43. Microscopic Protonation/Deprotonation Equilibria of the Anti-inflammatory Agent Piroxicam

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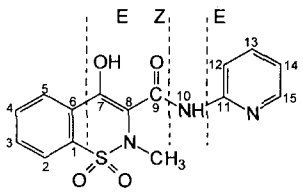
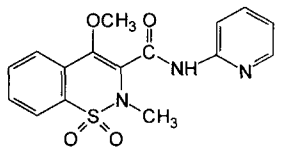
The microscopic ionization behavior of piroxicam was investigated using two different approaches, *i.e.*, direct UV spectroscopy and an indirect analogue approach (deductive method). The best microscopic pK_a values (pK_{a12} = 4.60, pK_{a21} = 5.40, pK_{a22} = 2.72, and pK_{a11} = 1.92) were obtained by the deductive method using as pK_{a22} the pK_a of the enolic *O*-methylated piroxicam **2**. The results show remarkable electrostatic effects in the protonation/deprotonation equilibria, a marked increase in the acidity of the enolic function (2.68 pK_a units) being caused by the pyridinium group. The electronic structure of piroxicam was studied based on ^{1}H-NMR chemical shifts at various ionization states, indicating an extended electron conjugation through the molecule. The partition measurements in octan-1-ol/H_2O of zwitterionic compound **3** (the pyridyl *N*-methyl derivative of piroxicam (**1**)) suggest that the two opposite charges in zwitterionic piroxicam are indeed in a close intramolecular proximity.

Introduction. – Oxicams (= 4-hydroxy-1,2-benzothiazine-3-carboxamides) are a class of long-lasting non-steroidal anti-inflammatory drugs (NSAIDs), which act by inhibiting enzymes involved in the biosynthesis of prostaglandins [1] [2]. To date, piroxicam (= 4-hydroxy-2-methyl-*N*-(pyridin-2-yl)-2*H*-1,2-benzothiazine-3-carboxamide 1,1-dioxide; **1**, see *Fig. 1*) and tenoxicam are among the top-ten NSAIDs on the market [3].

Of particular chemical interest among the oxicams are those bearing a weakly basic pyridyl group, resulting in compounds of a zwitterionic nature such as piroxicam, tenoxicam, and lornoxicam. For these zwitterionic oxicams, the acidity of the enolic function



Piroxicam (**1**)[a])



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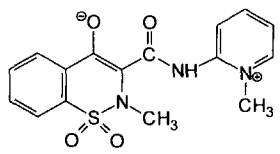

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[a]) Trivial numbering. The planar conformation shown is defined as *EZE* (for the sake of simplicity; *not* according to the definition of (*E*)- and (*Z*)-notation for isomerism around double bonds) referring to the bonds C(8)–C(9), C(9)–N(10), and N(10)–C(11), respectively.

(pK_a = 1–2) is remarkably enhanced due to the presence of the basic pyridyl function, the pK_a of non-zwitterionic oxicams such as isoxicam and meloxicam being *ca.* 4 [4]. In the case of piroxicam (**1**), the fast internal proton transfer has been shown to be unusually sensitive to chemical substitution, solvent, and temperature [5].

With a reduced population of the neutral form in the aqueous phase, the octan-1-ol/water distribution coefficients (log D) of the three zwitterionic oxicams (piroxicam, tenoxicam, and lornoxicam) at isoelectric pH were found to be smaller than those of isoxicam and meloxicam in the neutral state [4]. Overall, the structural and physicochemical properties of oxicams are highly sensitive to their acid-base behavior. That electrostatic effects and charge delocalization combine in zwitterionic oxicams to draw close the two opposite charges was put forward as a hypothesis to explain their unusual acid-base behavior.

While, in a previous study [4], the microscopic ionization constants of piroxicam were estimated from the pK_a value of isoxicam and meloxicam (enolic function), they, however, remained to be validated. The first goal of this study is thus to investigate the microscopic protonation/deprotonation behavior of piroxicam using two different approaches, namely UV spectroscopy and the study of analogues (deductive method). In the latter approach, the synthesis of piroxicam derivatives, *i.e.*, enolic O-methylated (**2**) and pyridyl N-methylated piroxicam (**3**), was undertaken, and their acid/base chemistry studied. The second goal of this study is to understand better the electronic structure of piroxicam. ^{1}H-NMR spectroscopy was used to gain insight into the electronic properties of piroxicam (**1**) in different ionization states, while semiempirical quantum-chemical calculations were used to study the conformational behavior and proton affinity of compound **2**. The partition coefficient in octan-1-ol/H_2O systems of zwitterionic compound **3** was measured to help understand the lipophilicity of zwitterionic piroxicam. The results obtained shed light on the intercharge distance and electrostatic effects in influencing the acid-base chemistry of piroxicam.

