90. Ionization Behavior and Ionization-Dependent Conformation of Raclopride, a Dopamine D_2 Receptor Antagonist

by **Pierre-Alain Carrupt, Ruey-Shiuan Tsai, Nabil El Tayar,** and **Bernard Testa***

Institut de Chimie Thérapeutique, Ecole de Pharmacie, Université de Lausanne, BEP, CH–1015 Lausanne

and **Tomas de Paulis**

Chemistry Department, Vanderbilt University, Nashville, TN 37235, USA

and **Thomas Högberg**[1])

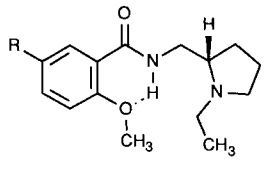
Astra Research Centre AB, CNS Research & Development, S–15185 Södertälje

(2.V.91)

Raclopride, an antipsychotic 6-methoxysalicylamide (= 2-hydroxy-6-methoxybenzamide) derivative, was shown by titrimetry and UV-photometry to exist in zwitterionic form at physiological pH. Calculations revealed that the neutral and zwitterionic forms differ considerably in their conformational behavior, the latter form being energetically favored by an intramolecular phenolate–ammonium ionic bond. These findings indicate that raclopride and other halogenated 6-methoxysalicylamides with a highly acidic phenolic group may not resemble other *ortho*-methoxybenzamides in their stereoelectronic structure and mode of binding to the dopamine D_2 receptor.

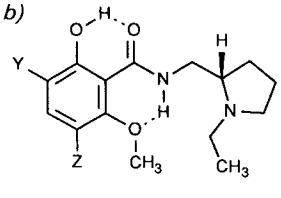
Introduction. – Orthopramides (2-methoxybenzamide derivatives) such as sulpiride and sultopride (*Fig. 1, a*), are atypical antipsychotic drugs which act as selective antagonists of the dopamine D_2 receptor [1–3]. Iodopride, in which the sulfonyl moiety is replaced by an I-atom, is sharing the same properties [4]. The 2-MeO group in orthopramides has been shown to be an essential structural feature for D_2 receptor affinity, being critical in the formation of an intramolecular H-bond [3] [5–11]. This H-bond,

a)



R	Name
SO_2NH_2	(*S*)-Sulpiride
$SO_2CH_2CH_3$	(*S*)-Sultobride
I	Iodopride

b)



Y	Z	Name
Cl	Cl	Raclopride
Et	Cl	Eticlopride
Br	H	FLA797

Fig. 1. a) *Chemical structure of (S)-sulpiride, (S)-sultopride, and iodopride, three representative orthopramides.* b) *Chemical structure of raclopride, eticlopride, and FLA 797, three representative 6-methoxysalicylamides.*

[1]) Present address: *Astra Draco*, Preclinical Research and Development, Box 34, S–22100 Lund.

which exists predominantly in media of low polarity, closes a six-membered pseudo-ring and renders the amide group coplanar with the aromatic ring.

de Paulis et al. [12] [13] have developed a series of 6-methoxysalicylamides (= 2-hydroxy-6-methoxybenzamides) by introducing an OH group in the *ortho*-position of the aromatic ring. Compounds of this type (*e.g.* raclopride, eticlopride, and FLA 797 (*Fig. 1, b*)) are potent and selective dopamine D_2 antagonists [3]. The additional *ortho*-OH group in these compounds was suggested to further stabilize the amide-methoxy intramolecular H-bond by forming a second H-bond between the carbonyl O-atom and the phenolic OH group (*Fig. 1, b*) [12].

^{13}C-NMR chemical shifts in $CDCl_3$ solutions [14] and X-ray crystallographic studies of some 6-methoxysalicylamides [15–18] have confirmed a planar benzamide conformation stabilized by the two intramolecular H-bonds. Based on modelling studies with conformationally constrained compounds, *i.e.* piquindone [17] and Du 122290 [18], the coplanar benzamide conformation and a folded or half-folded side-chain conformation were thus considered as the pharmacologically active form for 6-methoxysalicylamides acting on the D_2 receptor [3] [17] [18]. Raclopride was used as prototype for the substituted 6-methoxysalicylamides in the force-field calculations and modelling. In these studies, raclopride was treated in its neutral form [17] [18]. The present study shows that raclopride in fact exists in zwitterionic (inner salt) form at physiological pH, an observation that prompted a re-examination of its conformational behavior at different ionization states. The pK_a values of raclopride were determined by potentiometric titrations and UV-spectrophotometric analysis.

Experimental. – *Chemicals.* Raclopride (+)-tartrate was provided by *Astra* (Södertälje, Sweden). Buffers and salt were of anal. grade. Distilled H_2O was used throughout.

Determination of Ionization Constants by Potentiometry. A 3-mM soln. was prepared in dist. H_2O which had been boiled to remove O_2 and CO_2 and saturated with N_2. The ionic strength was fixed at 0.1M using KCl. An excess of HCl was added, and the soln. was back-titrated with 0.01N NaOH using a *Metrohm* 670 titroprocessor. The temp. was 25 ± 1°. Titration curves were determined in triplicate, and the pK_a values were calculated using a non-logarithmic linearization of the titration curve proposed by *Benet* and *Goyan* [19] and modified by *Leeson* and *Brown* [20] to overcome the problem of dilution during titration.

