47. Physicochemical and Structural Properties of Non-steroidal Anti-inflammatory Oxicams

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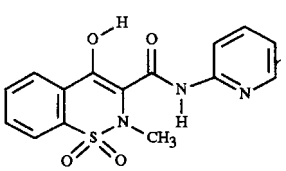
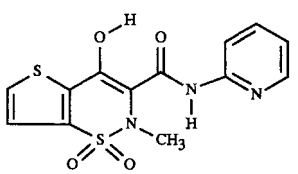
(1.XII.92)

Using the five therapeutic oxicams **1–5**, we showed that isosteric replacements result in remarkable changes in the physicochemical and structural properties of congeners. Thus, the acidity of the phenolic OH group is relatively higher in the oxicams containing a pyridinyl moiety, *i.e.* in piroxicam (**1**), tenoxicam (**2**), and lornoxicam (**3**), due to their zwitterionic nature. This consequently influences their lipophilicity profile at different ionization states. Furthermore, partitioning behaviour in octan-1-ol/H_2O and heptane/H_2O systems suggests an internal H-bond between the enolic OH and the amide C=O group. The anionic oxicams readily partition into the octanol phase at pH 7.4 and not at all into the heptane phase. Only the partition coefficients of oxicams measured in the heptane/H_2O system, but not in the octanol/H_2O system, correlate with their transfer across the blood-brain barrier. This implies that only the neutral form of oxicams crosses the blood-brain barrier.

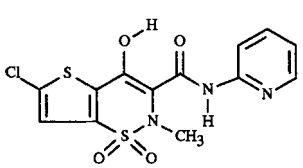
Introduction. – Oxicams have emerged as a novel, long-acting class of non-steroidal anti-inflammatory drugs (NSAID's) [1] [2]. Not only have these drugs aroused pharmacological and therapeutic interest, they also provided profound chemical insights in terms of their dynamic structural features. *E.g.*, piroxicam (= 4-hydroxy-2-methyl-*N*-(pyridin-2-yl)-2*H*-1,2-benzothiazine-3-carboxamide 1,1-dioxide; **1**) was shown to have 12 possible tautomers, the tautomeric shifts resulting from fast internal proton transfers being unusually sensitive to changes in chemical substitution, solvent, and temperature [3].



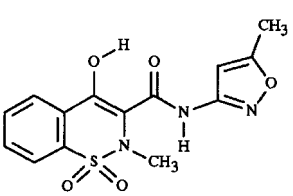
1 piroxicam



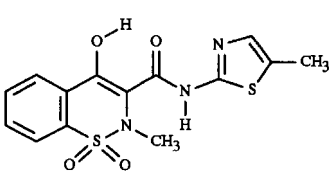
2 tenoxicam



3 lornoxicam



4 isoxicam



5 meloxicam

Most oxicams are congeneric compounds generated by the concept of *isosteric replacement* in drug design [4]. In this approach, groups or fragments of the lead compound are substituted with moieties of similar stereoelectronic features in order, *e.g.*, to improve pharmacokinetic properties such as a higher resistance to chemical or enzymatic degradation. The prerequisite of these group substitutions is that the congeners should have similar, or even better, pharmacodynamic properties. Thus, replacement of the benzo ring of piroxicam (**1**) with a thieno ring yielding tenoxicam (**2**) or with a 2-chlorothieno ring yielding lornoxicam (**3**) should not, in principle, dramatically influence the lipophilicity (conventionally expressed as partition coefficient in octan-1-ol/H_2O, log P_{oct}), since the log P_{oct} values of thiophene (1.81) and 2-chlorothiophene (2.54) are of similar magnitude to that of benzene (2.13) [5]. However, electron-withdrawing effects of the S-atom may change the electronic properties of neighbouring groups to some extent. Replacement of the pyridin-2-yl ring of **1** with a 5-methylisoxazol-3-yl ring yielding isoxicam (**4**) or with a 5-methylthiazol-2-yl ring yielding meloxicam (**5**) also appears to be 'isolipophilic', since the liphophilicity of 5-methylisoxazole (log $P_{oct} = 0.45$) or 5-methylthiazole (log $P_{oct} = 0.71$) is similar to that of pyridine (log $P_{oct} = 0.65$) [5]. Apparently, this substitution also leads to a dramatic decrease in the basicity of the N-atom of the carboxamide-substituting heterocycle and hence a change in electronic features.

It should be noted that the above qualitative descriptions and predictions of physicochemical properties based on additive group contributions may not be valid, if subtle intramolecular interactions exist. In such a case, physicochemical measurements are needed to understand better the structural properties of oxicams in solution. The formation of zwitterions of piroxicam (**1**) in polar solvent as demonstrated by ^{13}C-NMR spectroscopy thus suggests intramolecular interactions [6]. Surprisingly, few studies regarding the physicochemical properties of oxicams were reported. Contradictory attributions of their ionization constants can be found in the literature [7] [8]. As repeatedly demonstrated, the partitioning behaviour of solutes in different biphasic solvent systems can unravel valuable structural information. To understand better the physicochemical and structural properties of the five therapeutic oxicams piroxicam (**1**), tenoxicam (**2**), lornoxicam (**3**), isoxicam (**4**), and meloxicam (**5**), we studied their acid-base behaviour, the partitioning in octan-1-ol/buffer and heptane/buffer systems in different ionization states using centrifugal partition chromatography (CPC), the solvation behaviour of anionic species in the octanol phase using first-derivative UV spectrophotometry, the H-bond donating capacity using the parameter $\Delta \log P_{oct\text{-}hep}$ (*i.e.* log P_{oct} minus log P_{hep}), and the H_2O-accessible surface area. The present study should help shed light on the structure-property relationships of oxicams and perhaps also on their property-activity relationships.

