143. Ionization and Partitioning Profiles of Zwitterions: The Case of the Anti-Inflammatory Drug Azapropazone

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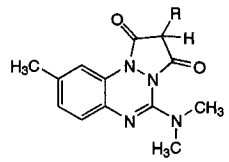
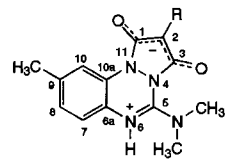
Azapropazone (**1**) is a non-steroidal anti-inflammatory drug (NSAID) whose chemical structure is markedly different from that of other agents in this class and challenges our understanding of structure-activity and structure-permeation relationships. Using a variety of experimental and computational techniques, we studied **1** for its molecular structure in the gas phase and non-protic polar solvents, protonation/deprotonation equilibria, tautomerism, and pH-lipophilicity profiles (octan-1-ol/H_2O and dodecane/H_2O). Other NSAIDs and model compounds were also examined for comparison. Due to its very low acidic pK_{a1}, **1** exists in the physiological pH range as a zwitterion and as an anion. Some pharmacological implications of these findings are discussed.

Introduction. – Non-steroidal anti-inflammatory drugs (NSAIDs) represent a therapeutic class that displays remarkable similarities in therapeutic actions and side effects, but is markedly heterogenous in structural terms [1] [2]. A number of NSAIDs bear an acidic function and can be classified as salicylic acids (*e.g.* aspirin and diflunisal), anthranilic acids (*e.g.* mefenamic acid and tolfenamic acid), arylacetic acids (*e.g.* diclofenac and indomethacin), arylpropionic acids (*e.g.* ibuprofen and naproxen), enolic pyrazolidinediones (*e.g.* phenylbutazone, see *Fig. 1*) and enolic oxicams (*e.g.* piroxicam and tenoxicam). A few drugs, *e.g.* the benzotriazine azapropazone [3] and some oxicams, also contain a basic group and can, therefore, exist as zwitterions.

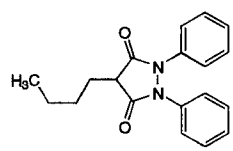


neutral

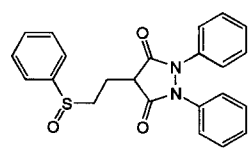


zwitterion

1 R = Pr: Azapropazone
2 R = Me: Model compound



3 Phenylbutazone



4 Sulfinpyrazone

Fig. 1. *Molecular structures of the compounds under study*

The pharmacokinetic behaviour of NSAIDs markedly influences their therapeutic properties as well as the type and incidence of side effects such as gastrointestinal lesions [1]. Thus, a classification of NSAIDs, based not only on their chemical structure but also on their partition behaviour, could help provide criteria to select the most appropriate agents for a particular therapeutic indication. Whereas a classification of this kind is relatively easy for acidic NSAIDs, recent studies with the zwitterionic piroxicam have underlined the difficulty to obtain a clear understanding on its partition behaviour [4] [5]. Indeed, zwitterionic NSAIDs possesses peculiar physicochemical properties distinct from those of acidic analogues, and it is, therefore, not surprising that they are believed to be less prone to inducing side effects and particularly gastrointestinal damage [1] [6].

In this context, azapropane (= *5-(dimethylamino)-9-methyl-2-propyl-1*H*-pyrazolo[1,2-a][1,2,4]benzotriazine-1,3(2*H*)-dione;* **1**) has two ionizable groups, namely a basic guanidino-type group and an acidic methinedicarbonyl group. Depending on the respective basicity and acidity of these two ionizable groups, the drug could exist at physiological pH as an anion, a cation, a zwitterion, or a neutral form. In addition, a number of tautomeric equilibria increase the number of possible states.

Azapropazone (**1**) is considered to have a low toxicity and a good gastrointestinal tolerance [3]. However, a recent study [7] concluded that azapropazone therapy is associated with a relatively high incidence of gastrointestinal side effects. These contradictory results call for a better understanding of the physicochemical properties of azapropazone (**1**). Indeed, even if its zwitterionic character in solution [8] [1] [6] [3] and in the crystalline state [9] is well-known, no comprehensive study has been published to shed light on the molecular and distribution equilibria of this drug, and on their possible relation with some of its pharmacokinetic and pharmacodynamic properties.

In the present study, a variety of experimental and computational methods were used to examine the multiple protonation/deprotonation equilibria, tautomerism and pH-lipophilicity profiles of azapropazone (**1**). Semiempirical MNDO calculations and ^{13}C-NMR spectroscopy helped to clarify the acid/base behaviour of azapropazone and the relative stability of its tautomers in the vacuum and in aprotic solvents, respectively. The pH-metric method [10–12] was applied to characterize its acid/base behaviour in aqueous media and (in combination with centrifugal partition chromatography, CPC) to investigate its distribution profiles in octan-1-ol/H_2O and dodecane/H_2O systems.