Determination of Ionization Constants by UV Spectrophotometry. Solns. (0.24 mM) were prepared in phosphate buffers of pH 2.05, 5.04, 5.75, 6.41, 7.31, and 7.99. Spectra over the range 200–400 nm were recorded using a *Philips* UV spectrophotometer, and the pK_a values calculated from spectral changes using the *Henderson-Haselbach* equation [21].

Conformational Calculations. Conformational analyses were performed using the SYBYL software running on a *Sun SPARC 1* workstation, as well as using the MOPAC 5.0 package (QCPE No. 445 [22]) running on a *Silicon Graphics Personal Iris 4D/25* workstation and on the *CRAY 2* and *CRAY XMP* computers of the Federal Institutes of Technology in Lausanne and Zürich. Cartographic representation was performed using the UNIRAS software running on a *VAX 8550* computer of the University of Lausanne.

Results and Discussion. – *Acido-basic Behavior of Raclopride.* The potentiometric titration curve of raclopride (*Fig. 2*) clearly illustrates the existence of two macroscopic pK_a values. Using a non-logarithmic linearization of the titration curve [20], pK_a values of 5.81 ± 0.01 and 9.21 ± 0.01 were obtained. Assignment of these values was achieved by UV spectrophotometry at various pH values (*Fig. 3*). From the changes of spectra at 314.4 nm, a pK_a value of 5.89 ± 0.03 was calculated.

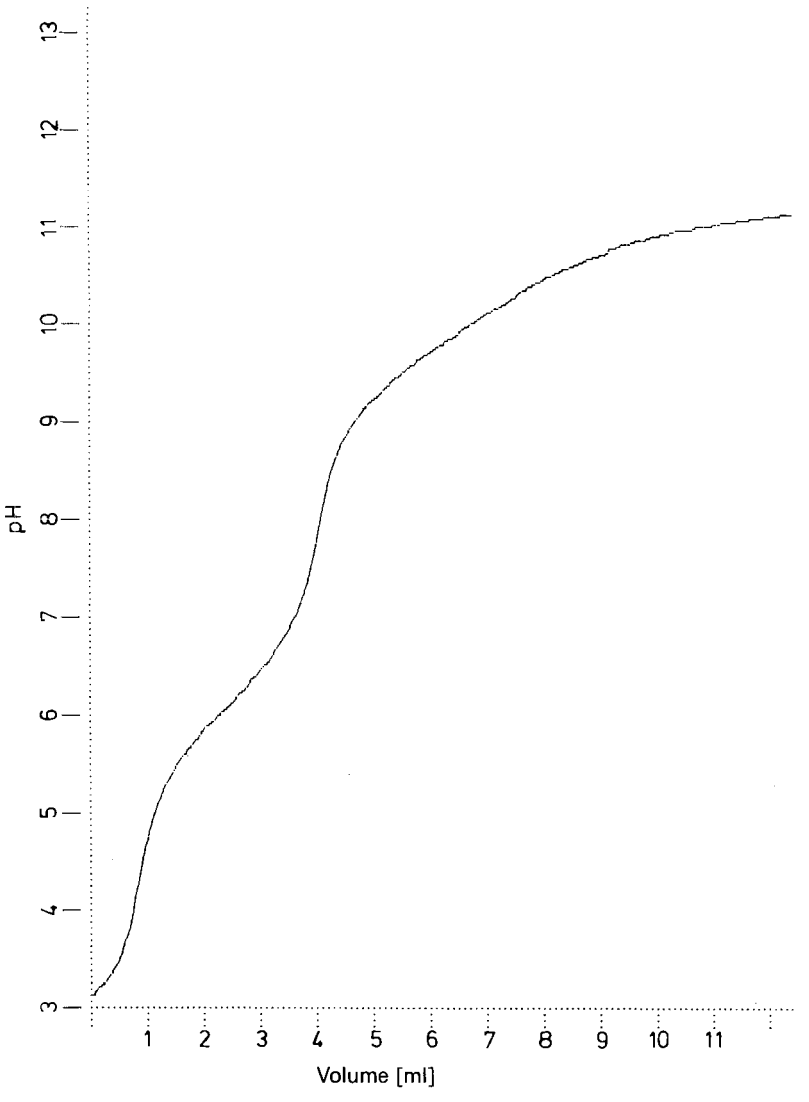


Fig. 2. Potentiometric titration curve of raclopride illustrating the existence of two pK_a *values, 5.8* (phenol) *and 9.2* (tertiary amine)

The changes in the UV spectrum are typically those of a phenolic group. Furthermore, previous studies have demonstrated that the 1-ethyl-2-pyrrolidinyl group of orthopramides has a pK_a value in the range of 9.0–9.4 [23]. Therefore, it can be concluded that the pK_a values 5.81 and 9.21 of raclopride correspond to the ionization of the phenolic and 1-ethyl-2-pyrrolidinium group, respectively. Raclopride at the physiological pH thus exists predominantly (96.0%) as a zwitterion, strongly suggesting this form to the biologically active one. However, all published electronic [24] and conformational calculations [17] [18] of raclopride have considered only the neutral form.

