JOURNAL OF PHYSICAL ORGANIC CHEMISTRY
J. Phys. Org. Chem. **12**, 486–492 (1999)

Acid–base equilibria in Mannich bases assessed by UV absorption spectroscopy

M. Przesławska,[1] A. Koll[1]* and M. Witanowski[2]

[1]Faculty of Chemistry, University of Wrocław, F. Joliot-Curie 14, 50-383 Wrocław, Poland
[2]Institute of Organic Chemistry, Polish Academy of Sciences, Kasprzaka 44, 01-224 Warsaw, Poland

Received 6 April 1998; revised 21 October 1998; accepted 21 October 1998

ABSTRACT: Acid–base equilibria in selected *o*-methylamino-*N*-dialkylphenols in aqueous solutions are discussed. Analysis of the pH dependence of the UV absorption spectra with application of the multi-channel least-squares procedure allows the estimation of some microscopic dissociation constants. Results are discussed on the basis of intramolecular interactions between different substituents in phenol rings and protonation of the methylamino groups in amino acids. Copyright © 1999 John Wiley & Sons, Ltd.

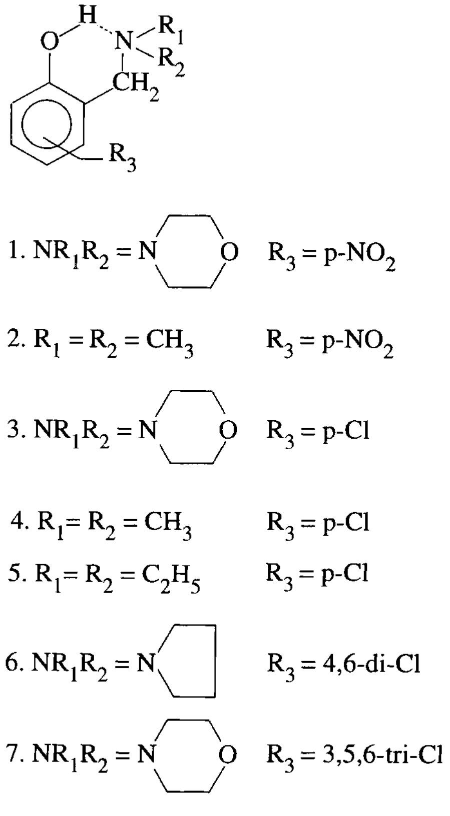
KEYWORDS: acid–base equilibria; amino acids; multi-channel least-squares method; intramolecular interactions; UV–Vis spectra

INTRODUCTION

Mannich bases produced by *ortho*-condensation of phenols with secondary amines and formaldehyde, as shown in Scheme 1, contain a proton donor moiety (the phenol) and a proton acceptor (the amino group) which are separated from each other by a methylene bridge. The latter quenches $\pi-$electron conjugation between the donor and the acceptor. Intramolecular hydrogen bonds can form between the OH group and the amino nitrogen atom, and the acid–base properties of such molecular systems can be modified to a considerable extent by varying either the structure of the amino moiety or the substituent on the phenyl ring.

In view of these properties, Mannich bases are employed as model compounds in studies of the nature of hydrogen bonds.[1] Any comparisons between intramolecular and intermolecular hydrogen bonding effects require the assessment of pK_a values for the donor and for the acceptor groups involved in the bonding.

The problem of acid–base equilibrium in ampholites is certainly non-trivial, as depicted in Scheme 2, and is a topic of recent studies.[2–4] Knowledge of the specific (microscopic) dissociation constants of A^+ to A and $A^\pm$ and those of A and $A^\pm$ to A^- as well as $K_{PT}(= [A^\pm]/[A])$ is necessary to describe the partition coefficients of ampholites between water and an immisible lipid, e.g. an organic solvent such as *n*-octane. The partition coefficient depends on the concentration of the neutral form (A in Scheme 2) in the two immiscible solvents. From the

