The Equilibria of 5-Deoxypyridoxal in Water–Dioxane Mixtures

Jose M. Sánchez-Ruiz, Salvador Asensio, Juan Llor, and Manuel Cortijo *
Departamento de Química Física, Facultad de Ciencias, Universidad de Granada, 18071 Granada, Spain

The electronic absorption spectra of 5-deoxypyridoxal have been obtained together with its macroscopic pK values in water–dioxane solution at 25 °C. These spectra have been resolved using log-normal curves, enabling the determination of the spectra of the formyl, hydrated, and tautomeric forms. The proportions of these forms could thus be ascertained and numerical values for the hydration and tautomeric equilibrium constants obtained. The microscopic protonation constants have been calculated from these equilibrium constants and from the macroscopic pK values, which were determined potentiometrically. The variation of these equilibrium constants according to solvent polarity fits Marshall's model satisfactorily.

We have recently studied the influence of solvent polarity on the tautomerism and ionization constants of pyridoxine, the simplest member of the vitamin B_6 group.[1] We have been able to calculate true thermodynamic constants which can be fitted to the Marshall model.[2] The interactions through the pyridine ring have been shown to be very important in pyridoxine, and the probability of the 'switching mechanism' postulated by Ivanov and Karpeisky,[3] may increase as the media polarity decreases.[1] Thus it would appear interesting to follow up our previous studies[4] with more complex vitamin B_6 molecules such as pyridoxal or pyridoxal 5′-phosphate. Nevertheless we preferred at this juncture to study a related molecule with fewer complications, such as 5-deoxypyridoxal. This molecule is a very good vitamin B_6 model as it possesses the three chemical groups which are considered to be fundamental to catalytic activity (CH=O, OH, =N–) but does not involve the complication of the formation of hemiacetal as does pyridoxal.

In this report we describe the influence of the solvent polarity on the thermodynamic equilibrium constants of ionization, tautomerization, and hydration of 5-deoxypyridoxal in water–dioxane mixtures at 25 °C.

Experimental

The materials and the purification methods used have been described previously.[1,5] The measurement of pH and the consequent determination of the macroscopic protonation constants in water–dioxane mixtures are the same as those previously used.[1,5,6] All measurements were made at 25 ± 0.1 °C and at low ionic strength, allowing us to estimate the activity coefficients as previously described.[1,5,6] The concentrations of the stock solutions of 5-deoxypyridoxal were calculated from their absorption at 390 nm after dilution in 0.1M-NaOH (ε 6 300).[7]

The u.v.–visible spectra were recorded with a Cary 210 spectrophotometer and they were resolved by the weighted least-squares minimization computer program, developed by Metzler *et al.*[8,9]

Results and Discussion

5-Deoxypyridoxal has two protonable groups and therefore shows two macroscopic pK values (Scheme). Owing to the existence of the hydration reaction of the formyl group, twice the number of ionic forms exist in this molecule than in pyridoxine or 3-hydroxypyridine.[1] The macroscopic pK values obtained are given in Figure 1. These values are the average of at least three independent measurements, using different initial concentrations of 5-deoxypyridoxal (5—15mM). The mean deviations are always equal to or less than 0.03. The pK values in water (4.00 and 8.15) are close to those reported at ionic strengths of I = 0.2 (4.15 and 8.05)[9] and 0.1 (4.17 and 8.14)[10] and the differences can easily be explained on considering the activity coefficients at these ionic strengths. We could not find values for pK values of this compound in dioxane–water mixtures or thermodynamic pK values in water in the literature. The two pK values are well separated in all the mixtures studied and thus, by simply controlling the pH of the medium, it is possible to have a single ionic species in solution (all those forms given in a horizontal line in the Scheme).

The cationic species (PH_2^+) shows two ionic forms, aldehyde and hydrated, and the corresponding hydration equilibrium constants in each media can be evaluated from their u.v.–visible spectra by the method we have described to calculate the tautomeric equilibrium constants of pyridoxine and 3-hydroxypyridine.[1] The low-energy region of the electronic spectra of 5-deoxypyridoxal shows two bands at *ca.* 29 500 and 34 000 cm^{-1} which can be ascribed to the aldehyde and hydrated forms respectively (see Scheme).