Results and Discussion. – 1. *Molecular Structures in the Gas Phase (MO Calculations).* The molecular structure of azapropazone (**1**) in the crystal shows unambiguously that a zwitterionic form exists in a crystalline environment with the negative charge delocalized over the keto-enol system and the proton attached to the N(6) of the triazine ring (see *Fig. 1*) [9]. To examine whether these protonation features are molecular or crystalline characteristics of **1**, semiempirical molecular orbital calculations were performed with model compound **2** where the Pr substituent at C(2) of **1** was replaced by a Me group. Two semi-empirical *Hamiltonians*, namely MNDO [13] and AM1 [14], were used to calculate the zwitterionic geometry. Although the two methods correctly reproduced the general molecular structure and particularly the non-planarity of the tricyclic moiety, the geometry predicted by the MNDO approximation was closer to the experimental geometry [9] than the one obtained by the AM1 method (*Fig. 1*). Thus, the MNDO *Hamiltonian* was chosen. The relative stability (ΔH_f, heat of formation) of the

calculated geometries of all tautomers and ionic forms is given in *Table 1*. The acid/base equilibria in the vacuum were obtained as deprotonation enthalpy (*DPE*) for acidic functions, and as proton affinities (*PA*) for basic functions.

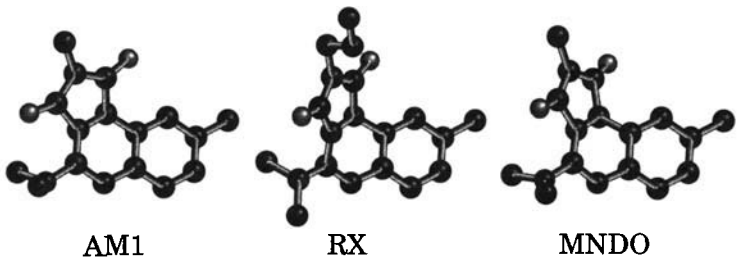


Fig. 2. *Comparison between the crystal structure* (RX) *of azapropazone* (**1**) (centre) *and geometries obtained from the two semi-empirical* Hamiltonians *AM1* (left) *and MNDO* (right)

Table 1. *Calculated MNDO* ΔH_f *Values* [kcal·mol^{-1}] *for Various Forms of Model Compound* **2**

	2A	**2Na**	**2Nb**	**2Nc**	**2Ca**	**2Cb**	**2Cc**	**2Cd**	**2Z**
ΔH_f	−50.01	−1.95	1.32	2.09	151.31	156.72	150.85	173.97	19.94

Tautomeric Equilibria. MO Calculations indicate that the diketonic neutral form of model compound **2** (*i.e.*, **2Na** in *Fig. 3*) is slightly more stable (by 3.2 and 4.0 kcal·mol^{-1}, respectively) than the corresponding keto-enolic forms **2Nb** and **2Nc**. In the case of the cationic forms **2Ca**–**2Cc** (*N*(6)-protonated as observed in the crystal), the keto-enolic tautomers **2Cb** and **2Cc** were slightly more and less stable (by 0.4 and 5.4 kcal·mol^{-1}, respectively, see *Fig. 3*) than the diketonic tautomer **2Ca**. These small differences in gas-phase energies suggest that the diketo/keto-enol ratio may change markedly in condensed phases due to difference in solvation energies.

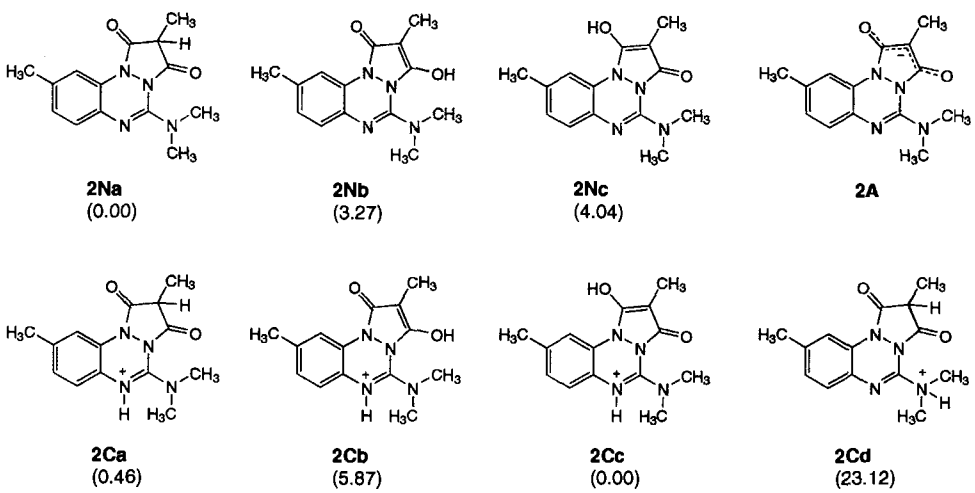


Fig. 3. *Possible electric states of model compound* **2**. Relative energies of each state are given in parentheses [kcal·mol^{-1}].

Acid/Base Equilibria. MNDO Calculations also demonstrate that the most basic atom of the guanidine-type fragment of compound **2** is the N(1) atom, the difference between the two diketonic cation **2Ca** and **2Cd** being of 22.7 kcal·mol^{-1}. This result is in agreement with the observed protonation site in the crystal, allowing calculations with the zwitterionic species to be restricted to the form **2Z** (*Fig. 1*).

The difference in MNDO-calculated ΔH_f values between zwitterion **2Z** and the most stable tautomer of neutral form **2Na** (*ca.* 21 kcal·mol^{-1}) contrasts with the difference calculated between the zwitterion and neutral forms of α-amino acids such as glycine (*ca.* 61 kcal·mol^{-1}). The compared differences suggest a stabilization of azapropazone (**1**) in the zwitterionic form presumably caused by charge delocalization.

