Formation of Schiff Bases of 5′-Deoxypyridoxal and Hexylamine in Aqueous and Non-aqueous Media

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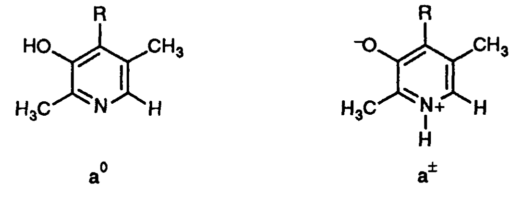
We have studied the formation of Schiff bases of 5′-deoxypyridoxal (DPL) and hexylamine in various non-aqueous solvents (dioxane, pentanol/dioxane mixtures, pentanol, butan-2-ol, propanol, ethanol, methanol and ethane-1,2-diol) at 25 °C. The results are interpreted in terms of the tautomer forms of DPL present in the medium, and the rate constant of formation of the Schiff base has been obtained for the neutral species of DPL and the zwitterionic form ($k_{1_n} = 1.7 \times 10^2$ dm^3 mol^{-1} min^{-1} and $k_{1_z} = 3.6 \times 10^3$ dm^3 mol^{-1} min^{-1}, respectively). We also determined the constant of the tautomeric equilibrium between the corresponding forms of the uncharged species of DPL in the different solvents. These results and those obtained in water/dioxane media indicate that the intramolecular acid catalysis involved in the formation of Schiff bases is also governed by the occurrence of a charged transition state.

Pyridoxal-5′-phosphate (PLP) takes part in a wide variety of biological processes, particularly those involved in amino-acid metabolism.[1] In every PLP-dependent enzyme studied so far, PLP is bound to the ε-amino group of an L-lysine residue of the polypeptide chain *via* its carbonyl group, forming a Schiff base (aldimine), which exists in a fairly hydrophobic medium.[2,3]

5′-Deoxypyridoxal (DPL) is a close analogue of PLP as it possesses the three chemical functions involved in the catalysis of amino-acid metabolism (–CH=O, –OH, =N–) as well as different protonatable groups and tautomeric forms (see Scheme 1).[4,5]



R = —CHO	DPL
R = —CHOH-NH—(CH$_2$)$_5$-CH$_3$	Carbinolamine (DPL + hexylamine)
R = —CH=N—(CH$_2$)$_5$-CH$_3$	Schiff base (DPL + n-hexylamine)

Scheme 1

Earlier studies have been made of the formation, hydrolysis and stability of the Schiff bases formed by PLP and DPL with hexylamine in aqueous media.[6–9] We have investigated also the influence of the solvent polarity on intramolecular catalysis in the formation of the Schiff bases of PLP and hexylamine in water/ethanol media[10,11] and those of DPL and hexylamine in water/dioxane media.[12] The results obtained showed that solvent composition plays a major role in the process and prompted further investigations using different non-aqueous solvents, both alone and as mixtures.

This paper discusses the influence of the polarity and the composition of non-aqueous media on the formation and stability of the Schiff bases of 5′-deoxypyridoxal and hexylamine at 25 °C. The results obtained are compared with those reported for the Schiff bases of PLP and DPL. The non-aqueous media used were as follows: dioxane, pentanol/dioxane mixtures, pentanol, butan-2-ol, propanol, ethanol, methanol and ethane-1,2-diol. Scheme 1 shows the tautomeric forms of the uncharged species of DPL in these media. Since reactivity might vary from form to form, we determined the proportion of each tautomer, *i.e.* the tautomeric equilibrium constant, in the different media used (methanol, ethanol, propanol, butan-2-ol, ethane-1,2-diol, pentanol, pentanol/dioxane mixtures and dioxane) at 25 °C.

Experimental

Materials.—5′-Deoxypyridoxal was synthesized from pyridoxine hydrochloride (Merck) according to Iwata. The correct analytical values for C, H and N are obtained. (Found: C, 63.25; H, 6.0; N, 8.85. Calc. for $C_8H_9NO_2$: C, 63.58; H, 5.96; N, 8.89). The infrared and ultraviolet spectra agree with that published previously.[13] All other reagents used were Merck reagent-grade chemicals.