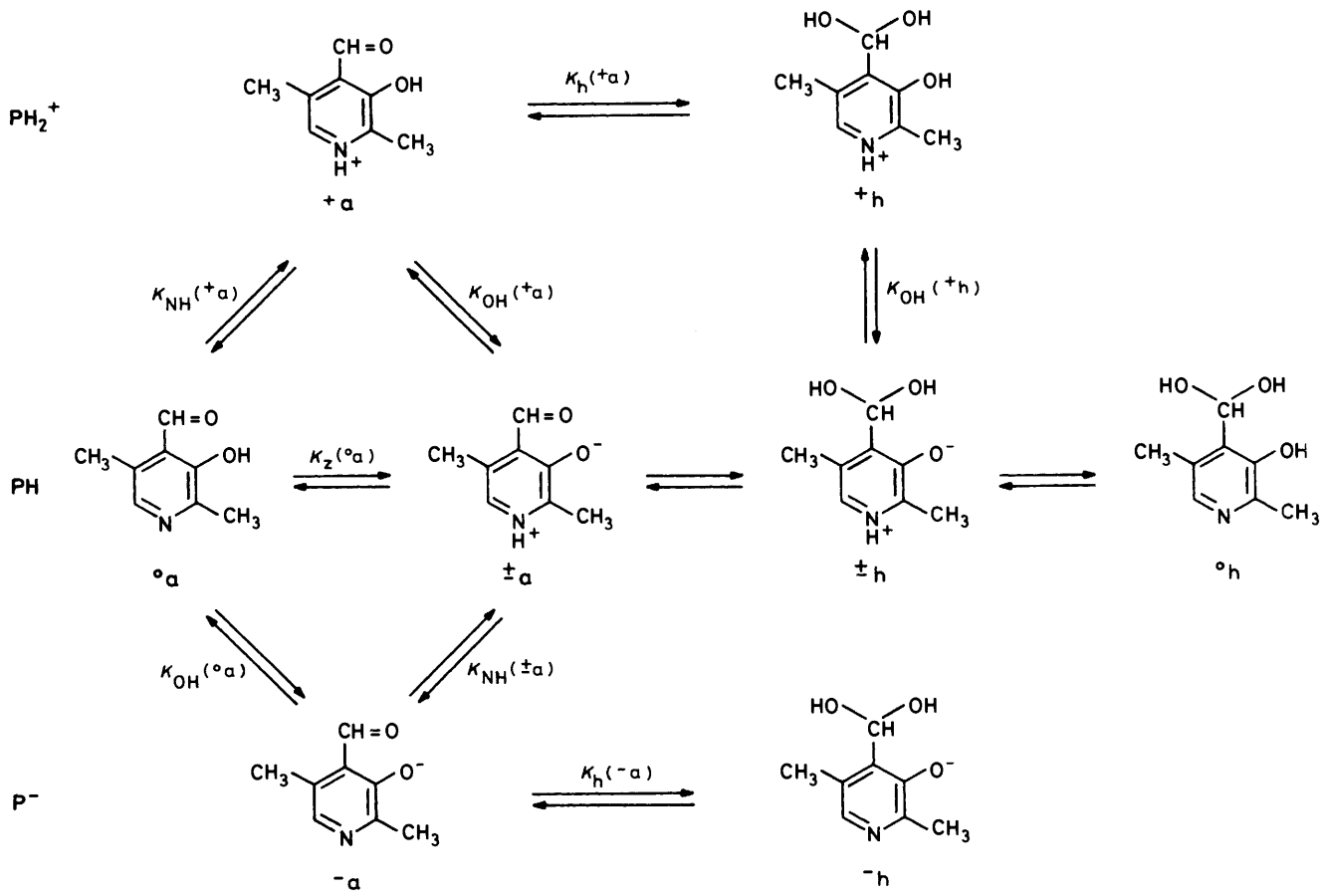
The computer resolution and fitting of the spectra to log-normal curves (see Experimental section and refs. 8 and 9) leads to the integrated intensities given in Table 1. The molar areas $a^\circ(^+a)$ and $a^\circ(^+h)$ are determined by fitting equation (1) to the

$$a(^+h) = a^\circ(^+h) - \frac{a^\circ(^+h)}{a^\circ(^+a)} \cdot a(^+a) \qquad (1)$$

data and the hydration constants can then be calculated. In order to decrease the experimental error not only the data given in Table 1 but those obtained at other temperatures (10—50 °C) have been used for this adjustment. All data can be fitted to a single straight line with a correlation index of 0.814, which indicates that the molar areas remain constant with changes both of temperature and solvent composition and confirms previous results.[5,8,9] The values obtained for the molar areas and their standard deviations are $a^\circ(^+h)$ 292 ± 9 and $a^\circ(^+a)$ 340 ± 66 Mm mol^{-1}. These values compare quite well with those given by Metzler *et al.* (300 and 350, respectively), which were obtained by analogy with similar compounds and some assumptions.[9]