Experimental. – *Materials.* Piroxicam (**1**) was generously supplied by *EGIS Pharmaceutical Works* (Budapest, Hungary) and used without further purification. 1,4-Dioxane was of HPLC grade (*Aldrich*), while all other reagents were of anal. grade. Compounds **2** and **3** were synthesized according to the method of *Hammen et al.* [6].

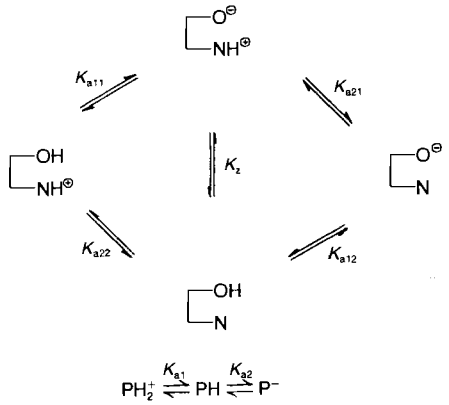
*4-Methoxy-2-methyl-*N*-(pyridin-2-yl)-2*H*-1,2-benzothiazine-3-carboxamide 1,1-Dioxide* (**2**). Enolic O-methylation was performed with diazomethane. A soln. of **1** (1 g, 3 mmol) in 20 ml of MeOH was treated with an ethereal soln. of diazomethane using a conventional method [7]. The reaction under continuous agitation proceeded overnight in the dark at ambient temp. The solvent was evaporated under vacuum, and the residue was dissolved with acetone and then filtered. The filtrate was further concentrated to give a yellow oil, which was then crystallized from acetone to yield a pale-yellow solid (0.7 g, 67%). M.p. 204–206°.

*4-Hydroxy-2-methyl-*N*-(1-methylpyridin-2-yl)-2*H*-1,2-benzothiazine-3-carboxamide 1,1-Dioxide* (**3**). To a mixture of **1** (0.83 g, 2.5 mmol) and K_2CO_3 (0.69 g, 5 mmol) in 15 ml of acetone, 0.6 ml of MeI (10 mmol) was added. The mixture was refluxed for 10 h. After cooling the soln., the insoluble inorganics were filtered off, and the solvent, was evaporated under vacuum. The residue was then dissolved with acetone and the insoluble fraction filtered off. The soln. was then purified by trituration with H_2O, yielding yellow crystals (0.52 g, 60%). M.p. 257–258°.

Determination of Microscopic Ionization Constants of Piroxicam. A general scheme describing the protonation/deprotonation routes of a diprotic molecule, the relevant macro- and microscopic pK_a, and their relationships are shown in (*Scheme 1*). To characterize the protonation/deprotonation processes in terms of microscopic pK_a, the knowledge of macroscopic pK_a values and at least one microscopic pK_a or equilibrium constant of tautomerism K_z is obligatory.

In the deductive method, the electronic properties of piroxicam derivatives used, *i.e.*, the enolic O-methyl and pyridinium N-methyl derivatives, must be similar to those of piroxicam. Furthermore, these derivatives should resemble the parent compound in their conformation and intramolecular interactions such as H-bonding. With

Scheme 1. *Protonation Scheme of a Zwitterionic Molecule*



$$K_{a1} = K_{a11} + K_{a22} \quad (1)$$

$$\frac{1}{K_{a2}} = \frac{1}{K_{a21}} + \frac{1}{K_{a12}} \quad (2)$$

$$K_z = \frac{K_{a11}}{K_{a22}} = \frac{K_{a12}}{K_{a21}} \quad (3)$$

these considerations in mind, the following two approaches were undertaken to determine the microscopic pK_a value of piroxicam: *1*) a direct UV spectroscopic method to determine the equilibrium constant of tautomerism K_z in mixtures of H_2O and apolar solvents; *2*) an indirect analogue approach (deductive method) using the methylated derivatives of piroxicam.

Determination of the Equilibrium Constant of Tautomerism K_z [8]. The determination of K_z for the zwitterionic/unionized equilibrium is based on the UV spectral differences between the zwitterionic form (existing predominantly at the isoelectric pH of the aq. soln.), and the unionized form (existing predominantly in org. solvent of low dielectric constant such as dioxane). The spectral changes with respect to the proportion of the two protonation isomers (zwitterionic and unionized) in various solvent mixtures allow K_z to be calculated according to *Eqn. 4*.

$$K_{z(\%)} = (A_\% - A_d)/(A_w - A_\%) \quad (4)$$

where $K_{z(\%)}$ equilibrium constant of tautomerism in a given solvent mixture;
$A_\%$ absorbance of the compound in a solvent mixture;
A_d absorbance of the compound in dioxane;
A_w absorbance of the compound in aqueous buffer solution at the isoelectric pH.