To allow comparison of *DPE* of compound **2**, two drugs (phenylbutazone and sulfinpyrazone; *Fig. 1*) and a few model compounds were also investigated at the MNDO level. *Table 2* summarizes *DPE* of acidic C–H bonds and of alcoholic or phenolic O–H bonds. For sulfinpyrazone, which contains a hypervalent S-atom, the MNDO/d algorithm [15] was used. The results indicate that the acidity of model compound **2** in its neutral form is close to the acidity of phenylbutazone and sulfinpyrazone (pK_a 4.61 and 2.37, respectively). As for *PA* of the guanidine-type group in model compound **2** (214.4 kcal·mol^{-1}), it proved close to that of methoxypyridine (214.1 kcal·mol^{-1}, with a pK_a = 6.62 [16]). It must be noted that only neutral forms were used in the calculations of *PA* and *DPE* of **2** in order to avoid artifacts due to the presence of other pre-existing charges. These results are in marked disagreement with previous interpretations which assigned a lowered acidity and a lowered basicity to the acidic and basic groups of azapropazone (**1**), and thus excluded the possibility of it existing as a zwitterion [6].

Table 2. *Calculated* DPE *Values for Different Compounds as Calculated by MNDO*

	pK_a	ΔH_f(N)[a]	ΔH_f(A)[b]	*DPE*(CH)[c]	*DPE*(OH)[d]
1	–	−1.95	−50.01	319.14	–
3	4.61	−4.58	−52.92	318.86	–
4	2.37	13.68	−37.87	315.65	–
MeOH	15.5	−57.35	−39.74	–	384.81
2-(*t*-Butyl)phenol	10.6	−26.64	−46.53	–	347.31
PhSH	6.5	27.82	4.25	–	343.63
PrOH	13.6	−67.53	−52.07	–	283.66

[a]) ΔH_f [kcal·mol^{-1}] of the neutral form. [b]) ΔH_f [kcal·mol^{-1}] of the anionic form. [c]) *DPE* (see text) concerning C–H bond. [d]) *DPE* (see text) concerning O–H bond.

2. *Behaviour of Azapropazone* (**1**) *in Aqueous Media and Biphasic Systems.* Delocalization effects may increase the respective acidity and basicity of the basic guanidino-type group and the acidic methinedicarbonyl group in azapropazone (**1**). In this study, a variety of techniques, including a modern pH-metric instrument, were used to examine the acid/base behaviour of azapropazone in aqueous solution and its distribution profile in two-phase systems. These are significant parameters in transport processes, as reviewed by *Dearden* [17].

*p*K$_a$ *Measurements.* The UV spectrum of **1** in aqueous solution showed a bathochromic shift of the λ_{max}, from 250 to 325 nm, when the pH changed from 3.2 to 8, yielding a pK_a value of 6.46 ± 0.14. However, this approach did not allow attribution of this pK_a value to an acidic or a basic group. A potentiometric method [10–12] was also

used to determine the pK_a values in various mixtures of H_2O/organic co-solvent, since pK_a variations under such conditions can indicate the acid-base nature of the ionizable group [11].

The results are compiled in *Table 3*. In H_2O/MeOH mixtures and in H_2O/dioxane mixtures, the pK_a decreased slightly as co-solvent concentration increased. This behaviour is typical of basic groups, whose basicity decreases with decreasing dielectric constant of the medium. Because the pK_a decrease observed for azapropazone (**1**) is rather modest, the acidic drug phenylbutazone (*Fig. 1*; $pK_a = 4.61$) was also examined under similar conditions and showed an increase in pK_a with increasing MeOH concentration (*Fig. 4*). This indicates the different nature of the two pK_a values and confirms the basic nature of the pK_a of **1** determined by UV spectroscopy ($pK_a = 6.46 \pm 0.14$) and by potentiometric method ($pK_a = 6.55 \pm 0.02$). This contradicts previous studies [1] [18] which concluded that the pK_a near 6.3 was that of the acidic group of **1**. Furthermore, all titration curves measured between pH 1.8 and 12.2 with the *Sirius PCA 101* titrator revealed the presence of only one protonation equilibrium, suggesting that the first pK_a of **1** should be lower than 1.5 as originally proposed by *Fenner* and *Mixich* [8].

Table 3. *Ionization Constants* (pK_{a2}) *of Azapropazone* (**1**) *in Mixtures of MeOH/H_2O and 1,4-Dioxane/H_2O, as Measured by Potentiometry*

	pK_{a2}	
Co-solvent [%] (*w*/*w*)	MeOH[a])	1,4-Dioxane[b])
0	6.55	6.55 ± 0.02
11–12	6.46	6.35 ± 0.01
23–24	6.41	6.28 ± 0.01
35–37	6.38	6.27 ± 0.03
50–51	6.33	–

[a]) $n = 2$. [b]) $n = 3$; ± SD.