Dioxane was purified by distillation over sodium under reflux and subsequent fractional distillation. Freshly distilled dioxane always gave a negative peroxide test (2% KI).

Ethanol was purified over sodium and ethyl succinate under reflux, after which it was distilled and stored over a molecular sieve, 4 Å. Methanol was purified by the same procedure, and propanol, butan-2-ol, pentanol and ethane-1,2-diol were dried over calcium hydride and subsequently treated like ethanol.

Methods.—DPL solutions were made daily and were stored in the dark. Their exact concentration was determined from their absorbance at 395 nm (ε = 64000 dm^3 mol^{-1} cm^{-1}) after dilution in 0.1 mol dm^{-3} NaOH.[14] The working concentrations ranged between 1×10^{-5} and 5×10^{-5} mol dm^{-3}. The hexylamine solutions were also prepared daily from hexylamine hydrochloride. The working concentrations ranged between 5×10^{-5} and 5×10^{-2} mol dm^{-3}.

The reaction was started by adding between 0.2 and 1.6 ml of

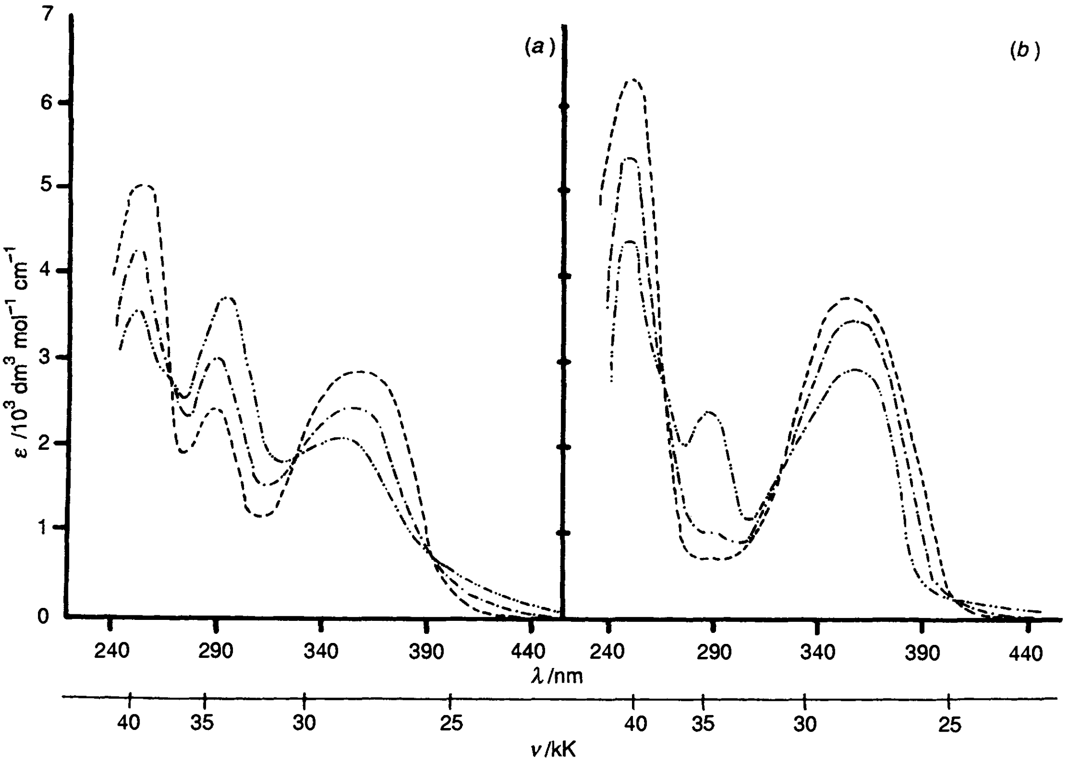


Fig. 1 Spectra of 5′-deoxypyridoxal in different non-aqueous solvents: (*a*) — — —, propanol; —·—·—, methanol; ··—··—, ethane-1,2-diol; (*b*) — ——, dioxane; —·—·—, dioxane–pentanol (50:50); —··—··—, pentanol