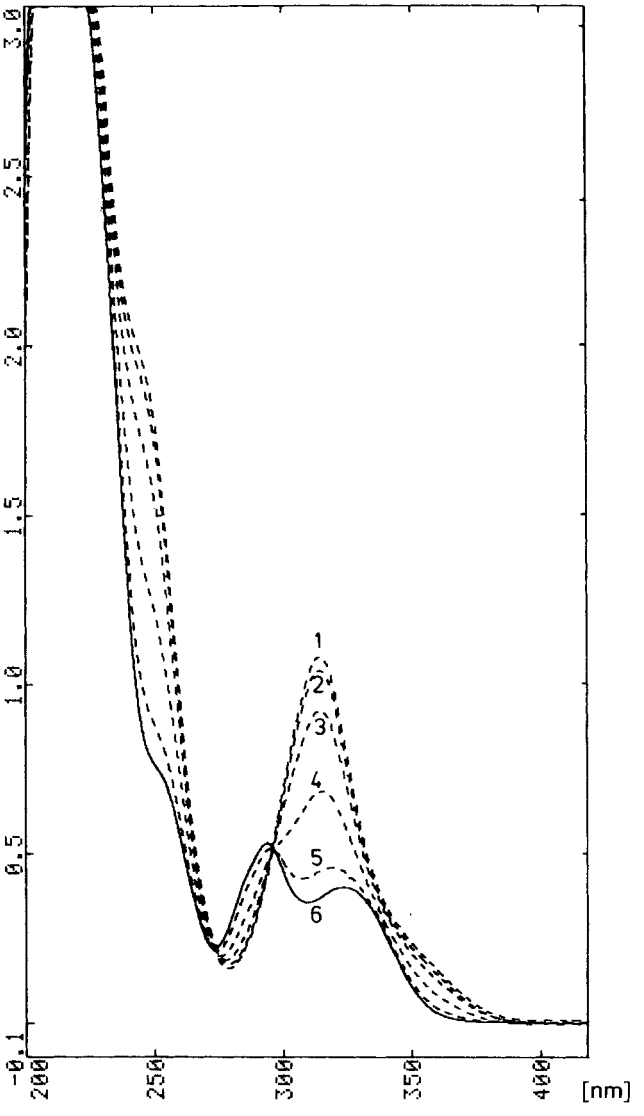


Fig. 3. *pH-Dependence of UV spectra of 0.24 mM solutions of raclopride in phosphate buffers:* 1: *pH 7.99*, 2: *pH 7.31*, 3: *pH 6.41*, 4: *pH 5.75*, 5: *pH 5.04*, 6: *pH 2.05*

Conformational Behavior of Raclopride As Investigated by the Tripos *Force Field.* The geometry of raclopride in its neutral and zwitterionic forms was minimized using the *Tripos* force field [25] with electrostatic terms using *Gasteiger* and *Marsili* atomic charges [26]. The conformational space generated by changing four torsional angles τ_1, τ_2, τ_3, and τ_4 (20° steps; *Fig. 4*) was explored as rigid rotor approximation (systematic search strategy), and the final structure was minimized again. For the *neutral form*, the complete minimization of conformers of low energy reveals two minima (*Table 1*).

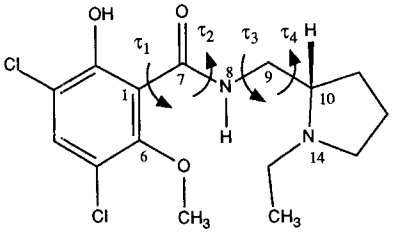


Fig. 4. *Definition of dihedral angles used in this study* (positive sign for counter-clockwise rotation)

Table 1. *Conformational Minima Found for Raclopride in the Neutral Form* (Energies in kcal/mol, angles in degrees, distances in Å)

Method	Conformer	ΔH_f	τ_1	τ_2	τ_3	τ_4	Ar–N[a])	N-aromatic plane[b])	N(8)H···OCH_3	OH···O=C(7)
SYBIL	S1	–	7.7	177.2	–148.2	–54.5	6.17	1.00	1.85	1.72
	S2	–	11.4	178.7	–176.1	–178.1	7.59	0.86	1.88	1.74
AM1	A1	108.8	44.5	–179.4	128.3	61.8	6.34	1.86	2.31	2.01
	A2	107.8	49.3	–175.2	136.0	–50.2	5.92	0.26	2.26	2.04
	A3	106.7	46.0	–178.0	114.4	172.3	7.24	0.82	2.25	2.03
PM3	P1	121.2	41.1	–175.6	126.6	64.5	6.44	1.85	2.35	1.80
	P2	121.4	42.9	–171.6	137.2	–53.2	5.98	0.01	2.32	1.81
	P3	120.5	42.2	–166.8	111.1	167.4	7.43	0.53	2.30	1.83

[a]) Distance between N(14) and the centroid of aromatic center.
[b]) Elevation of N(14) above the aromatic plane.