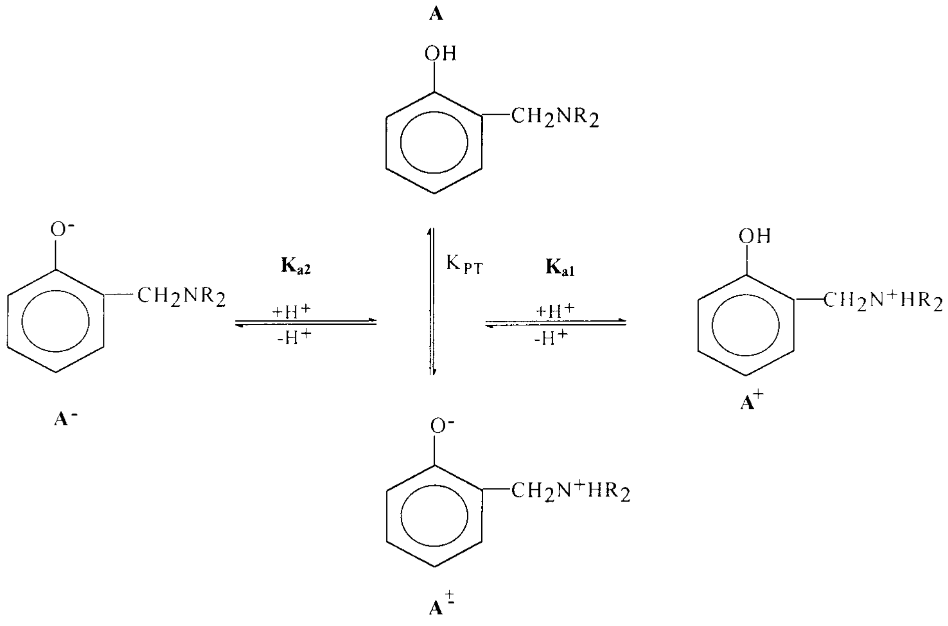


Scheme 1

**Correspondence to:* A. Koll, Faculty of Chemistry, University of Wrocław, F. Joliot-Curie 14, 50-383 Wrocław, Poland.

Contract/grant sponsor: KBN; *Contract/grant number:* 30T09A 116 10.

CCC 0894–3230/99/120486–07 $17.50



Scheme 2

experiment only average (macroscopic) K_{a_1} and K_{a_2} values are available. The lipophilicity characterized by this coefficient plays an important role in the biological activity of drug molecules.

Extensive *ab initio* calculations of the influence of solvents on the tautomeric equilibrium in nicotic and isonicotic acids at advanced levels such as MP2/6–31+G**//MP2/6–31G* have been reported.[2] The solvent effect on the tautomerization of *N*,*N*-dimethylglycine has been studied.[3] One popular method for solving the problem of determining the values of microscopic dissociation constants is to use the pK_a values of model compounds, namely the esters of acidic groups. This procedure introduces some uncertainty, however, even after using the correlations of the Hammett type.[5] Instead, analysis of the pH-dependent UV absorption spectra seems to offer a better means to provide insight into the protonation–deprotonation process concerned.

There have been some attempts in the literature[6,7] to explain the equilibria presented in Scheme 2 in Mannich bases. The influence of electronic interactions[7] as well as intramolecular hydrogen bond[6] on pK_a values has been discussed. Applying different assumptions to values of absorption coefficients of the particular forms, the authors estimated the microscopic dissociation constants and K_{PT}. Such an approach seems futile since only three of the equilibrium constants are independent, and the remaining ones become redundant. From the point of view of pH-dependent equilibria, the most reasonable choice for selecting a set of three independent equilibrium constants seems to be as presented in Scheme 2. In the cases of K_{a_1} and K_{a_2}, the corresponding equations include the concentration of H^+, whereas that for the tautomerization constant K_{PT} does not:

$$K_{a_1} = ([A] + [A^{\pm}])[H^+]/[A^+]$$
$$K_{a_2} = [A^-][H^+]/([A] + [A^{\pm}])$$