The hydration constants (also given in Table 1) have been calculated by using equation (2).

$$K_h(^+a) = \frac{a(^+h)/a(^+a)}{a^\circ(^+h)/a^\circ(^+a)} \qquad (2)$$



Scheme.

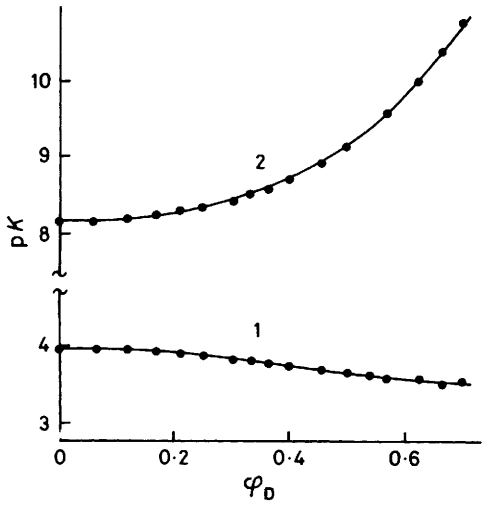


Figure 1. Macroscopic ionization constants (pK_1 and pK_2) of 5-deoxypyridoxal in dioxane–water at 25 °C where φ_D indicates volume fraction of dioxane

Metzler *et al.*[9] give two values for this constant in water, 2.8 and 2.2. The first one is considered by them to be less reliable but it is nevertheless closer to our value in water (2.8 ± 0.3). The calculated hydration constants slightly but definitively increase as the water content in the media decreases, except at very high dioxane contents. We are confident of the reliability of this result as it also occurs in water–*NN*-dimethylformamide mixtures[11] and with other vitamin B_6 compounds.[12]

Table 1. Areas (in Mm mol^{-1}) under bands I of the aldehyde [$a(^+a)$] and hydrated [$a(^+h)$] forms and the hydration equilibrium constants [$K_h(^+a)$] for the cationic species of 5-deoxypyridoxal in dioxane–water mixtures at 25 °C

φ_D(v/v)	$a(^+h)$	$a(^+a)$	$K_h(^+a)$
0	214.5	88.2	2.83 ± 0.30
0.2	221.5	77.2	3.34 ± 0.35
0.4	234.8	71.8	3.81 ± 0.40
0.6	236.0	68.4	4.02 ± 0.42
0.7	228.1	71.4	3.72 ± 0.39
0.8	217.6	80.4	3.15 ± 0.33
0.9	191.5	114.0	1.96 ± 0.21
$a°(^+h)$ = 292 ± 9		$a°(^+a)$ = 340 ± 66	

We were able to study the anionic species (Scheme) in a similar way, but the low percentage of the hydrated form and the small variation of this hydration equilibrium constant with both solvent and temperature prevented us from obtaining reliable values for the molar areas of the aldehyde and hydrated forms and therefore we do not report here the hydration equilibrium constants for this species. By using the molar area values estimated by Metzler *et al.*,[9] we found that these equilibrium constants were between 0.06 and 0.09 in all the conditions studied.

The 5-deoxypyridoxal species with a net zero charge is a mixture of four tautomeric aldehyde and hydrated forms in equilibrium (see Scheme). The electronic spectra were thus very complex and we were only able to resolve them unequivocally in the band I region (20 000—37 000 cm^{-1}), by using five log-

Table 2. Integrated intensities (in Mm mol^{-1}) of the bands I for the several forms of the net zero charge species of 5-deoxypyridoxal in dioxane–water mixtures at 25 °C and the tautomeric constants [K_z(°a)] for this compound (see Scheme)