Results and Discussion. – *Acid-Base Behaviour of Oxicams.* There are some contradictory reports in the literature as to attribution of the ionization constants of oxicams. While *Wiseman et al.* [7] assigned pK_a 6.3 measured in dioxane/H_2O to the enolic group of **1**, *Bernhard* and *Zimmermann* [8] attributed a remarkably different value of 1.86 to the acidic enolic group and 5.46 to the basic pyridin-2-yl group based on UV spectroscopic titrations. The latter value thus implies the zwitterionic nature of pyridine-containing oxicams, *i.e.* **1**–**3**. The fact that significant amounts (25%) of zwitterionic piroxicam were detected in DMSO at room temperature using ^{13}C-NMR spectroscopy [6] is also suggestive of the lower pK_a value of the acidic group as compared to that of the basic group. The

zwitterionic *N*-alkylated pyridin-2-yl derivatives were reported to resemble zwitterionic piroxicam in a number of physical properties such as high melting point and yellow colour.

The dissociation constants of oxicams **1–5** measured in this study or taken from *Bernhard* and *Zimmermann* are compiled in *Table 1*. Illustrated in *Fig. 1* are the spectral

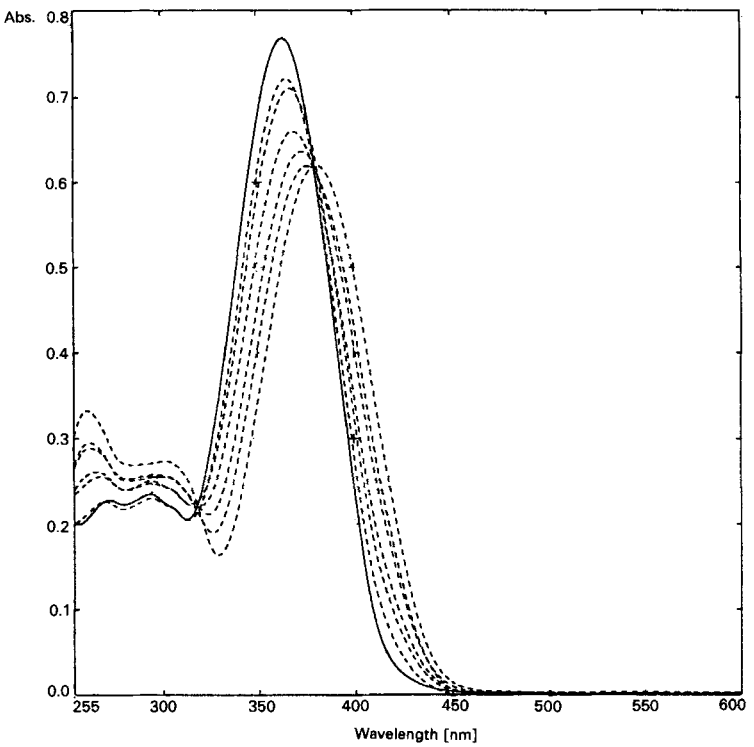


Fig. 1. *UV-Spectral changes of lornoxicam* (**3**) *due to protonation/deprotonation at pH 0.12* (full line), *0.46, 0.68, 0.85, 1.14, 1.40, and 2.79* (dotted lines increasingly removed from the full line)

Table 1. *Dissociation Constants of Oxicams* **1–5** *in* H_2O *and* H_2O/*EtOH*

	H_2O		H_2O/EtOH 1:1 (*v*/*v*)		H_2O/EtOH 1:4 (*v*/*v*)
	pK_{a1}	pK_{a2}	pK_{a1}	pK_{a2}	pK_{a1}
1	1.86[a])	5.46[a])	–	5.26 (±0.01)[d])	–
2	1.07[a])	5.34[a])	–	4.95 (±0.02)[d])	–
3	0.85 (±0.06)[b])	5.59[b])	–	4.69 (±0.01)[d])	–
4	3.93[a])	–	–[c])	–	4.85 (±0.01)[d])
5	4.08[b])	–	4.24 (±0.01)[d])	–	4.63 (±0.03)[d])

[a]) Data taken from [8] (UV-spectrophotometric titrations).
[b]) Measured using UV-spectrophotometric titrations.
[c]) Non measurable due to precipitation during titration.
[d]) Measured using potentiometric titrations.

changes due to the protonation/deprotonation of the enolic group of **3**, which are similar to those displayed by **1** and **2** upon protonation/deprotonation of their enolic group [8]. To confirm further the attribution and hence the zwitterionic nature of **1–3**, their pK_a values were determined by potentiometry in H_2O/EtOH. It is generally agreed that, when going from H_2O to media of lower polarity such as H_2O/EtOH, the pK_a of acidic and basic groups should increase and decrease, respectively, due to a less favourable solvation of charged species. Thus, correct pK_a attributions of the acidic and basic group of a zwitterionic molecule can also be performed by comparing the pK_a values in H_2O and in H_2O/EtOH. Indeed, the results (*Table 1*) show that the basicity of the pyridin-2-yl group (pK_{a2}) of **1–3** is lower in H_2O/EtOH 1:1 than in pure H_2O, confirming the pK_a attribution by *Bernhard* and *Zimmermann* [8]. On the contrary, the pK_a values of acidic isoxicam (**4**) and meloxicam (**5**) increase in H_2O/EtOH.