Thus, if one examines the UV absorption spectra of the system as a function of pH, there is a variable superposition of three component spectra, that of the anion, that of the cation and that of a tautomeric mixture ($A+A^{\pm}$), where the contributions of A and $A^{\pm}$ are related by the tautomerization constant K_{PT}. Thus, the latter spectrum does not change as such, and only its contribution to the total spectrum varies with pH. It is not possible, therefore, to obtain information on the tautomeric equilibrium between the zwitterion $A^{\pm}$ and the truly neutral species A if one simply examines the UV spectra concerned as a function of pH. However, in the present case of Mannich bases there is some way round, provided that we can extract the component spectrum ($A+A^{\pm}$) from the pH-dependent variation of the observed spectra. Since the UV absorption of the molecules concerned is related essentially to the presence of the phenol/phenolate chromophore, whereas the amino/ammonium moiety does not contribute directly, the spectral lineshape for the cation A^+ should be similar to that for the phenol A, and the spectrum of the anion A^- should be similar to that of the zwitterion $A^{\pm}$. In view of the foregoing in particular cases, the combined spectrum ($A+A^{\pm}$) can resemble the spectra of A^- or A^+ which are available at the border conditions of very high or very low pH. In such a case one is able to estimate some microscopic dissociation constants which can be com-

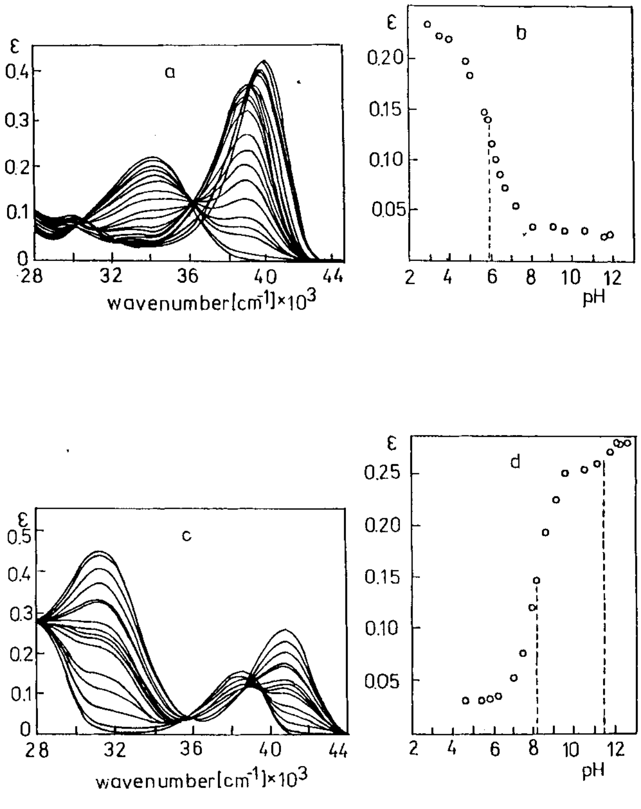


Figure 1. Examples of spectrophotometric titration of (a, b) 2-(*N,N*-dimethylaminomethyl)-4-nitrophenol and (c, d) 2-(*N,N*-dimethylaminomethyl)-4-chlorophenol

pared with pK_a values of related phenols and benzylamines, in order to analyze the intramolecular interactions. It provides also some unique information on intramolecular hydrogen bonding in aqueous solutions not available by other experimental methods.

EXPERIMENTAL

o-Methylamino-*N*-dialkylphenols (Mannich bases) were synthesized by standard techniques of Mannich condensation.[8] Their electronic absorption spectra at 25 ± 1 °C were recorded on a Specord M-40 spectrophotometer (Carl Zeiss Jena) at a resolution of 40 cm^{-1} for solutions in 1 cm quartz cells. Solutions within a concentration range from 1×10^{-4} to 12×10^{-4} mol dm^{-3} were prepared for each Mannich base. These solutions were diluted 10-fold by adding HCl or NaOH solutions of different concentrations, to give pH values ranging from 1 to 12. The pH of the solutions was determined with a combined glass–calomel electrode calibrated with standard buffer solutions.[9] This provided 20–33 different spectra as a function of pH for each compound, as shown in Fig. 1(a, c). The pK_a values were estimated from the plots of absorbance vs pH [cf. Fig. 1(b, d)].