Although our exploration of the conformational space was not complete (the rotation of the MeO group and *N*-Et substituent was not included in the search step), the qualitative comparison of structural salient features of the two minima with both X-ray structure and previous molecular-modelling studies [17] confirm that the *Tripos* force field provides a reasonable conformational assessment of salicylamide derivatives. Indeed, the MeO group is oriented out of the plane of the aromatic ring due to a steric interaction with the Cl-atom. Two strong intramolecular H-bonds stabilize and approximately planar arrangement of the benzamide moiety. As for the basic side chain, its most stable conformation is the folded one ($\tau_3 = -148.2$, $\tau_4 = -54.5$). However, the energy of the extended conformation ($\tau_3 = -176.1$, $\tau_4 = -178.3$) is very close to the global minimum (< 0.5 kcal/mol).

Table 2. *Conformational Minima Found for Raclopride in the Zwitterionic Form* (Energies in kcal/mol, angles in degrees, distances in Å)

Method	Conformer	ΔH_f	τ_1	τ_2	τ_3	τ_4	Ar–N[a])	N-aromatic plane[b])	O···HN^+(14)
SYBL	S3	–	–144.8	–160.4	106.9	–37.3	4.90	1.31	1.52
AM1	A4	–81.8	–113.9	–159.6	79.6	–41.0	5.00	0.80	1.87
PM3	P4	–92.1	–110.7	–153.1	84.4	–41.1	4.98	0.25	1.67

[a]) Distance between N(14) and the centroid of aromatic center.
[b]) Elevation of N(14) above the aromatic plane.

The *zwitterionic form* of raclopride reveals a totally different conformational behavior. One highly priviledged conformer was apparent corresponding to a global minimum preferred by 8 kcal/mol over the next minimum (*Table 2*).

As the calculation assumes gas-phase conditions, this global minimum arises from a strong electrostatic interaction between the two unsolvated opposite charges in the molecule. As a consequence, the two pseudo-rings no longer exist, and the amide bridge is almost perpendicular to the aromatic ring.

Conformational Behavior of Raclopride As Investigated by the AM1 and PM3 Methods. To verify the reliability of these results the conformational analyses for the neutral and ionized forms were repeated using the AM1 method [27]. In a recent study [22], AM1 was shown to be superior to MINDO/3 and MNDO parameterization for predicting molecular geometries. If AM1-calculated rotational barriers in conjugated molecules are found to be too low, conformational preferences are quite well calculated by this method [28]. For example, the AM1-calculated energy difference between the minimum-energy conformer and the perpendicular conformer for benzamide itself was only 4.0 kcal/mol (compared to 12.1 kcal/mol found with *ab initio* calculations), but the calculated twist angle in the most stable conformer was of 36° [28] (compared to 30° by *ab initio* calculations, 28° by estimation from correlations of ^{17}O-NMR shifts with MM2 torsional angles [29], and 39° by measuring the dipole moment and the molar *Kerr* constant [30]).

To keep the computation time within reasonable limits, we explored the conformational space generated by changing only the torsion angles τ_1 and τ_4. At each grid point, the other geometric parameters, were optimized to take also into account variations in angles τ_2 and τ_3.

Fig. 5 illustrates the energy surface generated by the rotation of τ_1 and τ_4 for the *neutral form* of raclopride.

The global minimum of this surface corresponds to an extended conformer, while two other folded minima of low energy can also be identified (*Table 1*). With respect to the minima located using molecular mechanics, differences in both τ_1 and τ_3 can be noted.

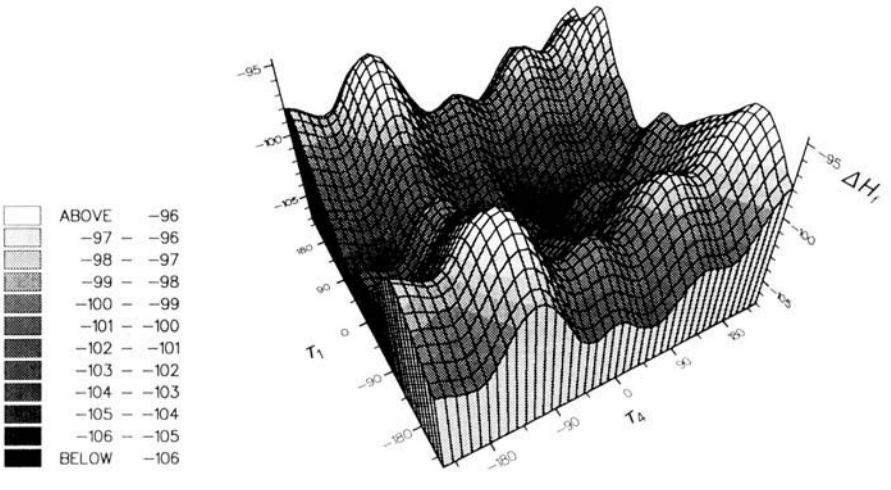


Fig. 5. *Three-dimensional conformational map of raclopride in the neutral form* (AM1 calculation)

Two reasons may explain the difference in τ_1: the inadequacy of AM1 to predict correct barriers for the rotation of single bonds in conjugated molecule [28] (underestimation of the resonance stabilization for planar conformers and thus, an overestimation of steric effects) or an uncorrect parametrization of force fields (based on crystallographic structures where crystal packing forces stabilize a planar conformation). The second change (τ_3) may come from the two different search strategies used in both molecular-mechanics and semi-empirical calculations. This change reflect a flip of the pyrrolidine ring above and below the CO–NH plane. The stability of the folded conformers is believed to arise from an electrostatic interaction and/or a partial H-bond between the basic N-atom and the amide proton.