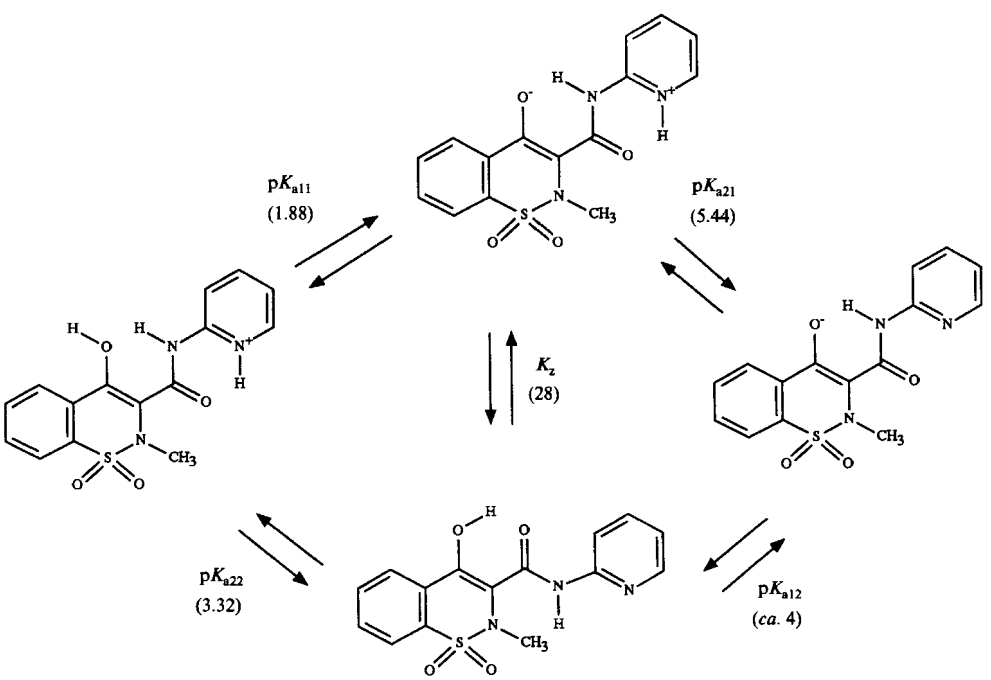
The remarkable acidity of the enolic group of **1–3** as compared to that of **4** and **5** is not straightforward to explain. A characterization of the microscopic ionization behaviour of **1** reveals that four rather than two deprotonation/protonation processes are involved (see *Scheme*). It follows that the macro- and microscopic ionization constants can be related as in *Eqns. 1–3* [9]

$$K_{a1} = K_{a11} + K_{a22} \tag{1}$$

$$1/K_{a2} = 1/K_{a21} + 1/K_{a12} \tag{2}$$

$$K_z = K_{a11}/K_{a22} = K_{a12}/K_{a21} \tag{3}$$

Scheme. *Microscopic Ionization Behaviour of Piroxicam* (**1**)



where K_z is the equilibrium constant describing the ratio of the zwitterionic to neutral form in aqueous solution, which is independent of pH. The pK_{a12} of **1** is estimated to be *ca.* 4 since substitution of the pyridin-2-yl group with a 5-methylisoxazol-3-yl ring (see **4**, pK_a 3.93) or a 5-methylthiazol-2-yl ring (see **5**, pK_a 4.08) does not significantly influence the dissociation constant of the enolic OH group. The other microscopic ionization constants can thus be calculated based on *Eqns. 1–3*. As shown in the *Scheme*, an enhanced acidity of the enolic OH group (*ca.* 2.1 pK_a units) is observed due to the presence of the charged ammonium group which exerts electrostatic effects upon the enolic OH group.

To support these interpretations, theoretical studies on the stability (heat of formation) and proton affinity of oxicams using semiempirical MO calculations were performed. It should be noted that the MNDO [10] or AM1 [11] method cannot be used in the calculations of anionic oxicams, the geometry optimization leading to a cleavage of the S–N bond. In contrast, the PM3 method [12] yields coherent geometries for all electrical forms of the oxicams. The results of PM3 calculations (*Table 2*) reveal that the gas-phase acidity of the enol function is enhanced by the pyridinium positive charge, $PA_1(N)$ being greater than $PA_1(Z)$ (PA = proton affinity; N, Z, A, and C = neutral, zwitterionic, anionic, and cationic form, resp.). Similarly, proton affinities of the pyridine ring are also enhanced by the presence of the enolate negative charge in zwitterionic oxicams.

Table 2. *Heat of Formation and Proton Affinity of Oxicams.* Energies in kcal/mol.

	$\Delta H_f(N)$[a])	$\Delta H_f(Z)$[b])	$\Delta H_f(A)$[c])	$\Delta H_f(C)$[d])	$PA_1(N)$[e])	$PA_1(Z)$[f])	$PA_2(N)$[g])	$PA_2(Z)$[h])
1	−68.31	−50.16	−118.59	77.80	316.88	298.73	221.05	239.20
2	−58.95	−41.82	−111.62	87.95	314.49	297.36	220.26	237.39
3	−60.88	−44.34	−115.13	86.11	312.91	296.37	220.17	236.71
4	−70.64	–	−123.21	–	314.59	–	–	–
5	−60.63	–	−111.50	–	316.29	–	–	–

[a]) Heat of formation of the neutral form (N).
[b]) Heat of formation of the zwitterionic form (Z).
[c]) Heat of formation of the anionic form (A).
[d]) Heat of formation of the cationic form (C).
[e]) Proton affinity (PA) of the enolate function in N, the heat of formation of H^+ being taken as 367.16 kcal/mol.
[f]) PA of the enolate function in Z.
[g]) PA of the pyridine function in N.
[h]) PA of the pyridine function in Z.