The same results were also processed by a multi-channel analytical method, the principles of which were described in detail elsewhere.[10,11] It is essentially a non-linear least-squares optimization method employed simultaneously over a large number (*N*) of wavelengths or frequencies (i.e. 'spectral channels') for the corresponding absorbances as a function of some experimental parameters (pH, in the present case). Additional details of using this method in describing the proton transfer equilibria in Mannich bases are discussed in other sections.

RESULTS AND DISCUSSION

Examples of different types of spectra dependence on pH are shown in Fig. 1. Figure 1(b) and (d) illustrate the method of determination of pK_a values which are described in Table 1 as 'spectroscopic–potentiometric' results.

In order unravel the UV absorption of the pH-depenent equilibria concerned, the three-component spectra A^+,A^- and $A{+}A^{\pm}$ we employed the so-called multi-channel least-squares fitting procedure.

The method, as used in the present work, performs simultaneously the least-squares fitting of 3*N* molar absorption coefficients ε three for each of the *N* channels, $\varepsilon(A^+)$, $\varepsilon(A^-)$ and $\varepsilon(A{+}A^{\pm})$, and also of pK_{a_1} and pK_{a_2}, using equations which relate the concentrations of the three species to pH. Since the approach is non-linear, one has to make starting approximations for all of the $3N{+}2$ variables fitted; for $\varepsilon(A^+)$s we used the spectrum run at the lowest pH, that at the highest pH for $\varepsilon(A^-)$s and for $\varepsilon(A{+}A^{\pm})$s we took a spectrum from the range of pHs which was inside the limits set by the starting approximations of pK_{a_1} and pK_{a_2} employed. Typically, we used the method over 300 spectral channels, so that the number of variables to be fitted simultaneously amounted to 902. This task seems to be numerically formidable, as the size of the corresponding matrix becomes 903 × 903, but the crucial point in the multi-channel method lies in a suitable factorization[10,11] which drastically reduces the sizes of the matrices involved and concomitant numerical instabilities. The method worked perfectly for all of the compounds studied and the corresponding UV spectra, and it turned out to be fairly insensitive to the quality of the starting approximations employed with respect to the optimized values obtained for the variables concerned.

The results obtained by this method for pK_{a_1} and pK_{a_2} values are listed in Table 1 as 'multi-channel' results.

The unraveled spectra in terms of molar absorption coefficients ε as a function of spectral frequency, are given in Fig. 2.

In each case, the component spectra $(A{+}A^{\pm})$, those which are linear combinations of the relevant neutral species A and $A^{\pm}$, provide some information on the tautomeric equilibria between the latter in spite of the fact

Table 1. Acid–base equilibria in Mannich bases

Compound	Phenol	Benzylamine	Concentration (mol dm^{-3})	Number of UV spectra at different pH	Fitted value of pK_{a_1}[a] ± std dev.	Fitted value of pK_{a_2}[a] ± std dev.	Value of pK_{a_1}[b]	Value of pK_{a_2}[b]	Literature values		Ref.
									pK_{a_1}	pK_{a_2}	
1	*p*-NO$_2$-	*N*-Morpholino-	2.12×10^{-5}	26	5.63 ± 0.01	7.82 ± 0.01	5.75	—	5.53	8.02	7
2	*p*-NO$_2$-	*N*-Dimethyl-	3.35×10^{-5}	33	5.91 ± 0.01	9.93 ± 0.01	5.85	—	—	—	
3	*p*-Cl-	*N*-Morpholino	4.34×10^{-5}	26	6.89 ± 0.01	9.74 ± 0.01	6.9	9.75	6.68	9.96	7
4	*p*-Cl-	*N*-Dimethyl-	4.60×10^{-5}	23	8.15 ± 0.01	11.11 ± 0.01	8.2	11.35	—	—	
5	*p*-Cl-	*N*-Diethyl-	1.18×10^{-4}	20	7.96 ± 0.01	11.79 ± 0.01	8.2	11.5	7.95	1.40	7
									7.75	1.17	6
6	*p*-Cl-	*N*-Pyrrolidino-	3.67×10^{-5}	21	8.30 ± 0.01	11.93 ± 0.01	8.3	11.5	—	—	
7	2,4-Di-Cl-	*N*-Morpholino-	3.05×10^{-5}	23	5.83 ± 0.01	8.73 ± 0.01	5.8	8.78	5.35	8.95	7
8	3,5,6-Tri-Cl-	*N*-Morpholino-	1.03×10^{-5}	22	4.83 ± 0.04	8.16 ± 0.04	5.0	8.3	4.84	8.33	7