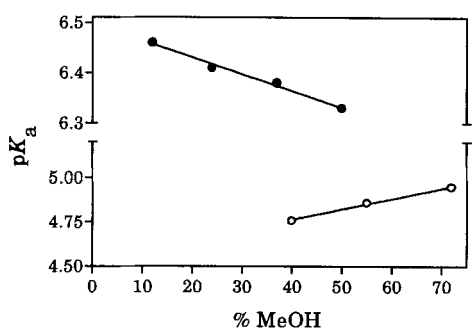
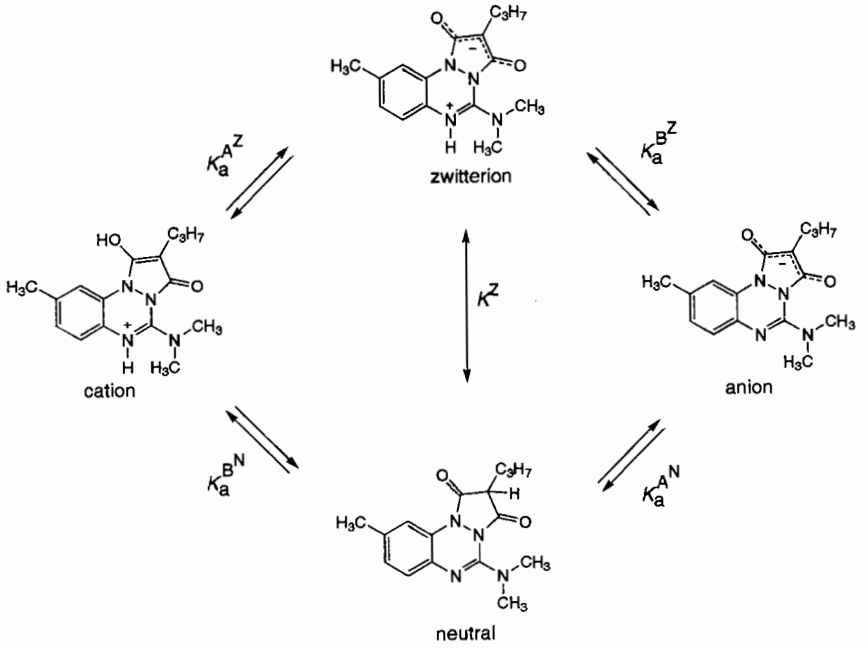


Fig. 4. pK_a *Variation in presence of MeOH.* pK_a of phenylbutazone (○) and pK_{a2} of azapropazone **1** (●) are compared.

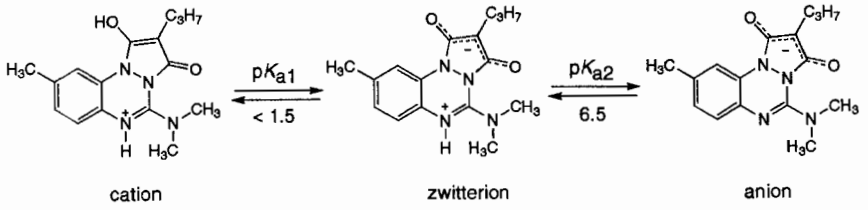
The two pK_a values thus determined, 6.5 and < 1.5, are macroscopic ionization constants and not the microscopic ionization constants shown in *Scheme 1*. At present, it is not possible to measure accurately the four microconstants. However, a large difference (> 5) between the two pK_a values of a zwitterionic compound results in a very high value

of K_Z, the equilibrium constant between the zwitterionic and neutral forms. This allows the full ionization scheme (*Scheme 1*) to be simplified as shown in *Scheme 2*. Due to the very low value of the acidic pK_a, it is obvious that **1** can exist only as a zwitterion in the gastrointestinal tract, and as a mixture of anion and zwitterion at physiological pH (7.4).

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



Scheme 2. *Stoichiometric Equilibria of Azapropazone* (**1**)



Partitioning Behaviour in Octanol/Buffer and Dodecane/Buffer Systems. Scheme 2 allows to interpret pH-distribution profiles in terms of the partitioning species and acid/base equilibrium constants. Using centrifugal partition chromatography (CPC), the pH-distribution profile of azapropazone (**1**) was measured in octan-1-ol/H_2O and dodecane/H_2O systems. The shape of the two profiles (*Fig. 5*) are similar, showing a plateau in the pH range 2–5, a large drop of distribution coefficients above pH 6, and a small decrease below pH 1. In the octan-1-ol/H_2O system, a second plateau is apparent for pH > 10. In the dodecane/H_2O system, lipophilicity became too low at pH > 7 to be measurable.

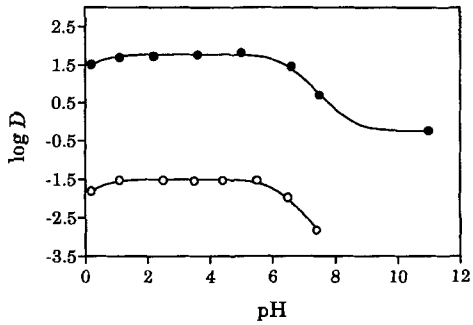


Fig. 5. *Distribution profiles of azapropazone* (**1**) *in octan-1-ol/H_2O* (●) *and dodecane/H_2O system* (○)

The bell-shaped curves in *Fig. 5* are characteristic of the partitioning of a zwitterionic compound. Interestingly, the difference between the two plateaux at pH 2–5 ($\Delta \log D_{oct\text{-}alk} = 3.3$) is much higher than the same difference shown by the acidic drug phenylbutazone at a pH above its pK_a, *i.e.*, when it partitions as the neutral species ($\Delta \log D_{oct\text{-}alk} = 0.68$). Thus, the very large $\Delta \log D_{oct\text{-}alk}$ value of azapropazone (**1**) can only mean that, in the pH range 2–5, it partitions in organic solvents as the zwitterion. In other words, the log *D* values at the plateau are the partition coefficients of the zwitterionic species (*i.e.*, log *P* written as log P^Z) in the octan-1-ol/H_2O and dodecane/H_2O solvent systems. This in turn is fully compatible with the simplified protonation scheme shown in *Scheme 2*.