0.1 ml of aliquots from a stock soln. of piroxicam (**1**; 1.5 mM in dioxane) was diluted to 10 ml with dioxane, *Sörensen* buffer (pH 3.65), or various mixtures of the two solns. The concentration of org. solvent was varied from 0 to 100 wt-% in steps of 10%. The UV absorption spectra of these solns. were recorded using *Hewlett-Packard 8452A* diode array spectrometer.

Determination of the pK_a *Values of Piroxicam Derivatives Using UV Spectroscopy*. Due to the poor water solubility of compound **2**, its pK_a value was measured using UV spectrophotometry. 0.2 ml of stock soln. in dioxane (1 mM) was diluted to 10 ml with HCl (1N) to have only the protonated form in the soln., with distilled water to have only the neutral form, or with seven different *Sörensen* buffers in the pH range from 2.5 to 4.5. The pK_a value was calculated from the UV spectroscopic data and pH values based on the *Henderson-Hasselbalch* equation [9].

The pK_a value of the enolic group of compound **3** was obtained similarly except that the solns. were prepared in the pH range 1–3.

^{1}*H-NMR Chemical Shifts of Piroxicam* (**1**) *at Various Ionization States*. A stock soln. of **1** (0.2 mM) was prepared in 0.1M DCl and the ionic strength adjusted to 0.2M with NaCl. Sixteen samples of pH vlaues ranging from 1 to 10 were prepared by adding different amounts of NaOD (0.02M). The ^{1}H-NMR spectra of these solns. were recorded on a *Bruker AC-400* spectrometer.

Measurement of Partition Coefficient Using Centrifugal Partition Chromatography (CPC). The partition coefficient in octan-1-ol/buffer (made of 0.02M 3-morpholinopropanesulfonic acid/NaOH, pH 7.2) of **3** was determined using flow-through CPC with a coil planet type centrifuge (*Ito Multi-layer Coil Separator-Extractor, P.C. Inc.*). The detailed experimental procedures have been described in [10] [11]. The measurements were performed using the org. phase as eluent with a total volume (V_t) of 265 ml in the columns, and the distribution coefficient was calculated using *Eqn. 5*.

$$\log D = \log \frac{V_t - U \cdot t_0}{(t_R - t_0)} \tag{5}$$

where t_0 and t_R are the retention time of the solvent front and of the solute, respectively, and U is the flow rate of the mobile phase. The volume of t_0 was measured using highly lipophilic (*e.g.* biphenyl) non-retained solutes.

Quantum-Chemical Calculations. The starting geometries of the uncharged and cationic forms of compound **2** were taken from X-ray crystallographic data [5] [6]. They were subsequently optimized with the molecular mechanical MM+ method and then the semiempirical PM3 method using the HYPERCHEM (Version 3.0) molecular modelling software package.

Results and Discussion. – *Microscopic Ionization Constants Using UV Spectroscopy to Determine* K_z. The determination of the equilibrium constant K_z in dioxane/H_2O solvent system was first described by *Metzler* and *Snell* [8] for pyridoxine and successfully applied by some of us to other drug molecules, also in MeOH/H_2O systems [12] [13]. An important criterion for the applicability of this method is the unambiguous assignment of the spectra of unionized and zwitterionic forms through the analysis of related compounds, namely *O*-methyl and pyridyl *N*-methyl derivatives. The spectrum of piroxicam in dioxane was identified as that of the unionized form with λ_{max} = 326 nm (*Fig. 1*) as compared to the very similar spectrum of compound **2** in buffered solution where the pyridyl *N*-atom is in the neutral state (*Fig. 2, a*). In contrast, the spectrum of zwitterionic piroxicam in aqueous buffer at isoelectric point pH (3.65) (λ_{max} = 360 nm) shows similar features to those of zwitterionic compound **3** (λ_{max} = 366 nm; *Fig. 2, b*).

Another criterion for the application of this method is that the spectra from different dioxane/H_2O mixtures must cross at isosbestic point(s), meaning that the spectral changes should result from a single chemical equilibrium, *i.e.*, zwitterionic/unionized tautomerism. As seen in *Fig. 1*, this criterion is not completely fulfilled in this case, the two isosbestic points at 280 nm and 344 nm not being sharp enough. This discrepancy indicates that other chemical equilibria in addition to the zwitterionic/unionized tautomerism must exist. The values thus obtained can be considered as approximations.