the hexylamine solution to a cell containing a thermostatically controlled solution of DPL in the same solvent. The concentration of the hexylamine was normally 50–1000 times higher than that of the DPL. The reaction was then monitored by measuring the absorbance changes at 340 nm using a Zeiss DMR11 spectrophotometer. Cells of 1.0 cm path length were used in all experiments and the temperature was kept at 25.00 ± 0.05 °C throughout.

The overall reaction between an aldehyde and an amine can be shown as in Scheme 2. Taking into account the Beer–Lambert

$$R^1\text{–CHO} + NH_2\text{–}R^2 \underset{k_2}{\overset{k_1}{\rightleftharpoons}} R^1\text{–CH=N–}R^2 + H_2O$$

Scheme 2

law, the rate equation of this process will be eqn. (1) where k_{obs} is given by eqn. (2). The parameters *a* and *b* are the initial

$$\ln\frac{A_\infty - A_0}{A_\infty - A} = -\ln\frac{ab - xx_e}{x_e^2} + k^t_{obs} \qquad (1)$$

$$k_{obs} = \{[k_2 + k_1(a + b)]^2 - 4\,abk_1^2\}^{\frac{1}{2}} \qquad (2)$$

concentrations of the aldehyde and the primary amine; x and x_e the concentrations of the Schiff base at time t and ∞; A_0, A and A_∞ are the absorbances at time 0; t and ∞; and k_1 and k_2 the overall rate constants of formation and hydrolysis of the Schiff base, respectively. Eqn. (2) includes the pseudo-first order expression for the reaction ($k_{obs} = k_2 + k_1b$) and $b \gg a$.

The constant k_{obs} was calculated from the slope of the plot of $\ln(A_\infty - A)$ *vs.* t since, in every experiment, $ab \gg xx_e$. The correlation coefficient obtained in the fittings was always $\geqslant 0.999$. The rate constants k_1 and k_2 were calculated from the k_{obs} values obtained at different $(a + b)$ values. Finally, the equilibrium constant, K_{pH}, was calculated as the k_1/k_2 ratio.

Fig. 1 shows the spectra given by DPL in the different media used over the wavelength range 500–250 nm. Absorbances were measured to within 0.001 units. The reference call was always filled with the same ingredients, omitting DPL.

Scheme 1 shows the tautomers of DPL present in each of the media used, where a^0 and $a^\pm$ denote the neutral and ionic dipolar (zwitterionic) forms of the aldehyde, respectively. The zwitterionic form of DPL gives three bands, centred at 389 (band I), 287 (band II) and 220 nm (band III), respectively. The neutral form also gives three bands, centred at 356 (band I), 254 band (II) and 217 nm (band III), respectively. The occurrence of a shoulder at 287 nm in the spectrum recorded in dioxane indicates the very small fraction of molecules of the zwitterionic form.

The experimental spectra were broken down by using three lognormal curves (Table 1),[15–17] namely those for (*i*) band I of the neutral form, (*ii*) band II of the zwitterionic form, and (*iii*) band II of the neutral form, in increasing order of energy. The spectra obtained in methanol, ethanol and ethane-1,2-diol, were broken down by using four rather than three lognormal curves—the fourth curve was for band I of the zwitterionic form (Table 1)—because of the greater proportion of the ionic dipolar form in these more polar media. Some bands corresponding to the presence of hemi-acetals of DPL and the alcohol medium were occasionally also introduced to improve the fittings; however, their contributions were always quite small (less than 1%) and imprecise. Consistent with the findings of Nakamoto and Martell,[18] $\bar{\nu}_0$ (the band position, see Table 1) decreased on passing from water to less polar media.