The new PM3 parametrization [31] [32] was shown to be superior to the AM1 parametrization for the calculation of H-bonds, but it seems inadequate for the calculation of rotational barriers. For this reason, PM3 optimizations were performed on

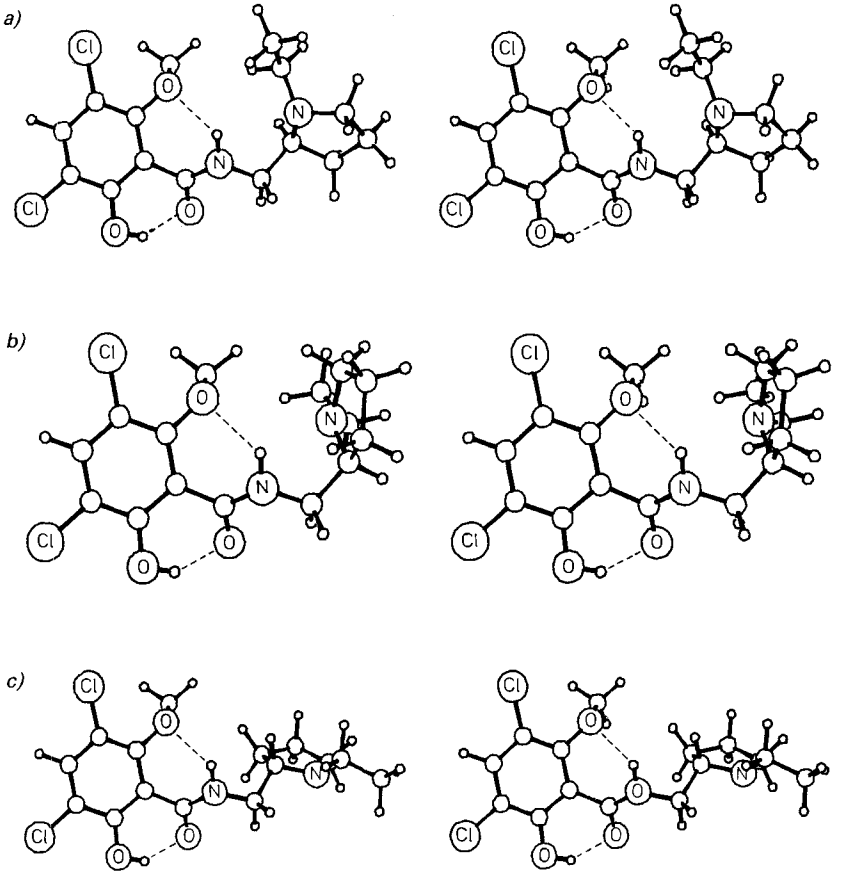


Fig. 6. *Stereoscopic view of raclopride in the neutral form.* The three conformational minima optimized by PM3 are: *a*) **P1**; *b*) **P2**; *c*) **P3**.

minima previously identified by the AM1 method (*Fig. 6* and *Table 1*). Qualitatively, the PM3 results are comparable to those obtained by AM1 with a slightly smaller deviation of the plane of the amide group from the plane of aromatic ring. The bond length of the intramolecular H-bond OH---O=C was closer to the experimental value than the AM1-calculated one, because the rotation of the OH group compensates the out-of-plane deviation of the amide group. The other intramolecular H-bond NH---OCH_3 can be seen but was calculated to be longer than the experimental value due to the smaller overlap between the lone pair of the MeO group and the amide proton due to the rotation of the amide group.

The AM1 conformational analysis of the *zwitterionic form* of raclopride confirms the results obtained by molecular mechanics. The energy surface resulting from the rotation of τ_1 and τ_4 (*Fig. 7*) reveals one region of low energy which corresponds to an electrostatic interaction between the phenolate and ammonium groups.