To estimate microscopic constants, we nevertheless calculated the K_z constants. From the spectroscopic data, $K_{z(\%)}$ values were calculated, and an empirical equation relating them to dioxane concentration ($C_{dioxane}$ [wt-%]) was obtained.

$$\log K_{z(\%)} = -0.032\, C_{dioxane} + 1.57 \tag{6}$$
$$n = 21 \qquad r = 0.999 \qquad s = 0.06$$

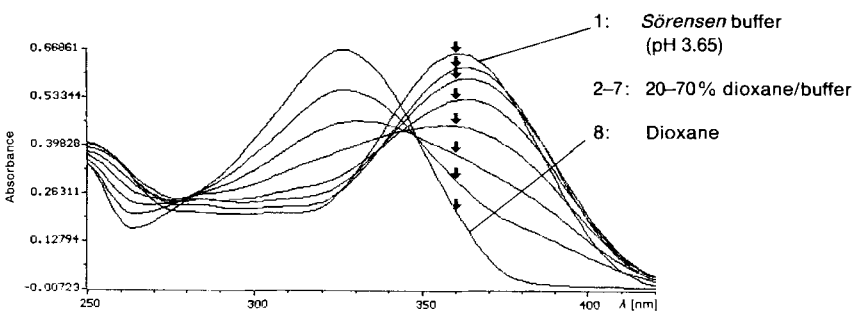


Fig. 1. *UV Spectra of piroxicam* (**1**) *in mixtures of* Sörensen *buffer* (pH 3.65) *and dioxane*

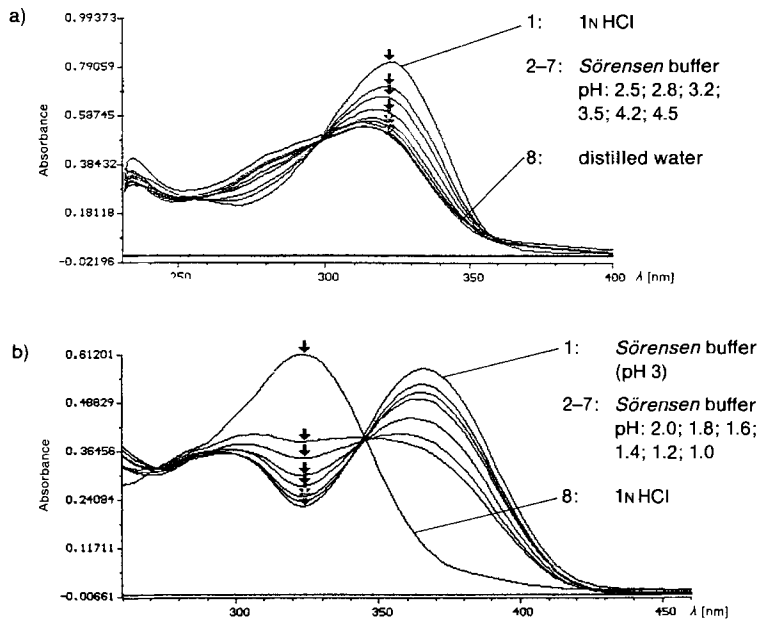


Fig. 2. *pH-Dependent UV spectra of piroxicam derivatives:* a) *compound* **2**, b) *compound* **3**

Table 1. *Micro- and Macroscopic p*K_a *Values of Piroxicam* (**1**)[a])

	Estimated values[b])	Values determined through K_z[c])	Values determined through pK_{a22}	Values determined by NMR method[d])
pK_{a12}	*ca.* 4	3.88	4.60	–
pK_{a21}	5.44	5.45	5.40	–
pK_{a22}	3.32	3.44	2.72[e])	–
pK_{a11}	1.88	1.87	1.92	–
pK_{a1}	1.86[f])	1.86[f])	1.86[f])	2.00
pK_{a2}	5.46[f])	5.46[f])	5.46[f])	5.45

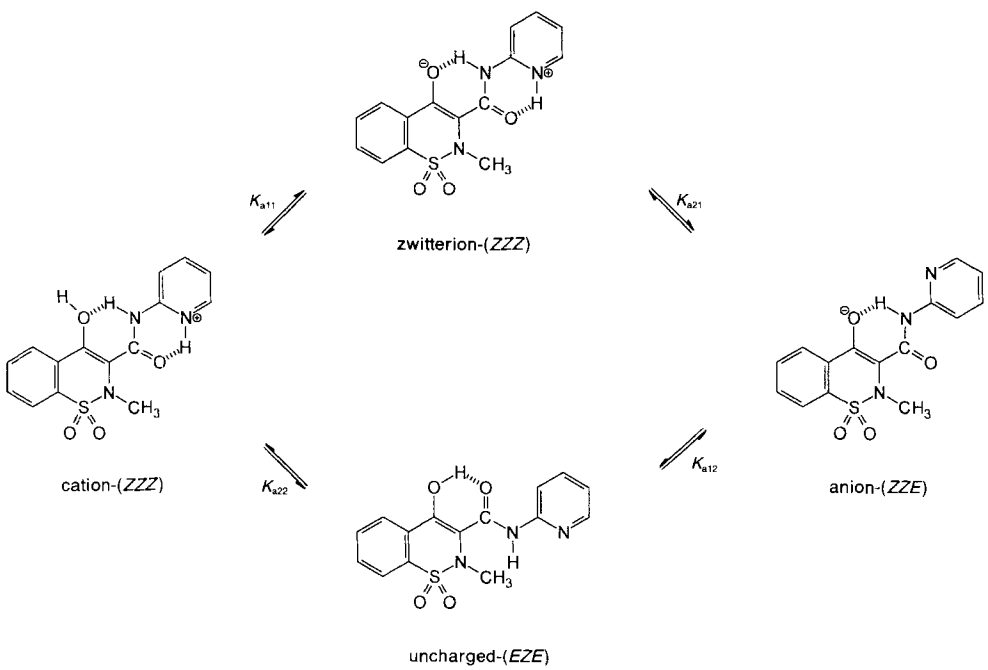
[a]) See *Scheme 1* for the denotation of microscopic pK_a.
[b]) Data taken from [4].
[c]) The values can be considered approximative only since the isosbestic points in *Fig. 1* are not sharp enough.
[d]) The macroscopic pK_a values are calculated based on the results from *Fig. 3, a.*
[e]) The value is taken from the pK_a of compound **2** and considered as the microscopic ionization constant in the neutral/cationic equilibrium.
[f]) Data taken from [15].