The pH-distribution profiles of **1** can be described by *Eqn. 1* for the octan-1-ol/H_2O system (where the log *P* of the anionic form is measurable) and by *Eqn. 2* for the dodecane/H_2O system, adapted from the general distribution function [12].

$$\log D = \log \left[\frac{P^Z \cdot (10^{pK_{a2} - pH}) + P^A}{1 + 10^{pK_{a2} - pH} + 10^{pK_{a1} + pK_{a2} - 2 \cdot pH}} \right] \quad (1)$$

$$\log D = \log \left[\frac{P^Z \cdot (10^{pK_{a2} - pH})}{1 + 10^{pK_{a2} - pH} + 10^{pK_{a1} + pK_{a2} - 2 \cdot pH}} \right] \quad (2)$$

where P^Z and P^A are the partition coefficients of the zwitterion and anion, respectively, and pK_{a1} and pK_{a2} are the ionization constants of the acidic and basic functions, respectively. It must be noted that the partition coefficient of the cationic species (P^C) was not measurable (pH < 0).

A nonlinear fit of the distribution profiles in octan-1-ol/H_2O and dodecane/H_2O systems using *Eqns. 1* and *2* gave the results presented in *Table 4*. This procedure led to a more precise estimate of pK_{a1} (0.15) and of the partition coefficients of the zwitterion ($\log P^Z_{oct} = 1.78$ and $\log P^Z_{alk} = -1.49$). These log *P* values are relatively high, suggesting a partial cancellation of the effects of the two opposite charges on solvation energies, presumably due to charge delocalization across the aromatic rings. Spectroscopic techniques were used to clarify this aspect of the molecular structure of azapropazone (**1**), as reported below.

3. *Molecular Structure in Aprotic Polar Solvents.* The zwitterionic nature of **1** explains its minimal solubility at pH 3.5, *i.e.*, near the isoelectric pH_i (3.2) [19], with marked

Table 4. *Physicochemical Parameters of Azapropazone* (**1**) *Determined by Nonlinear Fitting of pH-Distribution Profiles Using* Eqns. 1 *and* 2

	Octan-1-ol/H_2O system	Dodecane/H_2O system
pK_{a1}[a])	0.15 ± 0.36	0.21 ± 0.28
pK_{a2}[b])	6.46 ± 0.18	6.14 ± 0.12
log P^{Zc})	1.78 ± 0.08	−1.49 ± 0.06
log P^{Ad})	−0.24 ± 0.15	−[e])

[a]) Acidic group, see *Fig. 4*. [b]) Basic group, see *Fig. 4*. [c]) log *P* of the zwitterion. [d]) log *P* of the anion. [e]) Non determinable (see text).

increases for pH > 7.0 and pH < 2.0. This low aqueous solubility near the isoelectric pH made it difficult to use aqueous solutions for IR and NMR experiments.

UV Spectra in Buffer, DMSO and Dioxane. To choose a solvent able to solubilize **1** in zwitterionic form better than H_2O at pH 3.5, UV spectra in H_2O were compared with those obtained in DMSO and dioxane. The UV spectra of **1** in different mixtures of DMSO and buffer of pH 3.5 showed only bathochromic shifts (spectra not shown), demonstrating that the zwitterionic character of **1** was unchanged in DMSO. In contrast, the spectra in dioxane showed several ill-defined isosbestic points (*Fig. 6*), indicating the presence of at least two tautomeric equilibria, namely zwitterion-neutral [20] and keto-enol.

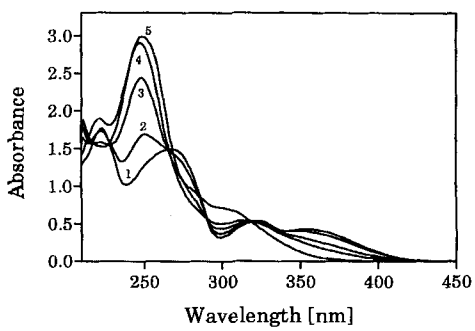


Fig. 6. *Azapropazone UV spectra in dioxane/buffer mixtures. 1*: 100% dioxane; *2*: 98% dioxane; *3*: 96% dioxane; *4*: 94% dioxane; *5*: 1% dioxane.

IR Spectra in DMSO. The IR spectra of azapropazone (**1**) and phenylbutazone in the range 1800–1600 cm^{-1} were compared (*Fig. 7*). The strong absorbance at 1720 cm^{-1} (C=O stretching) of phenylbutazone clearly indicates a diketo tautomeric structure, while the absence of this band in the spectrum of **1** suggests the predominance of a keto-enolate form in DMSO, as confirmed by the marked absorbance at 1643 cm^{-1} (C=C and/or C–O stretching).

NMR Spectra at Different pH Values in DMSO/Buffer Mixtures. The ^{13}C-NMR spectra of azapropazone (**1**) in (D_6)DMSO (conditions *B* in *Table 5*) were first examined by DEPT experiments and compared to calculated additive chemical shifts. This allowed assignments to be proposed, as shown in *Table 5*. To obtain conditions where compound **1** exists in cationic or anionic state, 5 equiv. of HCl (conditions *A*) or 5 equiv. of NaOH (conditions *C*), respectively, were added to the (D_6)DMSO solution. The resulting changes in chemical shifts allowed the sites of protonation/deprotonation to be identified (*Fig. 8*).