[a] pK_a Studied by multi-channel least-squares analysis of pH-dependent UV spectra.
[b] pK_a Studied by spectroscopic–potentiometric method.

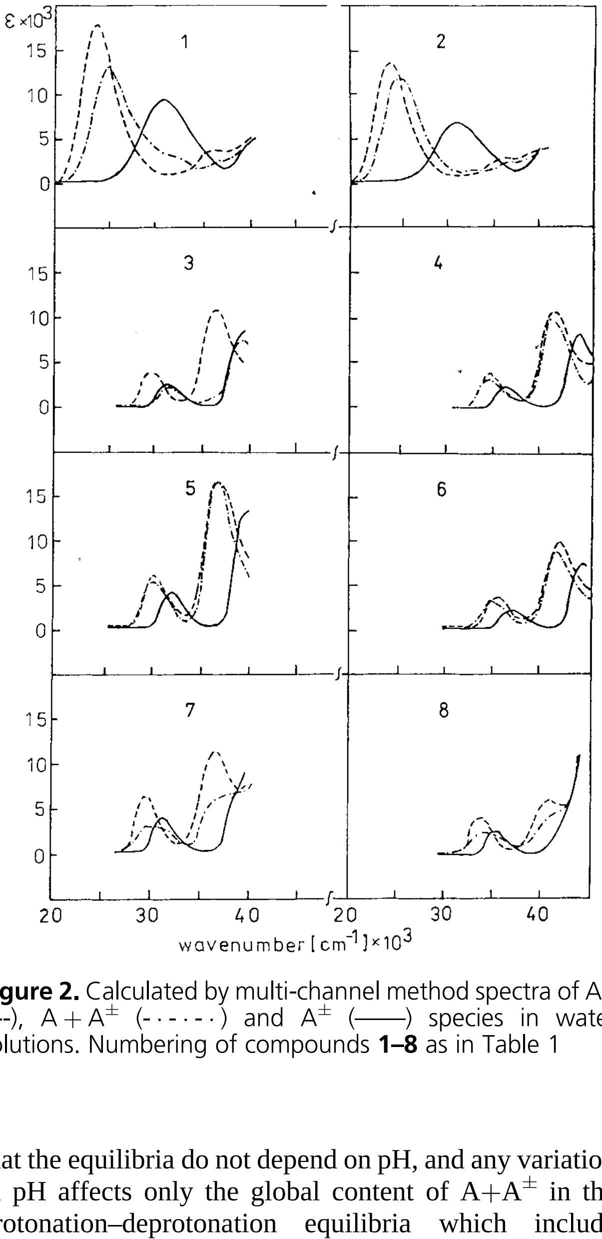


Figure 2. Calculated by multi-channel method spectra of A^- (---), $A+A^{\pm}$ (-·-·-·) and $A^{\pm}$ (——) species in water solutions. Numbering of compounds **1–8** as in Table 1