The aqueous log K_z value is the intercept of *Eqn. 6* (1.57), yielding the microscopic pK_a calculated based on *Eqns. 1–3*. Interestingly, the values thus obtained are very similar to those previously estimated (see *Table 1*).

Microscopic Ionization Constants Using the Deductive Method to Determine pK_{a22}. This method is in fact the most often used approach for the determination of microscopic pK_a values [14], the accuracy of these values being highly dependent upon the choice of

derivatives with a reduced number of ionizable groups. Generally, the use of a methyl-ether derivative has been shown to be appropriate for this purpose. UV Spectroscopic studies show that the electronic environment of the pyridyl N-atom in compound **2** is similar to that in neutral piroxicam (*Fig. 2, a*) as described in previous section. The pK_a value of compound **2** (2.72 from UV spectroscopic results in *Fig. 2, a*) was thus taken as the microscopic pK_{a22} of piroxicam referring to the cationic/unionized equilibrium and the other microscopic pK_a values calculated and compiled in *Table 1* according to *Eqns. 1–3*. The pK_a value of zwitterionic compound **3** (1.05 from UV spectroscopic results in *Fig. 2, b*) is, however, rather different from the microscopic pK_{a11} (1.92) as derived from the pK_a of **2**. It is unlikely that the pK_{a11} is smaller than the macroscopic pK_{a1} (1.86). This suggests that *N*-methylation might have altered to some extent the electronic structure in the vicinity of enolic group. Thus, it would be misleading to use the pK_a of **3** as pK_{a11} and calculate other microscopic pK_a values. The best microscopic pK_a values appear to be those obtained by the deductive method using the pK_a of compound **2** as pK_{a22}.

Scheme 2. *Microscopic Protonation/Deprotonation Equilibria of Piroxicam* (**1**)



As seen from all the microscopic pK_a values derived from different methods (*Table 1*), the main deprotonation route appears to be cation-(*ZZZ*)⇌zwitterion-(*ZZZ*)⇌anion-(*ZZE*). The route cation-(*ZZZ*)⇌uncharged-(*EZE*)⇌anion-(*ZZE*) is a minor one (see *Scheme 2*).

^{1}H-NMR Chemical Shifts of Piroxicam (**1**) *in Various Electric States.* The change in chemical shifts in ^{1}H-NMR spectroscopy upon protonation/deprotonation can often

provide insight into molecular electronic structure. *Fig. 3, a* and *b*, show the changes in chemical shift of several protons, when pH is increased from 1 to 10. Macroscopic pK_a values can be calculated using the results of chemical shifts, yielding $pK_{a1} = 2.00$ and $pK_{a2} = 5.45$ in accordance with the results from UV spectroscopic methods [15] (see *Table 1*).

All ^{1}H-NMR signals in the benzothiazine and pyridyl rings show significant shifts, when the enolic or pyridyl group changes its electric state, implying an extended electron