However, it should be noted that the order of proton affinity of oxicams does not parallel that of their pK_a values due to the differences in the stability of zwitterion and neutral forms in the gas phase and H_2O solution [13]. Taken together, these results corroborate the enhanced acidity of pyridine-containing oxicams due to electrostatic effects.

The electrostatic effects significantly influence the acid-base behaviour of zwitterionic molecules provided that the distance between the opposite charges is within 5 Å, as seen with the enhanced acidity of the COOH of α-amino acids (a decrease of *ca.* 2 pK_a units) due to the influence of the geminal NH_3^+ group [14]. The implication of our results is that the two opposite charges of zwitterionic oxicams are in close proximity in aqueous solution and that their hydration shells overlap. This may explain the low aqueous

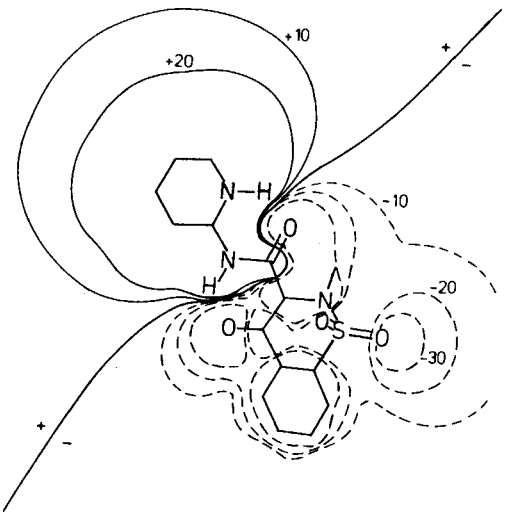


Fig. 2. *Charge delocalization in zwitterionic piroxicam* (**1**)

solubility of **1–3** at their isoelectric pH (around 3.7). The close proximity of these opposite charges was indeed supported by the molecular electrostatic potential of piroxicam (**1**) in zwitterionic form (*Fig. 2*), the distance between the centroid of positive and negative regions being less than 5 Å.

Partitioning of Oxicams in Different Ionization States in Octan-1-ol/Buffer. The distribution coefficients of the oxicams **1–5** in different ionization states in octan-1-ol/buffer are compiled in *Table 3*. The lipophilicity of the neutral (log P_{oct}) and zwitterionic (log P^{Z}_{oct}) forms cannot be obtained directly for **1–3**, since their maximal log D_{oct} values at a pH

Table 3. *Distribution Coefficients* (log D_{oct}) *of Oxicams* **1–5** *in Octan-1-ol/H_2O at Different pH's*[a])

	log D_{oct}						log P^{A}_{oct}[b])	log P_{oct}[c])
	pH 2.11	pH 3.08	pH 4.17	pH 5.24	pH 6.07	pH 7.40		
1	1.61 (±0.01)	1.76 (±0.01)	1.76 (±0.01)	1.76 (±0.02)	1.20 (±0.02)	−0.05 (±0.02)	−0.83 (±0.02)	–
2	0.81 (±0.01)	–[d])	0.84	0.63 (±0.01)	0.23 (±0.00)	−0.32 (±0.02)	−0.64 (±0.01)	–
3	1.71 (±0.01)	1.77 (±0.01)	1.72	1.66 (±0.00)	1.13 (±0.01)	0.61 (±0.01)	0.08 (±0.01)	–
4	–[d])	2.72 (±0.01)	2.44 (±0.03)	1.55 (±0.01)	0.79 (±0.02)	−0.32 (±0.04)	−0.64 (±0.01)	2.83[e])
5	3.01 (±0.14)	–[d])	2.67 (±0.00)	1.88 (±0.01)	1.31 (±0.01)	0.09 (±0.02)	−0.22 (±0.04)	3.02[e])

[a]) The log D_{oct} are measured using CPC. Values in parentheses are the standard deviations of at least three measurements.

[b]) Partition coefficient (log P_{oct}) of oxicams in anionic form (measured at pH 12).

[c]) Partition coefficient of oxicams in neutral form.

[d]) Not measured.

[e]) The value is calculated from log D_{oct} at pH 2.11, 3.08, and 4.17.

value close to the isoelectric point are due to the partitioning of a mixture of neutral and zwitterionic species. However, log P_{oct} of neutral piroxicam (**1**) may not be much different from 3 as deduced from the log P_{oct} of **5** (3.02) and **4** (2.88) and the similar fragmental values of pyridine, 5-methylthiazole, and 5-methylisoxazole [5]. It is noted that the log P_{oct} value of **5** or **4** is relatively higher than the maximal distribution coefficients of **1–3**. Clearly, the isosteric replacements among oxicams have changed their acid-base behaviour as well as their partitioning behaviour in biphasic systems.