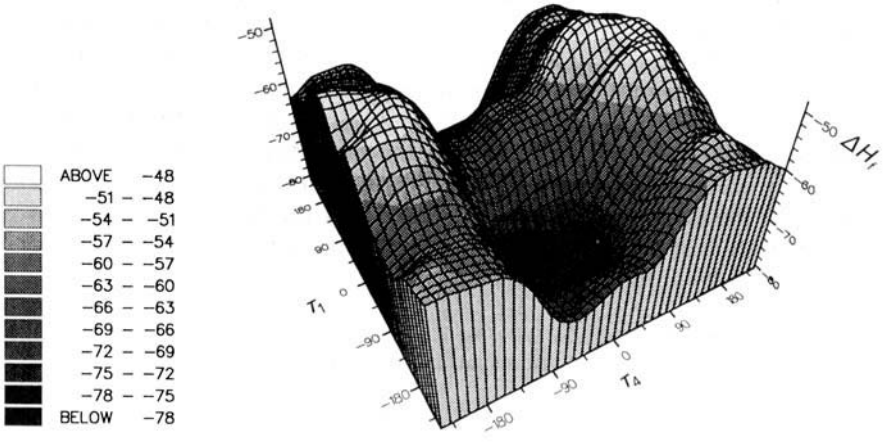


Fig. 7. *Three-dimensional conformational map of raclopride in the zwitterionic form* (AM1 calculation)

The calculated geometric parameters of the global minimum are reported in *Table 2*. The AM1 and PM3 calculations are close to those obtained by molecular mechanics (*Fig. 8* and *Table 2*) except for the differences in the angles τ_1 and τ_3. The larger partial atomic charges calculated by the semi-empirical parametrizations compared to those calculated by the *Gasteiger* and *Marsili* method may be responsible of a greater electrostatic repulsion between the carbonyl O-atom and the O-atom of the 6-MeO substituent and thus of the change in angle τ_1. The change in τ_3 is correlated to the variation in τ_1: when the τ_1 angle diminishes, the τ_3 angle must follow a similar variation to maintain the strong electrostatic interaction between the two charged groups.

A comparison between the energy surfaces generated by the rotation of τ_1 and τ_4 in the neutral and zwitterionic forms of raclopride clearly shows the conformational differences between both species, especially a complete inversion around the aromatic ring–amide substituent bond.

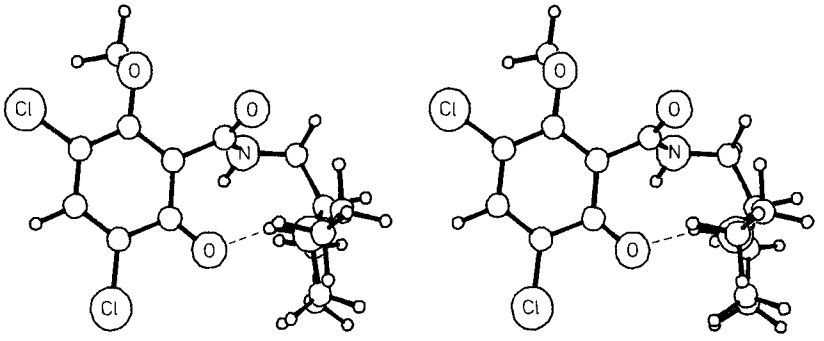
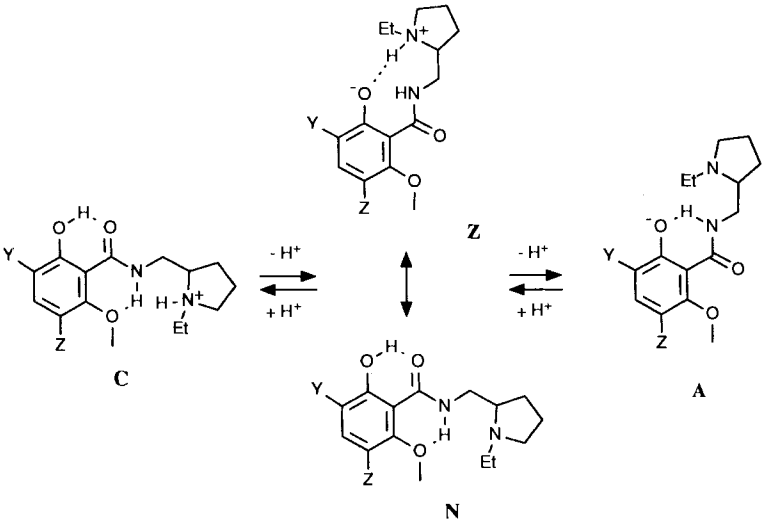


Fig. 8. *Stereoscopic view of raclopride in the zwitterionic form: the* **P4** *conformational minimum localized by PM3*

Mutual Interactions between Conformational and Ionic State in Raclopride. The acid-base behavior of salicylamides is summarized in the *Scheme.*

The population of different electrical forms of raclopride must result from a subtle balance between differents effects: *1*) The presence of electron-withdrawing substituents on the aromatic ring must increase the acidity of phenolic proton by stabilizing the phenolate species **Z** and **A**. *2*) The existence of intramolecular H-bonds (OH---O=C and NH---OCH_3) in the neutral and cationic forms would increase the pK_a of the phenolic group. *3*) The existence of a zwitterionic species **Z** stabilized by an ionic bond between the ammonium and phenolate group would increase the acidity of the phenolic function and the basicity of the amino function. *4*) In contrast, the resonance form **N** would tend to decrease both the acidity of the phenolic group and the basicity of the amino group. *5*) The stabilization of the anionic species **A** by an intramolecular H-bond (O---HN) may decrease the basicity of the amino function.