Results and Discussion

Table 2 lists the areas of the bands obtained by breaking down the spectra of DPL in the different media used. If one assumes the area of a band to be independent of the solvent composition,[16] and only two tautomer species to occur in the medium, then the constant of the tautomeric equilibrium, K_z, can be

Table 1 Band parameters for the resolved absorption spectra

Compound	$\bar{\nu}_0$/nm	ε_0/mol^{-1} cm^{-1}	W/nm	ρ	a/Mm mol^{-1}
Dioxane	356.2	3790	60.0	1.31	194.8
	287.4	530	27.0	1.45	18.5
	253.6	6420	30.6	1.29	326.0
75%–25%[b]	356.0	3760	60.0	1.32	192.8
	287.4	600	27.0	1.45	22.3
	254.3	6390	30.6	1.29	320.8
50%–50%[b]	356.0	3600	60.0	1.32	183.8
	287.4	900	27.0	1.45	37.5
	253.8	6360	31.0	1.29	311.8
25%–75%[b]	356.0	3310	60.0	1.33	175.3
	287.4	1450	27.0	1.45	52.3
	253.5	5950	31.0	1.43	301.4
Pentanol	354.5	2940	61.0	1.34	160.2
	287.4	2190	27.0	1.42	79.0
	253.5	5150	31.0	1.30	258.1
Butan-2-ol	354.5	3030	61.0	1.35	159.0
	287.0	2150	28.0	1.42	81.2
	253.5	5000	30.0	1.29	226.4
Propanol	354.5	2920	62.0	1.36	158.2
	287.0	2210	28.0	1.42	82.6
	253.5	4770	30.0	1.29	218.7
Ethanol	386.2	410	59.0	1.37	18.1
	356.6	2800	62.0	1.36	154.4
	287.0	2230	27.0	1.42	89.9
	253.5	4400	31.0	1.28	201.0
Methanol	386.2	350	59.0	1.37	14.8
	352.4	2620	62.0	1.36	145.1
	287.0	2600	26.0	1.41	106.4
	253.5	4100	31.0	1.28	184.7
Ethane-1,2-diol	383.3	450	59.0	1.37	19.0
	352.2	2220	62.0	1.37	134.3
	321.5	1000	37.0	1.42	34.2
	286.5	2950	28.0	1.42	123.9

[a] The position $\bar{\nu}_0$, height ε_0, width W and skewness ρ are those of the fitted lognormal curves. The area of a band is denoted by a. [b] %Dioxane–%pentanol.

Table 2 Areas, area increments and area–increment ratios for the neutral and zwitterionic form of DPL

Compound	a_n	a_z	Δa_n	$-\Delta a_z$	$-\Delta a_n/\Delta a_z$
Dioxane	194.8	18.5	2	3.8	0.526
75%–25%[a]	192.8	22.3	9	15.2	0.532
50%–50%[a]	183.8	37.5	8.5	14.8	0.574
25%–75%[a]	175.3	52.3	15.1	26.7	0.566
Pentanol	160.2	79.0	1.2	2.2	0.545
Butan-2-ol	159.0	81.2	0.8	1.4	0.571
Propanol	158.2	82.6	3.8	7.3	0.521
Ethanol	154.4	89.9	9.3	16.5	0.553
Methanol	145.1	106.4	10.8	17.5	0.617
Ethane-1,2-diol	134.3	123.9			

[a] %Dioxane–%pentanol.

Table 3 Tautomeric equilibrium constants and fractions of the DPL tautomers

Compound	K_z	f_n	f_z
Dioxane	0.053	0.95	0.05
75%–25%[a]	0.065	0.94	0.06
50%–50%[a]	0.114	0.90	0.10
25%–75%[a]	0.167	0.86	0.14
Pentanol	0.276	0.78	0.22
Butan-2-ol	0.286	0.78	0.22
Propanol	0.292	0.77	0.23
Ethanol	0.326	0.75	0.25
Methanol	0.411	0.71	0.29
Ethane-1,2-diol	0.517	0.66	0.34

[a] %Dioxane–%pentanol.