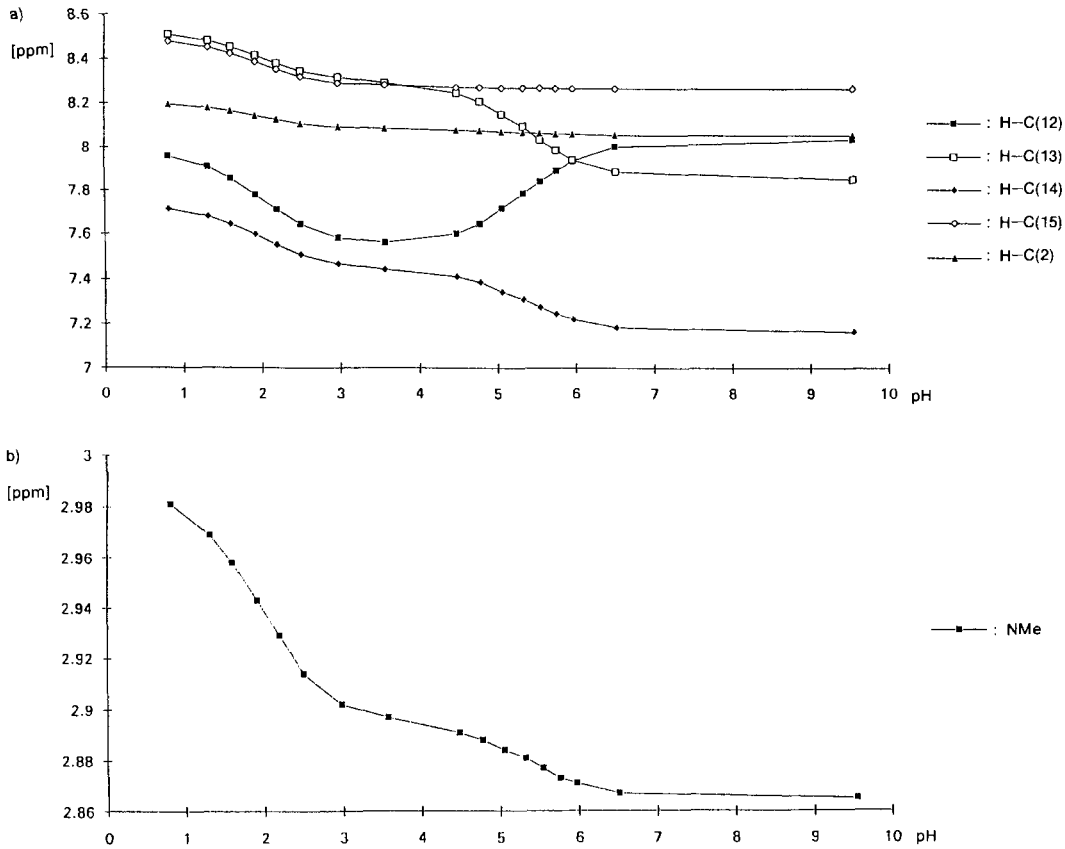


Fig. 3. *^{1}H-NMR Chemical shifts of piroxicam* (**1**) *as a function of pH* (see formula **1** for the numbering)

Table 2. *Partition Coefficient in Octan-1-ol/H_2O of Piroxicam* (**1**)[a]) *and Zwitterionic Compound* **3** *at Various pH*

	pH	log *D*	log *P*[b])
Piroxicam (**1**)	2.11	1.61	2.67
	3.08	1.76	2.65
	4.17	1.76	2.65
Compound **3**	7.20	0.18	0.18

[a]) The distribution coefficients of piroxicam at various pH were taken from [4].
[b]) The partition coefficient (log *P*) of piroxicam was calculated using *Eqn. 7*.

conjugation in the molecule. For example, the chemical shifts of pyridyl protons are sensitive to the protonation of its N-atom as well as the protonation of the enolate group (*Fig. 3, a*). Similarly, the Me protons of the sulfonamide group show upfield shifts in both protonation steps. It should be noted that all signals show expected upfield shifts upon the deprotonation of the enolic group. This is, however, not the case when the pyridinium function is deprotonated, H–C(12) displaying a downfield shift and H–C(15) a small change. This may be due to a conformational change from the zwitterion-(*ZZZ*) rotamer to the anion-(*ZZE*) rotamer concomitant with the deprotonation of the pyridinium group (see *Scheme 2*).

Conformational Behavior and Proton Affinity of Compound **2**. For the uncharged form of compound **2**, the lowest-energy conformer was found to take a planar (*ZZE*)-conformation with an internal H-bond MeO···HNC=O (heat of formation = −54.96 kcal/mol). This is in good agreement with the conformation of the ethanolamine salt of piroxicam found by X-ray crystallography [5] [6]. As for the protonated form of **2**, the planar (*ZZZ*)-rotamer with two internal H-bonds, MeO···HNC=O and HNC=O···$^+$HN (heat of formation = 85.44 kcal/mol), which has a similar conformation to the stable (*ZZZ*)-rotamer of zwitterionic piroxicam [4], appears to be the one with a minimum energy. Indeed, the calculated proton affinity of the pyridyl group of **2** (226.76 kcal/mol), by taking the heat of formation of H^+ as 367.16 kcal/mol, is similar in magnitude to that of neutral piroxicam (221.05 kcal/mol) [4]. This also justifies the use of **2** to determine the microscopic ionization constant pK_{a22} of piroxicam as discussed above.