that the equilibria do not depend on pH, and any variation in pH affects only the global content of $A+A^{\pm}$ in the protonation–deprotonation equilibria which include A^+,$A+A^{\pm}$ and A^- species. It is fairly obvious from the sample spectra presented in Fig. 2 that the composite spectrum of $A+A^{\pm}$ contains fragments which resemble the spectra of A^+ and of A^-. Since the UV absorption of the molecules concerned comes essentially from their phenol/phenolate chromophores, it is reasonable to expect that the fragment which resembles the spectrum of A^+ should represent the spectrum of A, and A^- should represent the spectrum of $A^{\pm}$. It is not feasible to extract the molar absorption coefficients $\varepsilon(A)$ and $\varepsilon(A^{\pm})$ from the pH-dependent spectra, since the $A/A^{\pm}$ ratio remains constant, but we can make a rough assumption that the ratio $\varepsilon(A)/\varepsilon(A^{\pm})$ for the coefficients at the corresponding absorption band peaks of A and $A^{\pm}$, respectively, should not depart significantly from the analogous $\varepsilon(A^+)/\varepsilon(A^-)$ ratio which is readily available from the unraveled spectra (Fig. 2). Using this assumption, one can qualitatively estimate the actual compositions of the $A+A^{\pm}$ mixtures of the neutral species involved; such estimates are presented in Table 2.

This seems to be virtually the only way in which one can estimate the individual contents of the truly neutral species A and that of the zwitterion $A^{\pm}$, in addition to those of the ions A^+ and A^-, in acid–base equilibrium systems which can include all of the foregoing species. It is interesting that in the molecules examined the zwitterion usually dominates over the truly neutral molecule A (Table 2), but there are some significant exceptions. In compound **3**, it is the A species which prevails, and in compounds **7** and **8**, the two species occur in comparable amounts at equilibrium.

On the basis of the results presented in Table 2, the proton transfer equilibria for the most of particular systems can be attributed to one of two possible reaction schemes:

$$A^- \overset{K_{a_2}(I)}{\rightleftharpoons} A \overset{K_{a_1}(I)}{\rightleftharpoons} A^+ \qquad (1)$$

$$A^- \overset{K_{a_2}(II)}{\rightleftharpoons} A^{\pm} \overset{K_{a_1}(II)}{\rightleftharpoons} A^+ \qquad (2)$$

The $K_{a_1}(I)$ value is related to dissociation of $R_1N^+H(R_2)_2$ cations whereas the $K_{a_1}(II)$ value is connected with phenolic OH group dissociation from A^+ species. $K_{a_2}(I)$ and $K_{a_2}(II)$ play an opposite role in the course of further stripping of the protons.

When the scheme of equilibrium is decided, one can attribute an average dissociation constant to one of the particular microscopic dissociation constants. By comparison of this values with K_a of related phenols and benzylamines (also given in Table 2) one can analyze the intramolecular interactions between acid–base centres of a molecule.

In the case of the Mannich base resulting from *p*-nitrophenol and morpholine (**1** in Table 1), the prevailing content of the $A^{\pm}$ form in the $A+A^{\pm}$ mixture suggests that a pK_{a_1} value of 5.63 can be attributed to dissociation of the OH group [reaction (2)].

A considerable decrease (-1.52) of pK_a in comparison with *p*-nitrophenol is found, which should result from the influence of the morpholine substituent. The electronic influence of an electron-donating subsitituent, however, should decrease the acidic properties of the phenol. Additionally the CH_2 group seriously reduces the electronic influence of the substituents. The observed influence of the morpholine group can only be explained by an electrostatic interaction of positive charge on the nitrogen of the protonated —$NH^+(R_2)_2$ group (see Scheme 2). The pK_{a_2} value, on the other hand, is increased in comparison with *N*-benzylmorpholine by 1.07 pK_a units. This can also be caused by the electrostatic interactions. Stripping of the proton from the —$NH^+(R_2)_2$ group proceeds in the electrostatic field

Table 2. Effective pK_a values obtained by the multi-channel method with related literature values for phenols and benzylamines and estimated composition of the $A + A^{\pm}$ mixture