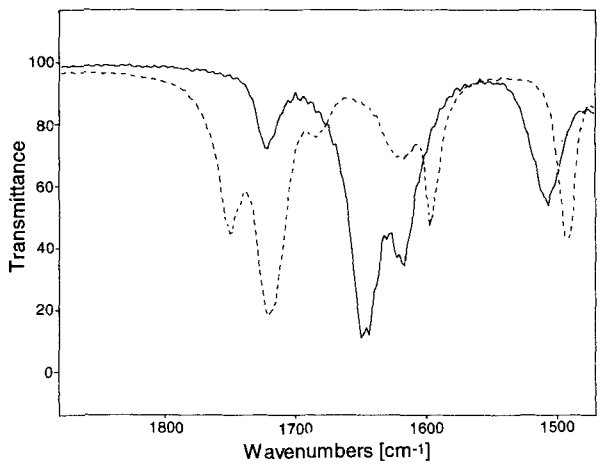
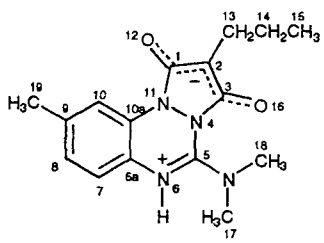


Fig. 7. *Comparison between the IR spectra of azapropazone* (**1**) (full line) *and phenylbutazone* (dotted line)

Table 5. *^{13}C-NMR Chemical Shifts*[a]) *of Azapropazone* (**1**) *in (D_6)DMSO (B), in (D_6)DMSO with 5 equiv. of HCl (A) and in (D_6)DMSO with 5 equiv. of NaOH (C).*
A non-systematic numbering of C-atoms is used.



	A	*B*	*C*
C(3)	168.23	169.83	169.09
C(5)	157.19	162.90	163.90
C(1)	167.70	146.06	148.86
C(10a)	128.55	135.31[b])	136.13
C(9)	134.44	134.60[b])	133.88
C(8)	129.55	123.51[c])	122.35
C(6a)	127.43	122.08[b])	131.07
C(7)	117.22	117.28[c])	113.20
C(10)	128.48	113.61[c])	113.20
C(2)	71.17	80.24	82.08
C(17)	39.40	40.62	39.18
C(18)	39.40	40.62	39.18
C(13)	30.56	23.23	23.93
C(14)	17.92	21.63	21.66
C(18)	20.77	20.87	21.60
C(15)	13.91	13.65	14.61

[a]) Chemical shifts (δ in ppm) relative to (D_6)DMSO. [b])[c]) May be interchanged.

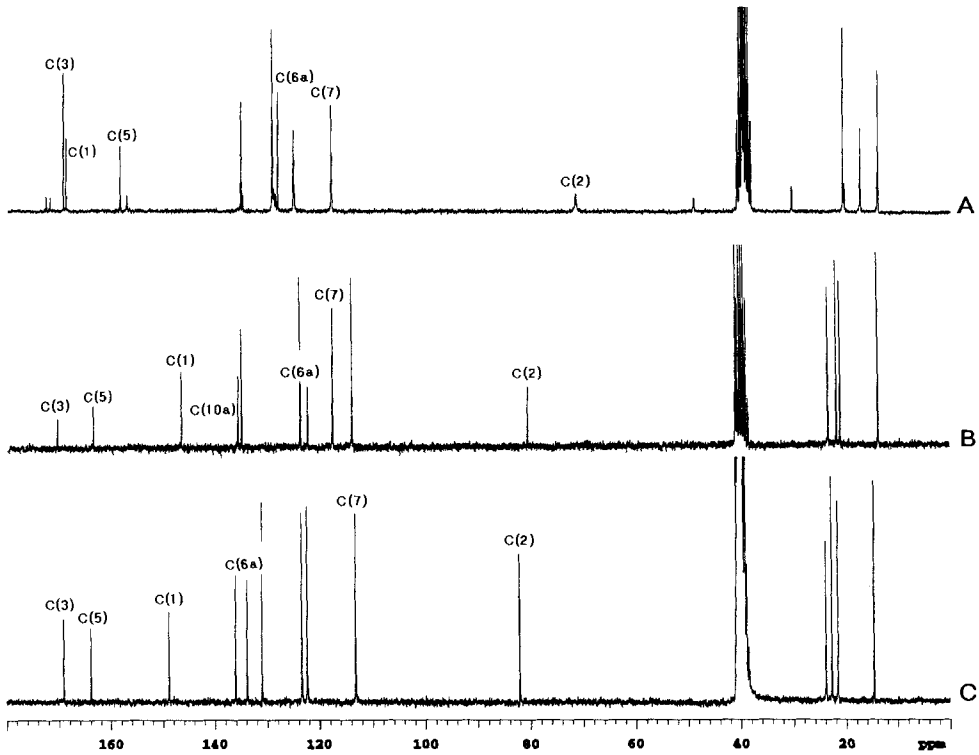


Fig. 8. *NMR Spectra of azapropazone* (**1**) *under acidic* (top), *neutral* (middle) *and basic* (bottom) *conditions*

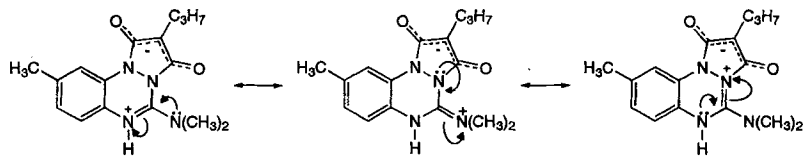
The UV and IR spectra discussed above have demonstrated that **1** exists in zwitterionic form when dissolved in DMSO. This interpretation is also supported by the ^{13}C-NMR spectra where the chemical shifts of C(1) and C(2) were similar under neutral and basic conditions, indicating a deprotonated methinedicarbonyl function in both cases. Moreover, a keto-enolate structure for the methinedicarbonyl function is compatible with the deshielding of C(2) (+10.09 ppm) under neutral and basic conditions, as compared with the acidic conditions.