Partitioning of Zwitterionic Piroxicam in Octan-1-ol/H_2O. The solvation/hydration of piroxicam in octan-1-ol/H_2O at various ionization states has been studied using its distribution coefficient-pH profile [4]. In view of the modest zwitterionic/neutral tautomeric ratio of piroxicam, it is reasonable to assume that the observed distribution coefficients (log *D*) in the pH range 2–4 are accounted for overwhelmingly by the neutral form, the contribution of the zwitterionic form being negligible. Thus, we assume that the partition coefficient of neutral piroxicam (log *P*) can be calculated using *Eqn. 7* [16].

$$\log P = \log D + \log[1 + 10^{(pK_{a22} - pH)} + 10^{(pH - pK_{a12})} + K_z] \quad (7)$$

where pK_{a22} and pK_{a12} of piroxicam are values from the study of analogue **2** (*Table 1*, third column). The log *D* values thus calculated yield an average log *P* of 2.66 ± 0.01, the negligible SD validating the assumption (see *Table 2*).

The partition coefficient of zwitterionic compound **3**, while not being identical to that of zwitterionic piroxicam, should in principle have a value similar to that of zwitterionic piroxicam. The log *P* of compound **3** as determined from the CPC method shows a value of 0.18 ± 0.02 (*Table 2*). If this value is taken as an estimate of the log *P* of zwitterionic piroxicam, the difference in log *P* between zwitterionic and neutral piroxicam would be −2.48. Interestingly, *Abraham* and *Leo* [17] have concluded that a similar value, −2.3, should be taken into account when calculating the log *P* of α-amino acids as compared to the neutral form in the CLOGP algorithm. Since the lipophilicity of zwitterionic molecules is highly sensitive to the intercharge distance [18], this result can be interpreted to mean that the two opposite charges in piroxicam are in close proximity as in α-amino acids.

Implications on the Intercharge Distance of Zwitterionic Piroxicam in Solution. The electrostatic influence on the pK_a of an ionizable group is a well-documented chemical

phenomenon. *Westheimer* and *Kirkwood* [19], and *Westheimer* and *Shookhoff* [20] have followed the theoretical treatment of *Bjerrum* and formulated a new theory by replacing the dielectric constant of the solvent with an 'effective' dielectric constant, which considers molecules as cavities of low dielectric constant. The electrostatic effect in a zwitterionic molecule could in fact be represented by interactivity parameter, ΔpK_a ($= pK_{a12} - pK_{a11} = pK_{a21} - pK_{a22}$; see *Scheme 1*). In the cases of zwitterionic amino acids, the authors found the calculated intercharge distances derived from ΔpK_a comparable to those determined from the measurement of dielectric increments [20]. While it is not obvious how this theory may be applied to flexible molecules of complex structure, the nature of the electrostatic effect as opposed to the through-bond inductive effect is probably operating through space by altering the local neighboring solvent structure. If the ΔpK_a value of several zwitterionic amino acids is plotted against their intercharge distance (R in Å) determined from dielectric increment measurements, an empirical exponential equation can be fitted.

$$\Delta pK_a = 14.92 \exp\left(-\frac{R}{1.6}\right) \qquad (8)$$

$$n = 7 \qquad r^2 = 0.985$$

To test the applicability of this equation to other types of zwitterions, the intercharge distance of picolinic acid (2.5 Å), nicotinic acid (3.5 Å), and isonicotinic acid (4.1 Å) were calculated using their ΔpK_a values (*Table 3*) and *Eqn. 8*. These values are reasonable in view of their relatively rigid structure. In the case of zwitterionic piroxicam, an intercharge distance of 2.7 Å is obtained, if its ΔpK_a value (2.68) is introduced into *Eqn. 8*. This calculated short intercharge distance together with the results from ^{1}H-NMR spectroscopic studies and lipophilicity measurements corroborates the charge delocalization nature of piroxicam which draws the two opposite charges in close proximity.

Table 3. *Microscopic pK_a Values of Relatively Rigid Pyridine-carboxylic Acids*

	pK_{a1}[a])	pK_{a2}[a])	pK_{a11}[b])	pK_{a21}[b])	pK_{a22}[b])	pK_{a12}[b])	ΔpK_a[c])
Picolinic acid	1.07	5.39	1.10	5.36	2.21	4.26	3.16
Nicotinic acid	2.14	4.82	2.17	4.79	3.13	3.83	1.66
Isonicotinic acid	1.88	4.38	1.90	4.36	3.26	3.00	1.10

[a]) The values were taken from [21].

[b]) The microscopic pK_a values were calculated by taking the pK_a of methyl esters [21] as pK_{22} and using *Eqns. 1–3*.

[c]) $\Delta pK_a = pK_{a12} - pK_{a11} = pK_{a21} - pK_{a22}$.

Conclusion. – Most clinically useful non-steroidal anti-inflammatory drugs with a carboxylic function have pK_a values in the range of 3–5 [22]. These compounds exist predominantly at pH 2–3 as the neutral form which is absorbed in the stomach and thus might contribute to adverse gastric side-effects [23]. It was argued by *McCormack* and *Brune* [24] that the anti-inflammatory drug azapropazone, which is insignificantly absorbed in the stomach, is of zwitterionic nature and hence devoid of gastric side-effects due to this physicochemical character. The authors also proposed the use of zwitterionic agents as a logical approach towards a stomach-sparing NSAID therapy.