Lipophilicity of the anionic oxicams (log P^{A}_{oct}) in the form of ion pairs can be obtained from the log D_{oct} values measured at pH 12. Interestingly, the order of the log P^{A}_{oct} values does not parallel the log D_{oct} values measured at other pH values, implying that differences in the structural features among anionic oxicams do not parallel those among neutral ones. It is noted that the maximal log D_{oct} values of **1** and **3** are of similar magnitude (1.76), while the log P^{A}_{oct} value of **1** (−0.83) is significantly lower than that of **3** (0.08; see *Fig. 3a*). In contrast, the distribution coefficients of **4** and **5** are comparable at all pH's examined (*Fig. 3b*), implying the similar lipophilic expression of their charges. The distribution coefficients of **4** and **5** decrease significantly when the pH is increased

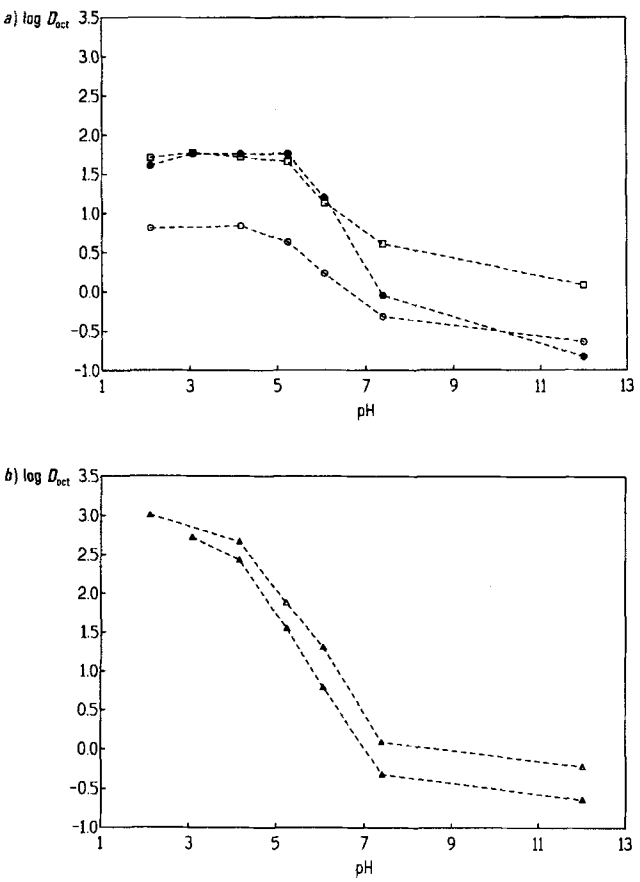


Fig. 3. *Distribution coefficients* a) *of piroxicam* (**1**; ●), *tenoxicam* (**2**; ○), *and lornoxicam* (**3**; □) *and* b) *of isoxicam* (**4**; ▲) *and meloxicam* (**5**; △) *at different ionization states*

from 4.17 to 5.24 (*Fig. 3b*) due to deprotonation of the enolic OH. The decrease is less marked for **1**–**3** (*Fig. 3a*) due to their predominant zwitterionic population in the aqueous phase in this pH range.

Solvation of Anionic Oxicams in Octan-1-ol/H_2O. To identify the electric forms partitioning into lipophilic media at physiological pH can offer insight into the nature of their protein or enzyme binding. Thus, the partitioning species in the octanol phase of an octanol/buffer (pH 7.4) system can be detected using first-derivative UV spectrophotometry which was shown to be far more sensitive than absorbance spectra in detecting the composition of different species [15]. It must be noted that the enolic group is completely ionized in highly basic solution (0.1M NaOH, pH 13), and hence the anionic species would be the only one partitioning into the octanol phase in the form of an ion pair. At pH near the isoelectric point (*ca.* 3.7), the species of zwitterionic oxicams partitioning into the octanol phase could well be a mixture of neutral and zwitterionic forms, their spectra being distinct from each other. As for **4** and **5**, the enolic OH group remains neutral when partitioning into the octan-1-ol phase from a highly acidic solution (0.1N HCl, pH 1). The percentage of anionic oxicams in the octanol phase at pH 7.4 can thus be determined by comparing and relating the spectra with those obtained under acidic and basic conditions. The results for piroxicam (**1**) at pH 7.4 are illustrated in *Fig. 4*, 67% of **1** being