Under acidic conditions, two protonated tautomeric forms became apparent for the methinedicarbonyl function, as indicated by the splitting of the signals for C(1) (167.79 and 170.78 ppm), C(2) (71.17 and 48.93 ppm), and C(3) (168.23 and 171.55 ppm). These chemical shifts correspond to the keto-enolic and diketonic forms of the methinedicarbonyl function, respectively (see *Fig. 3*), the keto-enolic tautomer being the most stable in DMSO. The upfield shift of the C(1) signal observed when going from acidic to basic conditions (−18.8 ppm) is compatible with an acidic behaviour of this function.

With the guanidino-type group, an upfield shift (−6.7 ppm) was observed for C(5) when changing from basic to acidic conditions. Such a shift, which is compatible with the effect on protonation of the analogous moiety in piribedil (−7.8 ppm) [21], confirmed the basic nature of this group. However, the C(5) signal was deshielded under neutral conditions with respect to the expected value for a protonated function in a zwitterionic form. Charge delocalization as well as bond order changes due to a different balance of

several resonance forms within the guanidino-type group could be responsible of this deshielding (*Scheme 3*). The presence of a zwitterionic form in DMSO, *i.e.*, a protonated guanidino-type group, was also confirmed by the chemical shifts of C(6a) and C(7) which were comparable under acidic and neutral conditions but different under basic conditions (see *Fig. 8*).

Scheme 3. *Several Resonance Forms within the Guanidino-Type Group*



Conclusion. – As previously mentioned, *McCormack* and *Brune* [6] using azapropazone (**1**) as an example suggested that molecules of zwitterionic nature could be an approach towards the design of stomach-sparing NSAIDs, while a recent pharmacovigilance compilation [7] reported that zwitterionic NSAIDs such as **1** and piroxicam have a relatively high risk of eliciting gastrointestinal side-effects. The physicochemical behaviour of **1** was clearly misinterpreted by *McCormack* and *Brune* [6] [1] who suggested the predominance of a cationic form near stomachal pH. The study we present here demonstrates that, at pH below 6.3, **1** exists as a zwitterion with a relatively high log P_{oct} (1.78), interestingly close to the log D_{oct} value of piroxicam (1.73). This value suggests that **1** could indeed permeate the gastric mucosa.

Octan-1-ol/H_2O distribution coefficients at physiological pH (log D_{oct}) are often well correlated with transport or metabolic processes [17] [22] but numerous exceptions exist. This appears to be the case for **1** and phenylbutazone, which have comparable log D_{oct} values at pH 7.4 (0.70 and 0.52, respectively) but are eliminated by different routes (predominant renal elimination for **1**, but hepatic excretion for phenylbutazone) [8]. In an attempt to find a suitable parameter able to account for their different route of elimination, the dodecane/H_2O distribution coefficient of the two drugs was considered. The two very different value obtained (the log D_{dod} at pH 7.4 are −2.83 for **1** and −0.50, for phenylbutazone) are intriguing and warrant further studies on the significance of log D_{dod} (or Δlog $D_{oct\text{-}alk}$) as a predictor of renal *vs.* hepatic elimination.

Finally, recent studies have demonstrated that pharmacodynamic properties such as selectivity for COX-1 (the constitutive cyclooxygenases) *vs.* COX-2 (the inducible cyclooxygenases) could also be of critical importance in eliciting some side-effects of NSAIDs [23]. In fact, it has been proposed [23] that the selective inhibition of COX-2 could reduce inflammation without gastric and renal toxicity. Preliminary results indicated [23] that piroxicam does not display an optimal COX-2/COX-1 ratio. Studies on the COX-2/COX-1 selectivity of azapropazone (**1**) would allow insights into the relation between this selectivity and the anionic *vs.* zwitterionic nature of NSAIDs.

Experimental Part

Materials. Azapropazone · 2 H_2O was kindly donated by *Siegfried* (Zofingen, CH). Octan-1-ol was from *Fluka* (Buchs, CH) and dodecane (99%) from *Aldrich* (Steinheim, G). 1,4-Dioxane and DMSO were for UV spectroscopy (*Fluka*). (D_6)DMSO (of 99.8% isotopic purity) was purchased from *Armar* (Döttingen, CH). All other reagents were of highest grade and used without further treatment.

Semiempirical MO Calculations. The geometry of azapropazone (**1**) in different electrical states was optimized by the quantum-mechanical semiempirical MNDO [13] and MNDO/d [24] methods using the Spartan 4.0 and 4.1 programs [25]. For the calculations of molecules containing hypervalent S-atoms, the MNDO/d parametrization of *Thiel* and *Voityuk* was used [26]. All calculations were run on a *Silicon Graphics* workstation *Indy R4400.*.