Scheme. *Acid-Base Behavior of Raclopride and Other 6-Methoxysalicylamides.* **C**: cationic form, **Z**: zwitterionic form; **N**: neutral form; **A**: anionic form.



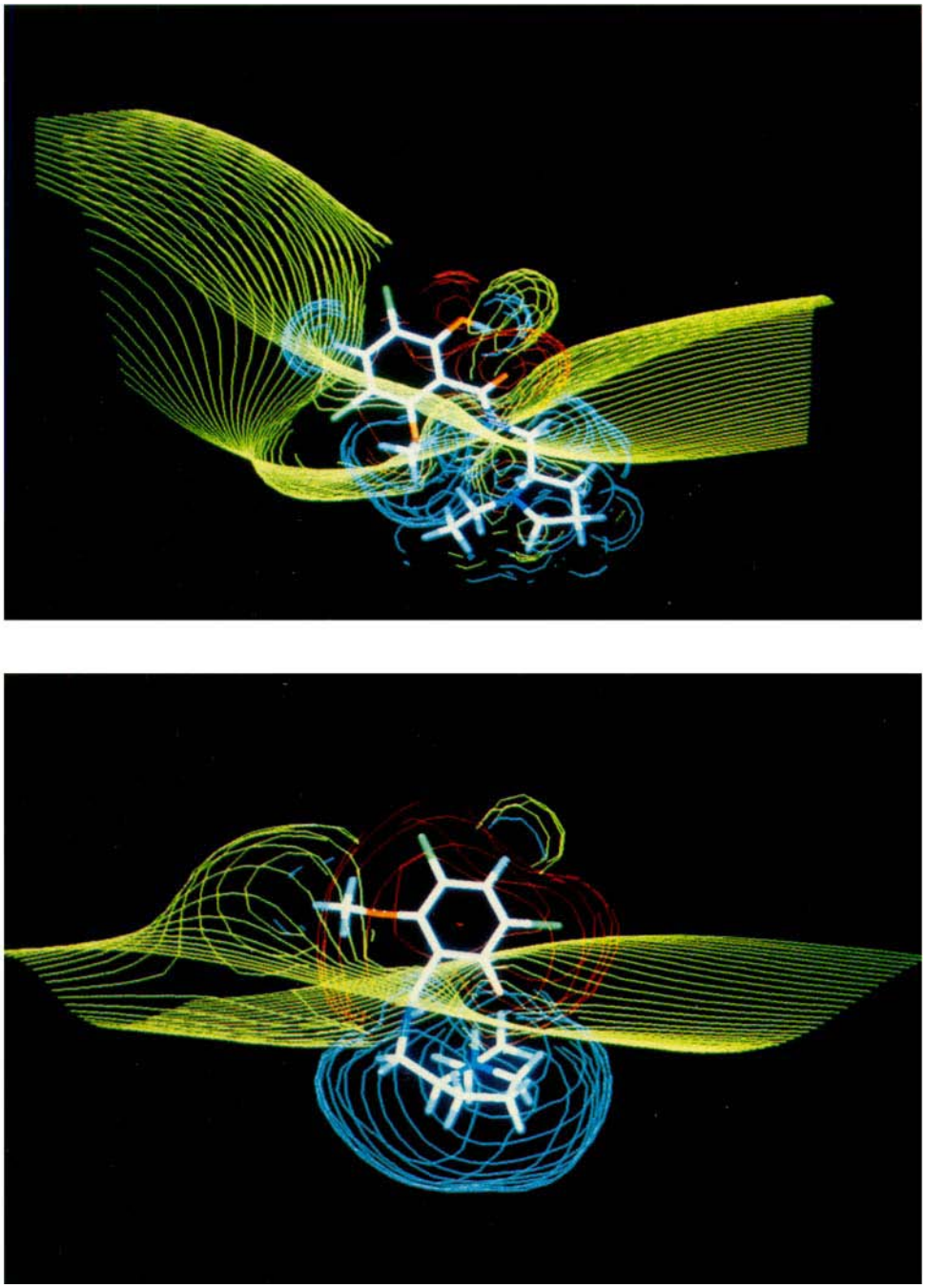


Fig. 9. *Representation of the 3-D electrostatic map of the raclopride* (**9A**) *and its zwitterionic form* (**9B**). Three isoenergy surfaces are represented: in red at −10 kcal/mol, in yellow at 0 kcal/mol, and in blue at +10 kcal/mol.

It is difficult to clarify the relative importance of these effects on the ionization of raclopride. Nevertheless, if their relative contributions change, so will the relative distribution of electrical states at physiological pH. In the 4-piperidinylbenzamide derivatives for example, the interaction between the phenolate and the ammonium group is possible only in high energy conformations of the piperidine ring. Thus, the zwitterionic species cannot be stabilized by the intramolecular ionic bond, and its pK_a enhancing effect would be lost. These variations could explain the different QSARs of aromatic substituents in salicylamides with an *N*-[(1-ethylpyrrolidin-2-yl)methyl] or *N*-(1-benzylpiperidin-4-yl) side chain [33] [34].