calculated as the ratio between the fractions of the zwitterionic (f_z) and neutral (f_n) forms, eqn. (3), where a_z and a_n are the

$$K_z = \frac{f_z}{f_n} = \frac{a_z/a_z^0}{a_n/a_n^0} = \frac{a_z a_n^0}{a_n a_z^0} \qquad (3)$$

areas of band II of the zwitterionic form and band I of the neutral form, a_z^0 and a_n^0 are the corresponding molar areas, and $(f_z + f_n) = 1$. Molar areas are usually difficult to obtain in the presence of tautomeric equilibria; however, provided the above assumptions hold, then the ratios can be readily calculated. On changing the solvent polarity, the area a_n will concomitantly change by Δa_n, just as a_z will change by Δa_z. Therefore eqn. (4) is obtained, since $(f''_z + f''_n) = (f'_z + f'_n)$, and this leads to eqn. (5).

$$\frac{-\Delta a_n}{\Delta a_z} = \frac{(f''_n - f'_n)a_n^0}{(f'_z - f''_z)a_z^0} = \frac{a_n^0}{a_z^0} \qquad (4)$$

$$K_z = (-\Delta a_n/\Delta a_z)(a_z/a_n) \qquad (5)$$

The data in Table 1 were used to obtain an average value of $-\Delta a_n/\Delta a_z = 0.56 \pm 0.04$ (Table 2). The ratio between the molar areas is virtually constant, which confirms the above assumptions. The $-\Delta a_n/\Delta a_z$ values obtained were of the same order of magnitude as those reported by Metzler *et al.*[16] for 3-hydroxypyridine (0.53–0.62) and pyridoxine (0.57 ± 0.04) in methanol–water mixtures. From eqn. (5) we estimated K_z values for the different media used and the fractions of the species present in each medium (Table 3).

The a_z^0/a_n^0 ratio can also be obtained from the slope of the plot of a_z *vs.* a_n, eqn. (7), which is obtained from eqn. (2) by taking into account that $f_n + f_z = 1$.

$$f_z + f_n = 1 = \frac{a_n}{a_n^0} + \frac{a_z}{a_z^0} \qquad (6)$$

$$a_z = a_z^0 - (a_z^0/a_n^0)a_n \qquad (7)$$

Eqn. (7) also allowed us to determine the molar areas of the tautomeric forms, namely $a_z^0 = 362 \pm 3$ Mm mol^{-1} and $a_n^0 = 205 \pm 2$ Mm mol^{-1}, respectively. The a_n^0 value found is similar to those reported by Harris *et al.*[17] (224) and by Sanchez Ruiz *et al.*[19] (216 ± 19). That of the zwitterionic form of DPL, a_z^0, is intermediate between those reported by Harris *et al.*[17] (404) and by Sanchez Ruiz *et al.*[19] (261 ± 37). In both cases, these values were calculated by comparison with other 3-hydroxypyridine derivatives, which might have introduced some errors.

The formation of Schiff bases takes place *via* an intermediate carbinolamine (Scheme 1), dehydration of which yields an aldimine in the rate-determining step under the conditions used.

The overall rate constants of formation (k_1) and hydrolysis (k_2) of the Schiff bases of DPL and hexylamine in the different non-aqueous media at 25 °C are listed in Table 4. As a rule, k_1 increases with the dielectric constant of the medium and with the acid character of the alcohol, *i.e.* with increasing proportion of the zwitterionic form of DPL. The stability constant, K_{pH}, consistent with the variation of k_2, increases with decreasing medium polarity. This behaviour is identical with that observed for the Schiff bases of PLP or DPL in water/ethanol[11] and water/dioxane[12] mixtures.

Table 4 Relative permittivities of the media. Overall rate constants of formation and hydrolysis of the Schiff bases of DPL and *n*-hexylamine.