Compound	Fitted value of pK_{a_1}	Fitted value of pK_{a_2}	Estimated composition of $A + A^{\pm}$
1	5.63 (7.15[a])	7.82 (6.75[b])	$A^{\pm}$ prevailing + some A
2	5.91 (7.15[a])	9.93 (8.9[b])	Large excess of $A^{\pm}$
3	6.89 (9.4[a])	9.74 (6.75[b])	Large excess of A
4	8.15 (9.4[a])	11.11 (8.9[b])	Large excess of $A^{\pm}$
5	7.96 (9.4[a])	11.79 (9.4[b])	Large excess of $A^{\pm}$
6	8.30 (9.4[a])	11.93 (9.66[b])	Large excess of $A^{\pm}$
7	5.83 (7.9[a])	8.73 (6.75[b])	Comparable amounts of A and $A^{\pm}$
8	4.83 (7.5[a])	8.16 (6.75[b])	Comparable amounts of A and $A^{\pm}$

[a] pK_a of particular phenols.[12]

[b] pK_a of related benzylamine.[6]

of the phenolate anion. Some influence of electrostatic interaction through the bond chain, and also an intramolecular NH^+O^- hydrogen bond, cannot be discarded. Similar behavior is observed with compound **2**, where the morpholine was replaced by dimethylamine. The pK_a of such an amine is different from the value for **1**, but the calculated ΔpK_a of -1.24 and 1.13 for the phenol and amine centers resemble the results found for **1**. This supports the explanations given in the case of **1**. As follows from Table 2, the character of the protonation/deprotonation scheme of derivatives of *p*-chlorophenol, and dimethyl-and diethylamine and pyrrolidine resembles that discussed above, i.e. following reaction (2). The decrease in pK_a of the phenolic part is 1.25, 1.44 and 1.21 in compounds **4, 5** and **6** respectively.

In derivatives of *p*-chlorophenol the increase in pK_{a_2} values is distinctly larger than that in the case of derivatives of *p*-nitrophenol; it is 2.11, 2.39 and 2.27 in compounds **4, 5** and **6**, respectively. This further increase in the stability of zwitterionc forms cannot be explained only by the electrostatic influence of a charge on the O^- atom.

One of the explanations may be the existence of intramolecular hydrogen bonding of the O^-HN^+ type in aqueous solutions. The reason for the observed differences may be the stronger destabilization of more ionic hydrogen bonds[13] in *p*-nitroderivatives than in *p*-chloro derivatives in polar solvents. There is no evidence in the literature for such intramolecular hydrogen bonding in aqueous solution.[7] It is not possible to distinguish clearly the hydrogen bonded and non-hydrogen-bonded zwitterionic forms, on the basis of the UV spectra.

An interesting exclusion appears with compound **3**, for which the protonation/deprotonation process follows reaction (1). The pK_{a_1} value is connected with the dissociation of amine cations. Fairly good agreement with the value for *N*-morpholinobenzylamine was obtained. Our value is 6.89 whereas the pK_a of *N*-morpholinobenzylamine is 6.75.

Also, the pK_a value of the phenolic part of the complex, 9.74, is not far from the value of 9.4 for *p*-chlorophenol. The 0.34 unit increase in pK_a in the Mannnich base can be explained by intramolecular hydrogen bonding in A, but the difference is too small for reliable disscussion, especially if one takes into account the comparatively low precision of the microscopic estimation of pK_a values. For compounds **7** and **8** comparable amounts of A and $A^{\pm}$ forms are observed in the mixture and the average pK_{a_2} and pK_{a_1} values cannot be attributed to any microscopic dissociation constants. The pK_{a_1} values are lower than either of the pK_a values for a phenol and benzylamine.

A similar lowering of pK_{a_1} values in Mannich bases with respect to the pK_a values of the parent phenols has been reported,[7] where a linear correlation of pK_{a_1} with pK_a was observed. Some points located off this correlation line are probably related to systems, where the pK_{a_1} values describe the dissociation of $-NH^+(R_2)_2$ groups. A good correlation of logK_{PT} with ΔpK_a of basic and acidic centers of the molecules was found despite the semi-quantitative procedure of K_{PT} estimation. Particular values of K_{PT} do not contradict the findings of our studies (cf. Fig. 2).