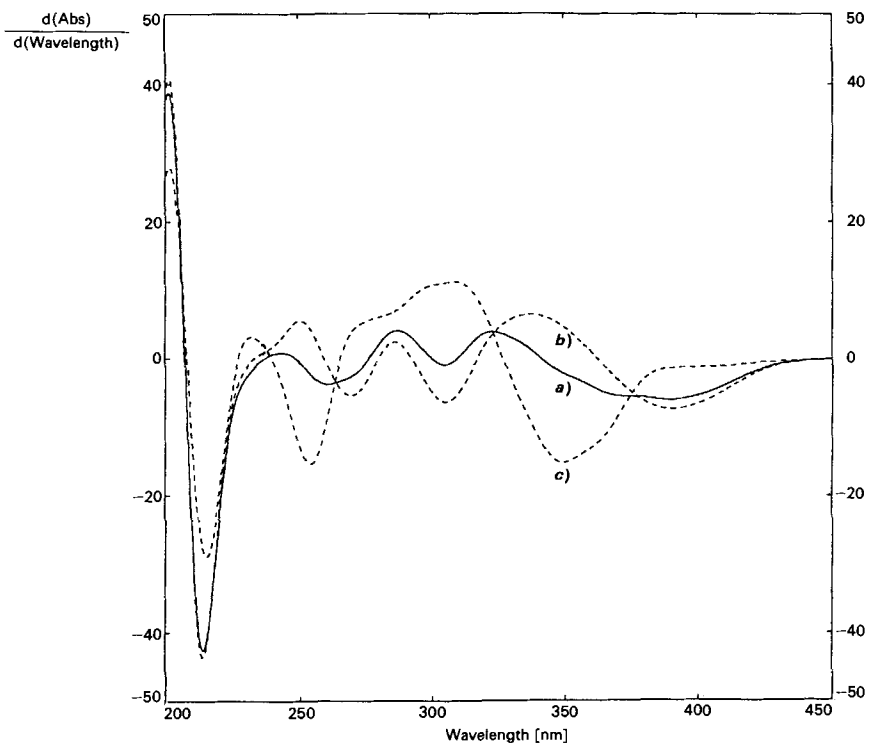


Fig. 4. *First-derivative UV spectra of piroxicam* (**1**) *in* a) *the octanol phase* (31.1 μM) *of octanol/buffer* (pH 7.40), b) *the octanol phase* (30.4 μM) *of octanol/buffer* (pH 13.1), *and* c) *the octanol phase* (31.2 μM) *of octanol/buffer* (pH 3.32)

detected as the anionic form and the rest (33%) in a mixture of neutral and zwitterionic states. Similarly, 89% of isoxicam (**4**) was detected in an anionic state and 11% in a neutral state (spectra not shown). On the other hand, **2**, **3**, and **5** appear to partition overwhelmingly as *anions* (*ca.* 100%) into the octanol phase at pH 7.4.

Taken together, these results suggest that the anionic oxicams can easily partition into octanol. This is of clear pharmacodynamic interest inasmuch as these compounds act by inhibiting the synthesis of prostaglandins, the inhibitors being mainly strong organic acids [16].

Polar Interactions of Anionic Oxicams with Solvent. Lipophilicity was successfully factorized into a cavity- or volume-related term V reflecting the energy required to create a cavity in the solvent (*i.e.* an endoergic term), and an exoergic interactive term A which results from polar solute-solvent interactions such as dipole-dipole and hydrogen bonding (see *Eqn. 4*) [17].

$$\log P = aV + A \tag{4}$$

Analysis of the structural information content encoded in A using *van-der-Waals* volume and solvatochromic parameters developed by *Kamlet*, *Taft*, *Abraham et al.*, namely the H-bond donating capacity α, the H-bond accepting capacity β, and the dipolarity/polarisability π^* of solutes, revealed that A derived from octan-1-ol/H_2O log P values (A_{oct}) is correlated mainly with β [18]. Although the structural information content of A_{oct} of anionic oxicams is yet to be examined, polar solute-solvent interactions reflecting the interactions of ion-dipole, dipole-dipole, and H-bonding must be major contributors. The markedly favourable hydration energy of a charge in comparison with that of neutral functional groups [19] implies that the main information content of A_{oct} of oxicams in anionic form may lie in the hydration feature of the charged enolic group. Thus, a plot of log P^A_{oct} against H_2O-accessible surface area (*Fig. 5*), the latter representing hydrophobicity, was used to examine differences in lipophilicity of anionic oxicams and hence in their charge characters. Interestingly, it was found that the oxicams with different rings in the amide side chain, *i.e.* piroxicam (**1**), isoxicam (**4**), and meloxicam (**5**), lie on the same line, while modifications at the benzo group, *i.e.* tenoxicam (**2**) and lornoxicam (**3**), are out of

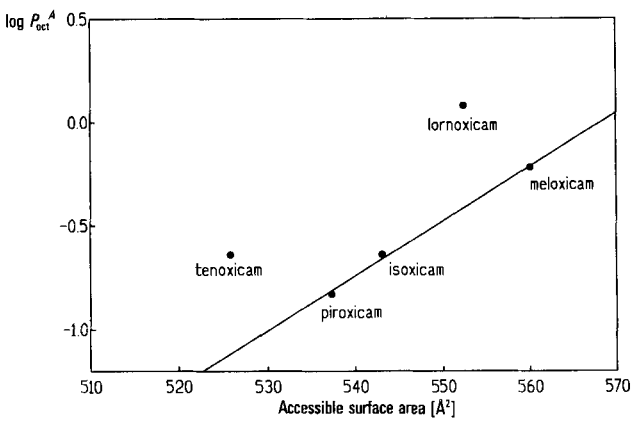


Fig. 5. *Plot of lipophilicity of anionic oxicams* (log P^A_{oct}) vs. *their* H_2O*-accessible surface area*

the line. These results suggest that differences in lipophilicity of the anionic form of **1**, **4**, and **5** are accounted for by their *van-der-Waals* interactions with the octanol phase, their charges eliciting similar interactions. The deviation of **2** and **3** from the line indicates that the interactions of the enolate with solvents must be perturbed due to through-space and/or through-bond electronic effects of the neighbouring S-atom, leading to an increase in their lipophilicity. Indeed, the lipophilicity of charged species was found to increase if a polar group is in close proximity to a charged group [20].

Partitioning of Oxicams in Different Ionization States in Heptane/Buffer. The distribution coefficients of oxicams in heptane/buffer (pH 6.0 and 7.4) are compiled in *Table 4*. For **4** and **5**, partition coefficients of their neutral form (log P_{hep}) are calculated since partitioning of the charged species must be negligible. The log P_{hep} values thus calculated from the distribution coefficients at pH 6.0 and 7.4 are relatively comparable (*Table 3*), confirming the non-significant contribution of ionic species to log D_{hep}. Assuming that the anionic and zwitterionic forms of **1** do not partition into the heptane phase, the log P_{hep} of neutral **1** can thus be calculated based on *Eqn. 5*:

$$\log P_{hep} = \log D_{hep} + \log [1 + 10^{(pK_{a11} - pH)} + 10^{(pH - pK_{a21})}] + \log K_z \qquad (5)$$

The calculated log P_{hep} values of **1** from log D_{hep} at pH 6.0 and 7.4 are of similar magnitude (1.86 and 1.95, resp.), indeed confirming the negligible partitioning of the anionic and zwitterionic species into the heptane phase. Assuming that the electrostatic effects enhancing the acidity of the enolic OH group in **1** (*i.e.* $pK_{a12} - pK_{a11}$), are of the same magnitude as in **2** and **3**, the microscopic ionization constants of **2** and **3** can thus be calculated using *Eqns. 1* and *2* (*Table 5*) and hence their log P_{hep} using *Eqn. 5* (*Table 4*).