φ_D	a(°a)	a($^{\pm}$a)	a($^{\pm}$h)	K_z(°a)
0	74.1	147.5	76.7	1.65
0.1	91.7	130.3	58.3	1.18
0.2	109.7	109.2	42.0	0.82
0.3	130.7	86.4	29.8	0.55
0.4	151.8	63.4	18.5	0.35
0.5	168.0	40.9	12.5	0.20
0.6	180.2	23.5	8.9	0.11
0.7	189.3	14.9	5.4	0.065

a°(°a) = 216 ± 19 a°($^{\pm}$a) = 261 ± 37 a°($^{\pm}$h) = 350 ± 140

normal curves corresponding to: (1) band I of $^{\pm}$a (*ca.* 25 000–25 500 cm^{-1}); (2) band I of °a (28 000 cm^{-1}); (3) band I of $^{\pm}$h (31 000 cm^{-1}); (4) band I of °h plus band II of $^{\pm}$a (35 000 cm^{-1}) which could not be resolved in both bands; (5) low-energy region of band II of °a. Therefore we have not attempted to take the uncharged hydrate form (°h) into account in the spectral resolution but rather give only the integrated intensities of the two aldehyde forms (with experimental error *ca.* 5%) and those of the dipolar hydrated form (with larger error due to its lower proportion) in Table 2.

Metzler *et al.*[9] ignore the existence of the neutral hydrated form due to its low percentage in water (they estimate that it was <7%).[9] This situation might however be different in solutions with a high dioxane content and we cannot rule out *a priori* the existence of this form in solution.

In order to evaluate the molar area, a°, of each form we carried out three approximations of increasing complexity. First, we assume that the hydrated forms were only present in negligible proportions in solution. This situation is then similar to the one we have just encountered for the cationic species or for pyridoxine[1] and a correlation index of 0.91 in the fit and values of 187 ± 4 and 231 ± 9 Mm mol^{-1} for the molar areas, a°, of the forms °a and $^{\pm}$a respectively were obtained. Very similar results and errors were arrived at when data at other temperatures were included in the fit. This drastic simplification leads to an increase in the values of both the aldehyde forms and therefore underestimates the molar areas and thus we obtain minimum values for these two molar areas.

Secondly, we considered that the proportion of the neutral hydrate, °h, was always negligible. The total molar fractions must be unity, or equation (3), which can be rearranged as

$$1 = \frac{a(^{\pm}\text{a})}{a^{\circ}(^{\pm}\text{a})} + \frac{a(^{\circ}\text{a})}{a^{\circ}(^{\circ}\text{a})} + \frac{a(^{\pm}\text{h})}{a^{\circ}(^{\pm}\text{h})} \quad (3)$$

(4), must hold.

Equation (4) has the form (5) where a, b, and c are the

$$a(^{\pm}\text{a}) = a^{\circ}(^{\pm}\text{a}) - \frac{a^{\circ}(^{\pm}\text{a})}{a^{\circ}(^{\circ}\text{a})}a(^{\circ}\text{a}) - \frac{a^{\circ}(^{\pm}\text{a})}{a^{\circ}(^{\pm}\text{h})}a(^{\pm}\text{h}) \quad (4)$$

$$z = a - bx - cy \quad (5)$$

unknowns, which can then be obtained by fitting the experimental data given in Table 2 (and those obtained at other temperatures) to a multivariant least-square method. A correlation index of 0.92 and values of 184 ± 3, 311 ± 30, and 460 ± 115 Mm mol^{-1} for the molar areas of the forms °a, $^{\pm}$a, and $^{\pm}$h, respectively, were obtained. By analogy with molecules with similar structures Metzler *et al.*[9] estimate two sets of values for the three molar areas. Their more reliable values (224, 404, and 352, respectively) are very different from ours. This could be due to the fact that we have only considered three forms in our calculations: dipolar ionic aldehydes, uncharged aldehydes, and dipolar ionic hydrates.

A third and perhaps more correct method is to estimate the proportion of the °h form and then to include it in equation (3). It can be evaluated from the molar fractions values for the form $^{\pm}$h simply by dividing these values by K_z (°h) for each solvent composition (see Scheme). The values for these tautomeric constants can be estimated from an interesting empirical relationship that we have recently discovered from studying 3-hydroxypyridine derivatives.[4] The plot of log K_z for a given vitamin B_6 compound [or log (dipolar form area/neutral form area)] *versus* log K_z for a model compound (we have used pyridoxine) is a straight line with a slope close to 1, that is equations (6) and (7) hold.