Compound	D	k_1/dm^3 mol^{-1} min^{-1}	k_2/min^{-1}	$K_{pH}/10^3$ dm^3 mol^{-1}
Dioxane	2	247	0.069	3.58
75%–25%[a]	5	540	0.170	3.18
50%–50%[a]	8	666	0.223	2.99
25%–75%[a]	11	718	0.258	2.79
Pentanol	14	781	0.294	2.65
Butan-2-ol	16	853	0.333	2.56
Propanol	20	897	0.353	2.54
Ethanol	24	944	0.389	2.43
Methanol	34	1146	0.510	2.25
Ethane-1,2-diol	38	1713	0.831	2.06

[a] %Dioxane–%pentanol.

The overall rate constant of formation of the aldimine can be expressed as a function of the individual rate constants of the tautomers present in each case, eqn. (8), where f_n and f_z

$$k_1 = f_n k_{1n} + f_z k_{1z} \qquad (8)$$

are the fractions of the neutral and zwitterionic forms of aldehyde, and k_{1n} and k_{1z} are the individual rate constants of formation of the respective tautomers of the aldimine.

By substituting the values of k_1 (Table 4), f_z and f_n (Table 3) into eqn. (8) we calculated the individual rate constants of formation of the tautomers of the aldimine, *viz.* $k_{1z} = 3.6 \times 10^3$ dm^3 mol^{-1} min^{-1} and $k_{1n} = 1.7 \times 10^2$ dm^3 mol^{-1} min^{-1}. Thus, the zwitterionic form reacted more rapidly than the neutral form, which accounts for the increase in the reaction rate with an increase in the medium polarity (*i.e.* with an increasing amount of the zwitterionic form in the medium), assuming that the process is a unimolecular reaction of neutral molecules.

The presence of a neutral 3-hydroxy group was used as support for the occurrence of intramolecular acid catalysis in the formation of Schiff bases of PLP in aqueous media.[9,20] The results obtained in non-aqueous media indicate the existence of other factors influencing such a catalysis, as the neutral form has a smaller rate constant.

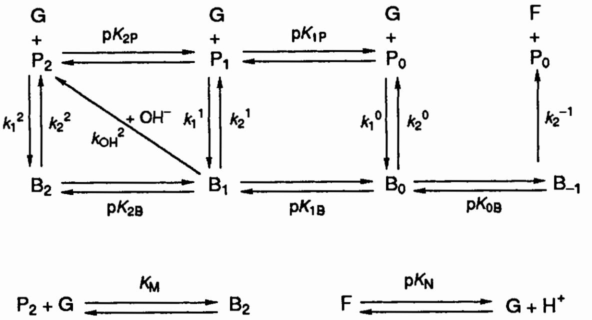
The formation of Schiff bases of DPL and hexylamine was also studied as a function of the pH in water/dioxane media containing 0, 50 and 70% of the organic solvent at 25 °C. The corresponding overall rate constants of formation and hydrolysis are listed in Table 5.

The overall rates of formation and hydrolysis can be expressed as a function of the individual rates of formation of the ionic species in solution, Scheme 3. P and B denote the aldehyde and the Schiff base, respectively, and the subscript of B_{-1} gives the net negative charge on the molecule. F denotes the monoprotonated form of the amine and G its unprotonated form. k_1^i (i = 0, 1 and 2) and k_2^i (i = −1, 0, 1 and 2) are the individual rate constants of formation and hydrolysis, respectively, of the Schiff base, while k_{OH}^2 is the individual rate

Table 5 Overall kinetic parameters for the formation of the Schiff bases as a function of pH in media with different dioxane contents (%v/v)[a]