Among anti-inflammatory oxicams, non-zwitterionic isoxicam, and sudoxicam had to be withdrawn from the market and discontinued from clinical assays, respectively, due to unwanted side-effects, while zwitterionic piroxicam and tenoxicam remain successful drugs [25]. One possible explanation for this discrepancy is the favorable tissue or organ distribution of zwitterionic oxicams. The physicochemical study of piroxicam in this paper thus sheds light on two aspects: on the one hand, it provides a molecular interpretation of the microscopic protonation/deprotonation behavior of piroxicam; and on the other hand, it offers a design concept to develop future NSAIDs with less potential for gastric mucosal damage.

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REFERENCES

[1] J. G. Lombardino, E. H. Wiseman, *Trends Pharmacol. Sci.* **1981,** 132.
[2] J. G. Lombardino, E. H. Wiseman, *Med. Res. Rev.* **1982,** *2,* 127.
[3] S. Winiwarter, H. J. Roth, *Pharm. Acta Helv.* **1994,** *68,* 181.
[4] R.-S. Tsai, P.-A. Carrupt, N. El Tayar, Y. Giroud, P. Andrade, B. Testa, F. Brée, J.-P. Tillement, *Helv. Chim. Acta* **1993,** *76,* 842.
[5] J. Bordner, P. D. Hammen, E. B. Whipple, *J. Am. Chem. Soc.* **1989,** *111,* 6572.
[6] P. D. Hammen, H. Berke, J. Bordner, A. C. Braisted, J. G. Lombardino, E. B. Whipple, *J. Heterocycl. Chem.* **1989,** *26,* 11.
[7] A. I. Vogel, 'Textbook of Practical Organic Chemistry', 4th edn., John Wiley, New York, 1989, p. 291.
[8] D. E. Metzler, E. E. Snell, *J. Am. Chem. Soc.* **1955,** *77,* 2431.
[9] A. Albert, E. P. Serjeant, 'Determination of Ionization Constants', Chapman and Hall, London, 1971.
[10] N. El Tayar, R.-S. Tsai, P. Vallat, C. Altomare, B. Testa, *J. Chromatogr.* **1991,** *556,* 181.
[11] R.-S. Tsai, P.-A. Carrupt, B. Testa, in 'Countercurrent Chromatography', Eds. W. D. Conway and R. Petroski, ACS symposium monograph, in press.
[12] K. Takács-Novák, M. Tókés-Kövesdi, G. Szász, B. Noszál, *Magy. Kém. Foly.* **1994,** *100,* 538.
[13] K. Takács-Novák, A. Avdeef, J. K. Box, B. Podány, G. Szász, *J. Pharm. Biomed. Anal.* **1994,** *12,* 1369.
[14] B. Noszál, in 'Biocoordination Chemistry: Coordination Equilibria in Biologically Active Systems', Ed. K. Burger, Ellis-Horwood, Chichester, 1989, pp. 18–55.
[15] E. Bernhard, F. Zimmermann, *Arzneim.-Forsch. Drug. Res.* **1984,** *34,* 647.
[16] K. Takács-Novák, M. Józan, I. Hermecz, Gy. Szász, *Int. J. Pharmac.* **1992,** *79,* 89.
[17] D. J. Abraham, A. Leo, *Proteins: Structure, Function, and Genetics* **1989,** *5,* 78.
[18] R.-S. Tsai, B. Testa, N. El Tayar, P.-A. Carrupt, *J. Chem. Soc., Perkin Trans. 2* **1991,** 1797.
[19] F. H. Westheimer, J. G. Kirkwood, *J. Chem. Phys.* **1938,** *6,* 513.
[20] F. H. Westheimer, M. W. Shookhoff, *J. Am. Chem. Soc.* **1939,** *61,* 555.
[21] A. Leo, C. Hansch, 'The Pomona College Medicinal Chemistry Project', Pomona College, Claremont, California, 1993.
[22] J. G. Lombardino, I. G. Otterness, E. H. Wiseman, *Arzneim.-Forsch. Drug. Res.* **1975,** *25,* 1629.
[23] K. Brune, H. Gubler, A. Schweitzer, *Pharmacol. Ther.* **1979,** *5,* 199.
[24] K. McCormack, K. Brune, *Arch. Toxicol.* **1990,** *64,* 1.
[25] E. Albengres, S. Urien, J. Barre, P. Nguyen, F. Brée, P. Jolliet, J.-P. Tillement, R.-S. Tsai, P.-A. Carrupt, B. Testa, *Int. J. Tiss. React.* **1993,** *15,* 125.