Table 4. *Distribution Coefficients of Oxicams in Heptane/Buffer*[a])

	log D_{hep} at pH 6.0	log P_{hep}	log D_{hep} at pH 7.4	log P_{hep}
1	−0.26 (±0.02)	1.86[b])	−1.46 (±0.04)	1.95[b])
2	−1.45 (±0.02)	1.47[b])	−2.88 (±0.04)	1.35[b])
3	−0.61 (±0.01)	2.58[b])	−1.84 (±0.07)	2.62[b])
4	−1.14 (±0.01)	0.93[c])	−2.51 (±0.29)	0.96[c])
5	−1.03 (±0.02)	0.90[c])	−2.32 (±0.17)	1.00[c])

[a]) The distribution coefficients are measured using CPC. Values in parentheses are the standard deviations of at least three measurements.

[b]) Partition coefficients of the neutral form in heptane/H_2O, calculated using *Eqn. 5* and assuming that the anionic and zwitterionic species do not partition into the heptane phase.

[c]) Partition coefficients of the neutral form in heptane/H_2O.

Table 5. *Microscopic Ionization Constants of* **1**–**3**[a])

	pK_{a11}	pK_{a21}	pK_{a22}	pK_{a12}
1	1.88	5.44	3.32	4
2	1.07	5.34	3.24	3.17
3	0.85	5.59	3.49	2.95

[a]) The microscopic ionization constants of **2** and **3** were calculated using *Eqns. 1* and *2*, assuming that the electrostatic effects on the pK_a of **1** are the same as those in **2** and **3**, *i.e.* $pK_{a12} - pK_{a11} = 2.1$.

These log P_{hep} values calculated from log D_{hep} at pH 6.0 and 7.4 are again comparable. It is thus concluded that the neutral form and not the anionic or zwitterionic form of oxicams is the only one partitioning into the heptane phase.

Polar Interactions of Neutral Oxicams with Solvent. The partitioning of solutes in octanol/H_2O is of a different nature from that in heptane/H_2O [21]. As a result, it was also found that the structural information content of the parameter Δlog $P_{oct\text{-}hep}$ (*i.e.* log P_{oct} minus log P_{hep}) is mainly a measure of the H-bond donating capacity of solutes (*Eqn. 6*), although H-bond accepting capacity also contributes to a small extent [21].

$$\Delta \log P_{oct\text{-}hep} = 3.54(\pm 0.36)\,\alpha + 0.37(\pm 0.15) \qquad (6)$$

$$n = 75;\ r = 0.915;\ s = 0.450;\ F = 325.6$$

where n is the number of solutes, r the correlation coefficient, s the standard deviation of the regression, and F the *Fisher* test for significance of the equation. The 95% confidence limits are given in parentheses. In this theoretical study, 75 compounds with zero, one, or two H-bond donor groups (*e.g.*, OH, NH_2, *etc.*) were shown to have Δlog $P_{oct\text{-}hep}$ values ranging from −0.79 (pentane) to 4.65 (sulfathiazole). In comparison, the Δlog $P_{oct\text{-}hep}$ values of isoxicam (**4**; 1.87) and meloxicam (**5**; 2.02) imply a weak H-bond donating capacity, probably because the two strong H-bond donor groups (enolic OH and amide NH) are internally bonded.

Biological Implications of the Physicochemical Properties of Oxicams. The physicochemical properties investigated in this study were used to search for correlation with the binding of oxicams to human serum albumin [22–24] and their transfer across the blood-brain barrier (b.b.b.) [25]. No correlations were found between the physicochemical and structural properties established here for **1**–**5** and thermodynamic binding parameters such as ΔG^0, ΔH^0, and ΔS^0 (manuscript in preparation). Regarding transfer across the b.b.b., only log P_{hep} (or log D_{hep} at pH 7.4) was found to be correlated with the *in vivo* maximal brain extraction values at time zero, $E(0)$ [25] (*Eqn. 7*).

$$E(0) = 0.20 \log P_{hep} + 0.03 \qquad (7)$$

$$n = 4;\ r^2 = 0.91$$

Eqn. 7 is limited to 4 compounds since no $E(0)$ value is available for lornoxicam (**3**).

Note that the neutral form of oxicams is the only one partitioning into the heptane phase. These results thus suggest that the neutral form of oxicams is the one partitioning into and crossing the b.b.b. Admittedly, no correlation was found with log D_{oct} because the anionic and zwitterionic forms were shown to partition into octanol.

As previously demonstrated, a high H-bond donating capacity of drugs is detrimental to their crossing the b.b.b. The weak H-bond donating capacity of neutral **4** and **5** revealed by the parameter Δlog $P_{oct\text{-}hep}$ is compatible with their permeability across the b.b.b., as indeed observed [25].