0%			50%			70%		
pH	log k_1	log k_2	pH	log k_1	log k_2	pH	log k_1	log k_2
4.23	0.430	−0.541	3.83	0.534	−0.986	4.32	0.503	−1.199
4.53	0.535	−0.515	4.09	0.602	−1.003	4.77	0.523	−1.224
4.84	0.616	−0.504	4.57	0.669	−1.021	5.18	0.537	−1.237
5.18	0.661	−0.506	4.96	0.692	−1.030	5.40	0.543	−1.249
5.47	0.697	−0.519	5.47	0.717	−1.055	5.78	0.563	−1.275
5.78	0.735	−0.560	5.65	0.729	−1.066	6.30	0.629	−1.345
6.23	0.811	−0.635	5.97	0.763	−1.100	6.62	0.722	−1.408
6.54	0.920	−0.712	6.26	0.816	−1.147	7.01	0.897	−1.482
6.84	1.043	−0.773	6.93	1.082	−1.285	7.37	1.131	−1.538
7.08	1.225	−0.818	7.17	1.234	−1.318	7.75	1.415	−1.563
7.32	1.375	−0.844	7.45	1.431	−1.346	8.21	1.769	−1.555
7.69	1.642	−0.849	7.78	1.680	−1.355	8.67	2.040	−1.505
7.91	1.777	−0.845	8.17	1.959	−1.329	8.99	2.153	−1.428
8.43	2.049	−0.760	8.51	2.170	−1.272	9.30	2.209	−1.289
8.69	2.185	−0.678	8.96	2.393	−1.112	9.63	2.226	−1.110
8.94	2.345	−0.560	9.25	2.523	−0.947	9.85	2.230	−0.957
9.27	2.547	−0.350	9.54	2.622	−0.746	10.05	2.229	−0.800
9.63	2.834	−0.060	9.77	2.696	−0.559			
9.96	3.090	0.228						
10.24	3.334	0.531						

[a] T = 25 °C. k_1 (dm^3 mol^{-1} min^{-1}); k_2 (min^{-1}).



Scheme 3

Table 6 Individual kinetic constants of formation and hydrolysis of the Schiff base of DPL and hexylamine in water/dioxane media

Kinetic parameter	Dioxane[a] 0	50	70
$\log k_1^0$	7.30	6.70	6.21
$\log k_1^1$	4.80	3.44	2.50
$\log k_1^2$	3.92	2.82	2.22
$\log k_2^{-1}$	−1.12	−0.761	−0.555
$\log k_2^0$	−0.485	−1.03	−1.23
$\log k_2^1$	−0.905	−1.40	−1.60
$\log k_{OH}^2$	1.93	0.996	0.413

[a] Dioxane (%v/v).

constant of hydrolysis of species B_1 (uncharged form of the Schiff base) by hydroxy ions. The method used to fit the experimental data and calculate the individual constants has been described elsewhere.[11,12] The individual rate constants of formation and hydrolysis obtained are given in Table 6.

The rate of formation of the uncharged species in water, log k_1^1 = 4.80, is much larger than expected (3.6×10^3 dm^3 mol^{-1} min^{-1}) from the virtually exclusive occurrence of the zwitterionic form of DPL.

The constant k_1^1 decreased markedly with an increase in the dioxane content of the medium. This behaviour can be analysed by using the Marshall model,[21] as the behaviour of DPL in water/dioxane mixtures does not conform to the Born model.[19] Accordingly, the slope of the plot of k_1^1 *vs.* the logarithm of the molar concentration of water will provide the difference between the number of solvation water molecules in the transition state and the reagents; in our case, the slope was close to 5. The fact that solvation increases by five molecules is not too surprising; a value of six has been reported for the Schiff base of 4-pyridinecarboxaldehyde and hexylamine in water/dioxane mixtures.[22]

The constant k_2^1 also decreases with increasing dioxane content. In this case, the slope of the Marshall plot is about four, *i.e.* one unit less than k_1^1. This correct taking into account that the k_2^1 values correspond to pseudo-first order constants that include the water concentration. In summary, the variation of k_1^1 and k_2^1 with the dioxane content can be ascribed to the need for a certain number of water molecules to stabilize charges in the excited state. The occurrence of a charged transition state may account for the fact that the rate constant of the zwitterionic form exceeds that of the neutral form.

Acknowledgements

This research was supported by the DGICYT (Project PB88-0284) and by the University of Alcalá de Henares.

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Paper 1/00397F
Received 28*th January* 1991
Accepted 6*th March* 1991