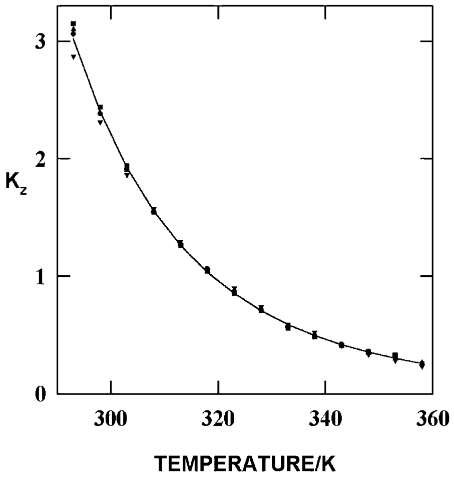

FIGURE 4. Tautomeric equilibrium constant versus temperature in 20% 1,4-dioxane/water mixture. Data obtained from the (●) C-3, (■) C-2, (▲) C-4, and (▼) C-6 signals using the parameters given in Table 3 (eq 2). Solid line from eq 8 and parameters given in Table 4.

C-6 experimental chemical shifts obtained from the multiple function curve fitting to eq 3 are also given. Similar calculations were carried out for all the 1,4-dioxane/water mixtures. The correlation coefficient and the standard error of the fit (SEF) are also included in Table 3 as criteria for the goodness of fit. The values for the chemical shifts of the pure forms of C-3, C-2, and C-4, together with the chemical shifts at 298 K given in Table 3, were calculated from the C-6 values and the intercept and slope of the linear correlations (Table 2). C-4 data have not been used in the calculations of 50% and 60% dioxane/water mixtures because the temperature effect on their chemical shifts is rather small (Figure 1). Comparison between the calculated chemical shift values at 298 K for every carbon with the measured values shows very good agreement. This result fully justifies the inclusion of the reference chemical shift as an adjustable parameter instead of using the measured value, since the use of the latter may introduce measurement error into the fitting. In addition, this excellent agreement is evidence of the accuracy of the values obtained from the calculation.

Tautomeric equilibrium constant values as a function of temperature were obtained from the data for C-3, C-2, C-4, and C-6 by using eq 2, and the parameters are given in Table 3. Figure 4 shows the K_z values obtained in 20% 1,4-dioxane/water. The plot clearly shows a high degree of coincidence with the results obtained from the different signals. In fact, it is difficult to distinguish between them over a broad range of temperature. Such a result confirms that the use of this method enables reliable thermodynamic data to be obtained from the ^{13}C NMR experimental results alone.

The chemical shifts of the pure forms and the enthalpy process have been considered temperature-independent in eqs 3 and 4. Although this assumption is not strictly exact, it can be done on the basis of several considerations. The excellent linear correlation between the experimental chemical shifts of any given pair of nuclei (eq 4), suggests that the pure form chemical shifts can be considered constant from a practical point of view. Moreover, a discussion about the temperature effect on the chemical shifts of the pure forms has been reported in a previous paper.[12] This effect would introduce a 0.1–0.2 ppm deviation for each chemical shift in the whole temperature range where measurements have been done. If this small error in the pure form chemicals shifts is assumed, i.e., $\Delta\delta_A$ and $\Delta\delta_B = \pm 0.2$, the maximum estimated error in K_z will also be ± 0.2. Thus, the calculated K_z values from the four different nuclei experimental data are coincident in the whole temperature range within a deviation error of the same order of the estimated one.

TABLE 4. Thermodynamic Characterization of the Tautomerization Equilibrium in Different 1,4-Dioxane/Water Mixtures[a]