Conclusion. – This study shows that isosteric replacements in piroxicam (**1**) lead to unexpected changes in the physicochemical properties of congeneric oxicams. Replacement of the pyridin-2-yl moiety transforms zwitterions into anions and, as a consequence, leads to a different partitioning behaviour in biphasic systems. Replacement of the benzo ring of **1** by a thieno or 2-chlorothieno ring remarkably influences the interactions of

enolate with solvent, which are much perturbed by the neighbouring S-atom. The results may be relevant to their pharmacological activity since a negative charge is an important structural requirement for binding to cyclooxygenase [26].

The heptane/H_2O system appears as a better model than the octanol/H_2O system in assessing the transfer of oxicams across the blood-brain barrier. The only correlations that emerge are between maximal brain extraction $E(0)$ and log P_{hep}, implying that only the neutral form, and not the anionic or zwitterionic form, crosses the blood-brain barrier. It should be noted that the physicochemical properties described in this study are global molecular properties. The binding of oxicams to human serum albumin may be governed by submolecular features, explaining why no correlations are found between physicochemical properties and thermodynamic binding parameters.

Experimental Part

Materials. The oxicams were obtained from the following sources: piroxicam (**1**) from *Pfizer* (Groton, Cincinnati, USA), tenoxicam (**2**) from *Hoffmann-La Roche Ltd.* (Basel, Switzerland), lornoxicam (**3**) from *CL. Pharma* (Linz, Austria), isoxicam (**4**) from *Warner Lambert* (Ann Arbor, Michigan, USA), and meloxicam (**5**) from *Thomae* (Biberach an der Riss, Germany).

Dissociation Constants Using UV Spectrophotometry. Since oxicams are poorly soluble in aq. soln. and precipitation is often encountered during the pK_a measurements using potentiometric titrations, UV spectrophotometric titrations were employed to determine the dissociation constants of **3** and **5**, oxicams **1**, **2**, and **4** having been measured by *Bernhard* and *Zimmermann* [8]. Using a *Philips* model *8700* UV spectrophotometer and the same condition as described in [8], except that the temp. was adjusted at 25 ● 1°, spectra over the range 255–600 nm were recorded and the pK_a values calculated from the spectra changes using the *Henderson-Haselbach* equation [27].

Dissociation Constants Using Potentiometry. pK_a values in H_2O/EtOH soln. were determined potentiometrically, because sufficient solubility (1 mM) of oxicams can be attained in soln. containing 50% EtOH. Sufficient time interval between stirring and potential measurement during the titration allowed to assure the protonation/deprotonation equilibrium in H_2O/EtOH. Titration curves were recorded using a *Metrohm* titroprocessor model *670* (Herisau, Switzerland) and pK_a values calculated using a non-logarithmic linearisation of the titration curve to overcome the problem of dilution during titration [28] [29].

Lipophilicity Using Centrifugal Partition Chromatography (CPC) [30]. Measurements of distribution coefficients in octan-1-ol/buffer (0.1M phosphate buffer) systems were performed using horizontal flow-through centrifugal partition chromatography with a coil planet type centrifuge (*Pharma-Tech Research Corp.*, Baltimore, Maryland, USA). The detailed experimental procedures were described elsewhere [31] [32]. Briefly, this method employs a liquid-liquid partition system with the aid of centrifugal and Archimedan hydrodynamic forces, which allows a maximal retention volume of the stationary phase. It was shown that the CPC method can circumvent those problems inherent in the traditional shake-flask method such as interference of impurities, instability of solutes, and imprecision due to a improper volume ratio of the org. and aq. phase. The distribution coefficient (log D) can be calculated by *Eqns. 8* or *9*,

$$\log D = \log \frac{(t_R - t_0) \cdot U}{V_t - U \cdot t_0} \quad \text{when using aq. phase as mobile phase,} \tag{8}$$

$$\log D = \log \frac{V_t - U \cdot t_0}{(t_R - t_0) \cdot U} \quad \text{when using aq. phase as mobile phase,} \tag{9}$$

where t_0 and t_R are the retention time of the solvent front and of the solute, resp., U is the flow rate of the mobile phase, and V_t the total capacity of the columns. The t_0 can be measured using highly polar (*e.g.* $K_2Cr_2O_7$) or lipophilic (*e.g.* biphenyl) non-retained solutes. For compounds with one ionizable group, partition coefficients of the neutral form (log P) can be determined from log D, pK_a, and pH [33]. As for compounds of zwitterionic nature, the partition coefficients of neutral forms and zwitterions cannot be calculated without the knowledge of microscopic dissociation constants and the ratio of zwitterionic to neutral form in the aq. and org. phases.

Semiempirical MO Calculations. The geometries of oxicams were optimized using the quantum-mechanical semi-empirical PM3 method [12] in the program MOPAC 5.0 (QCPE No. 445) [34]. The convergence criteria given

by the keyword PRECISE was used. The structure analyses were performed using SYBYL software (version 5.5) running on a *Silicon Graphics Personal Iris 4D/35* workstation. H_2O-Accessible surface areas were calculated using the program MOLSV (QCPE No. 509) and *van-der-Waals* radii described by *Gavezzoti* [35], except that the *van-der-Waals* radius of the H_2O molecule was taken as 1.5 Å. The molecular electrostatic potential was calculated with the point charge approximation using SYBYL and PM3 partial atomic charges. All geometries are available from the authors upon request.

B. T., P. A. C., and *N. E. T.* are indebted to the *Swiss National Science Foundation* for support. *F. B.* and *J. P. T.* want to thank the 'Réseau Français de Pharmacologie Clinique' and the 'Direction de la Recherche à l'Education Nationale' for financial support.

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