$$\log K_{z1} = n \log K_{z2} + a \quad (6)$$

$$K_{z1} = mK_{z2}^{n} \quad (7)$$

Equation (8) therefore can be obtained from equations (4)

$$a(^{\pm}\text{a}) = a^{\circ}(^{\pm}\text{a}) - \frac{a^{\circ}(^{\pm}\text{a})}{a^{\circ}(^{\circ}\text{a})}a(^{\circ}\text{a}) - \frac{a^{\circ}(^{\pm}\text{a})}{a^{\circ}(^{\pm}\text{h})}a(^{\pm}\text{h})\left(1 + \frac{1}{mK_z^{n}}\right) \quad (8)$$

and (7) where K_z values for pyridoxine at each of the solvent compositions were taken from ref. 1 and m and n are two new adjustable parameters. The results now obtained are: a°(°a) 216 ± 19; a°($^{\pm}$a) 261 ± 37 and a°($^{\pm}$h) 350 ± 140 Mm mol^{-1}; m 3.0 ± 6.6 and n 1.05 ± 0.67.

These a° values are very similar to those given by Metzler *et al.*[9] except for that ascribed to the dipolar aldehyde form. This value was established by these authors by making several assumptions which may involve large errors. Their value for the same form of 5-deoxypyridoxal *N*-methochloride (310)[9] is closer to ours and, being based upon different assumptions, is perhaps more reliable.

The molar fractions of each of these forms, which have been calculated from these latter values of a° and those given in Table 2, are presented in Figure 2. The molar fractions of the uncharged hydrate from (°h) were calculated from the molar fractions of the dipolar hydrate form ($^{\pm}$h) and from the corresponding tautomeric constants (K_{z1}) obtained by using equation (7) (m 3.0; n 1.05; and K_{z2} are the K_z values of pyridoxine taken from ref. 1). These K_z values might err by as much as 100%, although they do not give rise to significant errors in later calculations given the low percentage of this uncharged hydrate form in all the media we have studied. The hydration constant, $K_h(^{\pm}\text{a}) = {}^{\pm}\text{h}/{}^{\pm}\text{a}$, slightly decreases as the dioxane content increases, although the large experimental errors for dioxane contents >40% make these latter values unreliable. The value in water of 0.39 is similar to that estimated by Metzler *et al.*,[9] 0.57. The tautomeric constants, K_z(°a), for the aldehyde form, shown also in Table 2, have less experimental error. The value given by Metzler *et al.*[9] for this constant in water solution (1.01) is less reliable, in our opinion, than ours, due to their uncertainties in the a° values mentioned above.

From these data and the macroscopic pK values given in Figure 1 we obtained the microscopic pK values given in Table 3. The values given in Table 3 are those calculated with uncertainties < 0.15. These values cannot be fitted to the Born model because the plots of these pK values *versus* the inverse

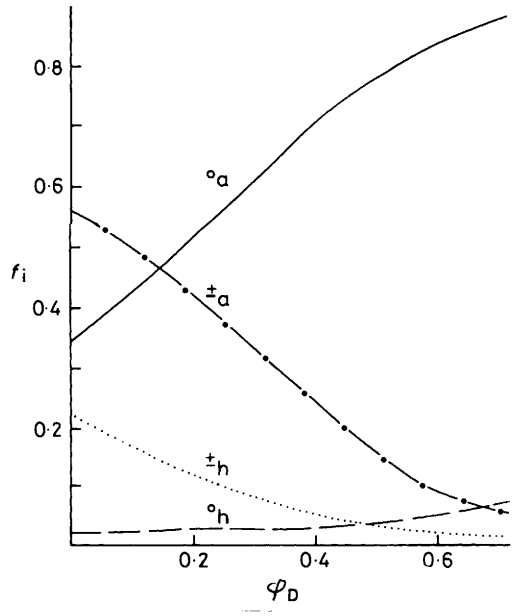


Figure 2. Molar fractions of the four molecular forms for the species with net zero charge of 5-deoxypyridoxal (see Scheme) in dioxane–water at 25 °C. φ_D indicates volume fraction of dioxane

of the dielectric constants of the media are markedly curved (not shown). They can, however, be fitted to the Marshall model by using the logarithm of water molar concentration in the medium, log c_w, as a solvent parameter. The slopes ($-k$) of the plots of pK *versus* log c_w are also given in Table 3 and they appear to be related to the value of the corresponding pK in water for each type of ionization. The logarithms of the tautomeric equilibrium constants, K_z, can be expressed as the differences between two microscopic pK values (see Scheme) and therefore they fit also the Marshall model. The pK values in water are a function of the corresponding substituent constants in the Hammett equation and thus the k and log K_z values must also be a function of these substituent constants, which explains relationships such as those indicated in equations (6) or (7) in this work.