% (v/v)	A	$\Delta H°$ (kJ/mol)	$\Delta S°$ (J/K·mol)	K_z (298 K)
0[b]	$2.12 \times 10^{-4} \pm 2 \times 10^{-5}$	-24.8 ± 0.2	-70.3 ± 0.8	4.67
10	$1.90 \times 10^{-5} \pm 2 \times 10^{-6}$	-29.1 ± 0.3	-89 ± 5	2.48
20	$3.73 \times 10^{-6} \pm 3 \times 10^{-7}$	-33.1 ± 0.2	-104 ± 3	2.38
30	$1.57 \times 10^{-6} \pm 1 \times 10^{-7}$	-34.8 ± 0.2	-111 ± 5	2.04
40	$4.36 \times 10^{-5} \pm 4 \times 10^{-6}$	-25.5 ± 0.2	-83 ± 9	1.30
50	$2.13 \times 10^{-4} \pm 1 \times 10^{-5}$	-21.3 ± 0.2	-70 ± 7	1.17
60	$3.01 \times 10^{-3} \pm 1 \times 10^{-4}$	-14.5 ± 0.1	-48 ± 9	1.03

[a] Parameters and standard errors obtained by fitting the calculated K_z values from C-3, C-2, C-4, and C-6 to the equation $K_z = A\,e^{-\Delta H°/RT}$. $\Delta S°$(J/K·mol) and K_z values at 298 K. [b] Data taken from ref 12.

All K_z values obtained for the four nuclei were adjusted to the equation

$$K_z = A\,e^{-\Delta H°/RT} \tag{8}$$

in order to obtain an empirical equation for K_z versus temperature for each dioxane/water mixture. The resulting parameters are shown in Table 4. The parametrized eq 8 allows the calculation of the equilibrium constant at any temperature and solvent. Accordingly, the solid line in Figure 4 is a plot of this function. It can be seen that the $\Delta H°$ values obtained from this calculation (Table 4) using the equilibrium constants agree very well with those obtained in the general fitting calculation (Table 3).

The values of the tautomerization equilibrium constant decrease with temperature in any given mixture, as one would expect for any equilibrium with a negative value for $\Delta H°$. Furthermore, the values at a given temperature diminish when the dioxane percentage in the mixture increases. This trend can be seen from the data obtained at 298 K (Table 4). Such behavior is logical since, intuitively, an increase in the percentage of the most apolar solvent of the mixture should lower the amount of the zwitterionic form of pyridoxine.

The standard entropy change, $\Delta S°$, was obtained from the tautomeric constant values at 298 K (Table 4). This magnitude has a negative value in all of the dioxane/water mixtures, a situation that is consistent with this kind of equilibrium, as a result of the increased order of the solvent caused by the dipolar form in all cases. The value of $\Delta G°$ is rather small, very close to zero, since the tautomerization equilibrium constants have small values that approach 1. Consequently, the enthalpic and entropic terms counterbalance each other. This behavior becomes more noticeable when the amount of dioxane in the mixture is increased. The values of $\Delta H°$ and $T\Delta S°$ are negative, and both have a maximum absolute value in the 30% dioxane/water mixture.

For this equilibrium an approximate value of $\Delta H° = -10$ kJ/mol, which is practically constant in all of the 1,4-dioxane/water mixtures,[31] was obtained by resolution of UV−visible spectra in log-normal curves, a method characterized by rather severe assumptions. In this work, values ranging from −14.5 kJ/mol (60% dioxane) to −34.8 kJ/mol (30% dioxane) were obtained (Table 4). Both values are calculated using different methodologies that have very different approximations. We consider that the results presented in this paper are more reliable as a result of the large number of highly consistent experimental data and the numerical data treatment and definitely because all nuclei led to the same result.

In the same way, we carried out a multiple function curve fitting of the signals for C-3, C-2, C-4, and C-6 using eq 6. This equation makes use of the chemical shifts in the same way as eq 3 but, in addition, requires data for the chemical shift derivative with temperature. This derivative was calculated through experimental data fitting to a second- or third-order polynomial (for an example see Table 1) and further mathematical derivation. Equation 6 does not require a reference experimental datum. This is an advantage because the number of fitting parameters decreases by one. In contrast, the use of eq 6 can introduce error into the derivative calculation. The results from eq 6 are very similar to those obtained from eq 3 (Table 3) in all mixtures except the 40% and 50% dioxane/water systems, where small discrepancies arise. These differences probably are a consequence of the method used for the calculation of the derivative values.