The deprotonation enthalpy (*DPE*) of a compound HB

$$HB \rightarrow H^+ + B^-$$

was calculated by *Eqn. 3*:

$$DPE(HB) = \Delta H_f(H^+) + \Delta H_f(B^-) - \Delta H_f(HB) \quad (3)$$

The proton affinity (*PA*) of a compound B is defined as *minus* the heat of reaction of protonation

$$B + H^+ \rightarrow HB^+$$

and was calculated by *Eqn. 4*:

$$PA(B) = \Delta H_f(H^+) + \Delta H_f(B) - \Delta H_f(HB^+) \quad (4)$$

Measurement of Ionization Constants Using UV Spectroscopy. Stock solns. of azapropazone (**1**; $2 \cdot 10^{-4}$ M) were prepared in a H_2O/MeOH mixture 50:50 (*v*/*v*) and diluted 10-fold with phosphate buffers of pH 3–8. Their spectra over the range 200–400 nm were recorded using a *Philips* model *8700* UV spectrophotometer. The pK_a values were calculated from the change in absorbance at $\lambda = 325$ nm using the *Henderson-Hasselbalch* equation [27].

Measurement of Ionization Constants Using Potentiometry. The apparent pK_a value of **1** in various mixtures of H_2O/MeOH and H_2O/dioxane were determined by potentiometry using the *Sirius PCA101* titrator (*Sirius Analytical Instruments Ltd.*, Forest Row, East Sussex, UK). The apparatus was equipped with a semi-micro combination pH electrode (*Orion 8103 SC*), a temp. probe, an overhead stirrer, a precision dispenser and a six-way valve for distributing reagents and titrants (0.15M KCl, 0.5M HCl and 0.5M KOH). The electrode was first calibrated by blank titration. A weighted sample (2–3 mg) was added manually to the titration vial. The temp. of the titration vial was maintained at 25° with a thermostated water bath. *Bjerrum* plots were calculated from the titration curves to obtain precise pK_a value. The detailed experimental procedures and data analyses can be found in [28] [29]. When titrating in the presence of an org. co-solvent, the measured pH was corrected with previously calibrated equations.

Measurement of Distribution Coefficients Using Centrifugal Partition Chromatography (CPC). Distribution coefficients in octanol/buffer were measured using the *Ito Multilayer Coil Separator-Extractor* (*P.C. Inc.*, Potomac, MD, USA), and those in dodecane/buffer using a horizontal flow-through centrifugal partition chromatograph with a coil planet-type centrifuge (*Pharma Tech Research Corp.*, Baltimore, MD, USA). The CPC technique employs a liquid-liquid partition system with the aid of centrifugal and *Archimedean* hydrodynamic forces, allowing a maximal retention volume of the stationary phase. It has been shown that CPC can circumvent problems inherent in the traditional shake-flask method such as impurities, instability of solutes, and low precision due to an unfavourable volume ratio of the org. and aq. phases [30]. The detailed experimental procedures and equations for the calculation of distribution coefficients can be found in [30] [31].

Determination of Distribution Coefficients from pH-Metric Two-Phase Titrations. The distribution coefficients of **1** in octan-1-ol/H_2O and dodecane/H_2O were also determined by pH-metric two-phase titrations with the built-in log *P* option of the *Sirius PCA101* instrument [32] [11]. Here, a weighted sample (2–3 mg) was added manually to the titration vial, and octan-1-ol added to the vial before titrating. The titration curve in the presence of octan-1-ol were used to calculate pH-lipophilicity profiles by using previously determined pK_a values.

UV Spectroscopy to Determine Zwitterionic Structure. UV Spectra were recorded in dioxane/buffer and in DMSO/buffer mixtures. Stock solns. of **1** was prepared in dioxane (0.01M) and in DMSO (0.05M); 0.1 ml of aliquots were diluted to 10 ml with a phosphate buffer (pH 3.6) and/or the co-solvent. The concentration of dioxane was varied from 100 to 92% in steps of 2% and then from 80 to 1% in steps of 20%. For the DMSO solns., the co-solvent concentrations were 100, 75, 50, 25 and 1%. The UV absorbtion spectra of these solns., were recorded using a UV/VIS *Perkin-Elmer Lamda 11* spectrometer (*Perkin-Elmer International*, Le Mont-sur-Lausanne, Switzerland).

FT-IR Spectroscopy. FT-IR Spectra were recorded using a *Galaxy* FT-IR spectrometer model *4020* (*Mattson Instruments, Inc.*, Madison WI, USA). The IR cell consisted in a demountable stainless steel holder on which two rectangular NaCl windows can be fixed. The path length of the cell could be varied by placing PTFE spacers of different thickness between the two NaCl windows. In the present study, spacers of 0.05-mm thickness were used. The spectra were collected from 4 scans at 4 cm^{-1} nominal resolution.

^{13}C-NMR Spectroscopy. The ^{13}C-NMR spectra in (D_6)DMSO (*ca.* 0.1M) were recorded at 50 MHz on a *Varian*

VXR-200 spectrometer. The assignment of chemical shifts was made with the aid of DEPT experiments [33], distinguishing the chemical shifts of CH, CH_2 and CH_3 groups, and of calculated ^{13}C chemical shifts using standard additivity rules with the VAMP 5.1 [34] software.

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