One last question that remains to be answered concerns the physical meaning of the k values of the Marshall model. Let us consider the deprotonation reaction (9) of a given acid where

$$AH + k\,H_2O \rightleftharpoons A^- + H^+ \qquad (9)$$

k is the assumed average net change in the number of water molecules of solvation during the reaction. A 'complete' equilibrium constant, K°, is then defined by equation (10) where K°

$$K^\circ = \frac{[A^-][H^+]}{[AH][H_2O]^k} = \frac{K}{[H_2O]^k} \qquad (10)$$

and k vary only with temperature.[2] In this model, the activity of water is defined as being equivalent to its analytical molarity. The standard states are therefore hypothetical 1 M solutions for all reacting species. Were this true, relationship (11) or (12)

$$\ln\frac{K^\circ(s)}{K^\circ(w)} = -\frac{1}{RT}[\Delta G_t^\circ(A^-) + \Delta G_t^\circ(H^+) - \Delta G_t^\circ(AH) - k\Delta G_t^\circ(H_2O)] = 0 \qquad (11)$$

$$\Delta G_t^\circ(A^-) + \Delta G_t^\circ(H^+) = \Delta G_t^\circ(AH) + k\Delta G_t^\circ(H_2O) \qquad (12)$$

Table 3. Microscopic ionization constants of 5-deoxypyridoxal in dioxane–water at 25 °C (see Scheme). k Values were obtained by fitting the pK values to the Marshall model: log K = log K° + k log c_w. All data were used in the fitting for phenols, and the data with φ_D equal to or lower than 0.4 in the fitting for pyridinium ions. r, Correlation coefficient. The figure alongside k indicates the 90% confidence limit.

φ_D	$pK_{NH}(^+a)$	$pK_{OH}(^+a)$	$pK_{OH}(^+h)$	$pK_{NH}(^\pm a)$	$pK_{OH}(^-a)$
0.000	3.91	3.70	4.52	7.94	7.72
0.063	3.82	3.70	4.58	7.91	7.78
0.118	3.74	3.70	4.64	7.92	7.88
0.167	3.68	3.71	4.71	7.93	7.96
0.211	3.60	3.70	4.73	7.95	8.05
0.250	3.54	3.71	4.77	7.95	8.12
0.302	3.45	3.71	4.82	7.98	8.25
0.333	3.39	3.71	4.85	8.02	8.35
0.362	3.33	3.71	4.88	8.07	8.45
0.4	3.26	3.72	4.93	8.13	8.59
0.455	3.17	3.76		8.24	8.83
0.5	3.12	3.82		8.38	9.08
0.538	3.05	3.85		8.51	9.31
0.571	3.02	3.91		8.64	9.53
0.625	3.00	4.02		8.94	9.97
0.667	2.95	4.07		9.24	10.36
0.7	2.97	4.16		9.56	10.75
k	−3.03	0.86	1.85	0.90	5.76
	± 0.06	± 0.1	± 0.09	± 0.16	± 0.2
r	0.998	0.926	0.990	0.893	0.991

between the free energies of transfer of the molecules involved in the process should hold good. K°(s) And K°(w) are the 'complete' equilibrium constants in a solvent mixture and in water respectively and ΔG_t°(X) the free energy of transfer of X from water to the solvent s. Ben-Naim[13] has argued that free energies of transfer in the molar scale alone can be regarded as being differences between the solvation free energies in the two solvents involved in the transfer process. This means that, according to equation (12), the k water molecules interchanged in the reaction should have the same structure, or at least the same interactions with the surrounding water molecules, when they are solvating the chemical species as when they are free, which is difficult to believe. Perhaps more likely is that this number, k, merely represents a numerical value necessary to balance both sides of equation (12) and is a consequence of the relative compensation of various competing effects. It would in fact appear that k has no real structural significance as those systems measured in several mixture solvents show different k values.[11]

In conclusion we have measured the hydration and tautomeric equilibrium constants of 5-deoxypyridoxal in water–dioxane mixtures at 25 °C. The corresponding microscopic pK values of this substance in the above mentioned media have been calculated from these equilibrium constants and from the macroscopic pK values, which were determined potentiometrically.

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