The solvent effects were characterized using the multiparameter approach introduced by Kamlet and Taft.[19−21] This method was applied to the $pK_z(s)$ data for the tautomerization process in the different 1,4-dioxane/water mixtures. A slightly modified version of eq 7 was used to explain the dependence of the pK_z values as a linear combination of the microscopic parameters of the solvent (π^*, α, and β):

$$pK_z(s) = pK_{zo} + s(\pi^* - 1.15) + a(\alpha - 1.08) + b(\beta - 0.41) \quad (9)$$

where pK_{zo} is the pK_z in pure water. The numerical parameters in eq 9 are the corresponding parameters for pure water. Multiple regression analysis was performed using all the possible combinations of the solvatochromic parameters. The standard error of the fitting and an *F*-test were used as criteria to determine the number and type of parameters that were statistically significant in each case. In this way eq 10 was obtained:

$$pK_z(s) = (-0.629 \pm 0.05) - (0.34 \pm 0.3)(\pi^* - 1.15) - (1.04 \pm 0.3)(\alpha - 1.09) \quad (10)$$

where $R = 0.976$ and the standard error of the fit = 0.062. The parameters π^* and α for each mixture decrease as the amount of dioxane in the mixture increases. The effect of this increase in pK_z can be considered as the sum of two different effects: the dipolarity and polarizability of the solvent and the ability of the solvent to act as a hydrogen-bond donor toward a solute. The ratio $a/s \approx 3$ shows that the effects of solvent on the pK_z values are mainly due to the HBD term. It is not necessary to use the three solvatochromic parameters given in eq 9. Similar fittings were obtained using only two parameters: π^* and α or β.

Similar relationships were also obtained for the solvent effect on the experimental chemical shift values for each nucleus. For instance, the chemical shift of C-6 at 298 K follows eq 11:

$$\langle\delta\rangle_{C-6}(s) = (125.00 \pm 0.3) - (29.6 \pm 4)(\pi^* - 1.15) - (7.1 \pm 2)(\alpha - 1.09) \quad (11)$$

where $R = 0.9956$ and the standard error of the fit = 0.43. The solvent effect on the C-6 chemical shifts can be understood in terms of this equation and is also the sum of the aforementioned two effects. Similar behavior was observed for the remaining nuclei.

As indicated in our previous article,[12] the method used here is mainly limited by two factors: (1) the narrow temperature range that is experimentally accessible and (2) the value of $\Delta H°$ itself. As far as the second limitation is concerned, it is clear that to quantify a thermically induced process with sufficient accuracy depends on the $\Delta H°$ value of the process in question. If the nature of the system does not allow direct measurement of the chemical shifts of the pure forms, it is necessary to use optimization procedures in order to estimate the thermodynamic and NMR parameters. The accuracy and reliability of the results can be markedly improved when the results are analyzed with the multiple function curve fitting technique and the relationships between the chemical shifts of the pure forms obtained from different nuclei are used as restriction conditions.

For the application of this method to the thermodynamic characterization of tautomeric processes, it is recommended that a large number of experimental data that are sensitive to the equilibrium should be obtained, and therefore it is suggested that measurements are carried out on different nuclei. However, in our example, the results obtained from the ^{13}C NMR signals alone are reliable enough, and it is not necessary to carry out ^{15}N NMR variable temperature measurements. Nevertheless, we did carry out some ^{15}N NMR chemical shift measurements on selected mixtures (results not shown), and the fitting results were fully coincident with the ones obtained from ^{13}C NMR spectroscopy.

In conclusion, consistent results were obtained with the ^{13}C NMR spectra alone, and it was not necessary in this case to measure the ^{15}N NMR chemical shifts. This conclusion is valid only in systems where the enthalpy of the process is sufficiently high; when the enthalpy is low, it would probably be necessary to obtain experimental results from both techniques together.

Acknowledgment. J.L. thanks the Junta de Andalucia (FQM-171) for financial support. Financial support from Xunta de Galicia (PGIDIT03PXIC30107PN) and Ministerio de Ciencia y Tecnología (BQU2002-02807) is also acknowledged. E.V. is grateful to the Xunta de Galicia for a fellowship.

JO0494533

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