CONCLUSIONS

The method of numerical analysis of the electronic spectra of ampholites measured as a function of pH proposed here allows the precise determination of effective dissociation constants on the basis of complete spectra, not just for selected wavenumbers. The study of the solutions with electrolyte concentrations no higher than 10^{-4}mol dm^{-3} reduces the effects of ionic strength on the pK_a values obtained.

The analysis allows the determination of the complete spectra of particular forms existing in equilibrium. Such spectra for the intermediate forms are not available in direct experiments.

A method for the determination of some microscopic dissociation constants has been proposed on the basis of the semiquantitative estimation of the amount of various forms in equilibrium.

It was found that, in most cases, the protonation/deprotonation reaction in water proceeds through the zwtterionic forms.

The first dissociation constants (pK_{a_1}) considers, in such a case, the phenolic OH group dissociation while the amine is totally protonated. These dissociation constants are 1.2–1.5 units lower than those for the parent phenols owing to the electrostatic field influence of the NH^+ group. The pK_{a_2} values, connected with NH^+ group dissociation, are increased by 1.1 ± 0.3 pK_a units, by the electrostatic influence of the O^- charge. In a few cases such a decrease is even higher, around 2.1–2.4 units. This can be explained by the stabilizing influence of the intramolecular hydrogen bond of the O^-NH^+ form. Such enhanced stability of NH^+ forms was not observed in the case of derivatives of *p*-nitrophenol. More polar $O^- \cdot\cdot\cdot HN^+$ hydrogen bonds in aqueous solutions are more strongly dissociated than less polar hydrogen bonds in derivatives of *p*-chlorophenol. A detailed pK_a analysis of ampholytes appears to be the method of choice for studying intramolecular hydrogen bonding in aqueous solutions of ampholites.

Acknowledgment

The authors acknowledge the financial support of KBN, grant No. 30T09A 116 10.

REFERENCES

1. E. Hasslinger and P. Wolschann, *Monatsh. Chem.* **111**, 536–549 (1980).
2. P. I. Nagy and K. Takacs-Novak, *J. Am. Chem. Soc.* **119**, 4999–5006 (1997).
3. A. D. Hadley, R. E. Corona and E. T. Cheung, *J. Phys. Org. Chem.* **10**, 898–900 (1997).
4. J.-C. Halle, J. Lelievre and F. Terrier, *Can. J. Chem.* **74**, 613–620 (1966).
5. B. van de Graaf, A. J. Hoefnagel and B. M. Wepster, *J. Org. Chem.* **46**, 653–657 (1981).
6. A. B. Teitelbaum, K. A. Dersturganova, N. A. Shishkina, L. A. Kudriavtseva, W. E. Bielskiy and B. E. Ivanov, *Izv. Akad. Nauk SSSR* **4**, 803–808 (1980).
7. H. Martinek and P. Wolfschann, *Bull. Soc. Chim. Belg.*, **90**, 37–43 (1980).
8. R. Benno, *Die Mannich-Reaction*. Springer, Berlin (1959).
9. D. J. Aluer, J. J. Greczek and A. G. Smeeth, *J. Chem. Soc. A*, 1205–1211 (1967).
10. M. Witanowski, S. Szymański, J. Sitkowski and S. Biernat, *Spectrosc. Int. J.* **6**, 73–81 (1988).
11. M. Witanowski, J. Sitkowski and S. Szymański, *Spectrosc. Int. J.* **6**, 213–220 (1988).
12. E. P. Serjeant and B. Dempsey, *Ionisation Constants of Organic Acids in Aqueous Solution*. Pergamon Press, Oxford (1978).
13. M. Rospenk and Th. Zeegers-Huyskens, *Spectrochim. Acta* **4**, 499–506 (1986).