Even more relevant may be the observation that the presence of an *ortho*-OH group induces large QSAR differences in orthopramides *vs.* 6-methoxysalicylamides having the same *N*-[(1-ethylpyrrolidin-2-yl)methyl] side chain [34], strongly suggesting different modes of binding to the dopamine D_2 receptor. Hovewer, this difference appears limited to the aromatic region since both chemical series are active as the eutomer of (*S*)-configuration or as the eutomer of (*R*)-configuration when an *N*-benzyl group replaces the *N*-Et group [35].

Conclusion. – In summary, our calculations indicate a dramatically different conformational behavior of the neutral and zwitterionic forms of raclopride. However, it must be emphasized that these calculations will overestimate the strength of ionic interactions, since solvation effects are neglected. Furthermore, the balance of resonance and H-bond contributions in relation to the ionic attraction is difficult to assess correctly.

To date, all theoretical interpretations [17] of the biological properties of raclopride and its analogues have completely neglected the fact that some of these compounds should exist and act predominantly as zwitterions. But as illustrated by 3-D electrostatic maps (*Fig. 9*) for raclopride and its zwitterionic form, the change in ionization state affects not only the distribution of minimum-energy conformers at physiological pH but also the stereoelectronic characteristics of the compound.

This implies that some of the published stereoelectronic studies on 6-methoxysalicylamides may have limited biological relevance and will have to be re-investigated taking the ionization behavior into account. Studies are in progress in our laboratories to better understand the origin of pK_a variations in substituted 6-methoxysalicylamides and to determine the effect of ionization on both stereoelectronic structures and biological behaviour of these derivatives.

We are grateful to Prof. *B. Vittoz* (EPF-Lausanne) and Prof. *H. Bühlmann* (ETH-Zürich) for generous gift of computing time. We thank also Prof. *M. Mutter* for the use of the Silicon Work Station. This study was supported by the *Swiss National Science Foundation* (grant No. 31-27531.89).

REFERENCES

[1] P. Jenner, C. D. Marsden, *Life Sci.* **1979,** *25,* 479.
[2] L. I. Goldberg, J. D. Kohli, *Commun. Psychopharmacol.* **1979,** *3,* 447.
[3] T. Högberg, *Drugs Fut.* **1991,** *16,* in press.
[4] T. de Paulis, A. Janowsky, R. M. Kessler, J. A. Clanton, H. E. Smith, *J. Med. Chem.* **1988,** *31,* 2027.
[5] A. Pannatier, L. Anker, B. Testa, P.-A. Carrupt, *J. Pharm. Pharmacol.* **1981,** *33,* 145.
[6] H. van de Waterbeemd, B. Testa, *J. Med. Chem.* **1983,** *26,* 203.
[7] H. van de Waterbeemd, B. Testa, *Helv. Chim. Acta* **1981,** *64,* 2183.
[8] L. Anker, J. Lauterwein, H. van de Waterbeemd, B. Testa, *Helv. Chim. Acta* **1984,** *67,* 706.
[9] C. R. Clark, L. E. Garcia-Roura, *J. Chromatogr. Sci.* **1989,** *27,* 111.
[10] M. Cesario, C. Pascard, M. El Moukhtari, L. Jung, *Eur. J. Med. Chem.* **1981,** *16,* 13.
[11] L. Y. Y. Ma, N. Camerman, A. Camerman, *Acta Crystallogr., Sect. B* **1982,** *38,* 2861.
[12] T. de Paulis, Y. Kumar, L. Johansson, S. Rämsby, L. Florvall, H. Hall, K. Angeby-Möller, S. O. Ögren, *J. Med. Chem.* **1985,** *28,* 1263.
[13] T. de Paulis, Y. Kumar, L. Johansson, S. Rämsby, H. Hall, M. Sällemark, K. Angeby-Möller, S. O. Ögren, *J. Med. Chem.* **1986,** *29,* 61.
[14] T. Högberg, T. de Paulis, L. Johansson, Y. Kumar, H. Hall, S. O. Ögren, *J. Med. Chem.* **1990,** *33,* 2305.
[15] A. Wägner, B. Stensland, I. Csöregh, T. de Paulis, *Acta Pharm. Suec.* **1985,** *22,* 101.
[16] T. Högberg, S. Rämsby, T. de Paulis, B. Stensland, I. Csöregh, A. Wägner, *Mol. Pharmacol.* **1986,** *30,* 345.
[17] T. Högberg, U. Norinder, S. Rämsby, B. Stensland, *J. Pharm. Pharmacol.* **1987,** *39,* 787.
[18] T. Högberg, S. Rämsby. S. O. Ögren, U. Norinder, *Acta Pharm. Suec.* **1987,** *24,* 289.
[19] L. Z. Benet, J. E. Goyan, *J. Pharm. Sci.* **1967,** *56,* 665.
[20] L. A. Leeson, M. Brown, *J. Pharm. Sci.* **1966,** *55,* 431.
[21] A. Albert, E. P. Serjeant, 'The Determination of Ionization Constants', 3rd edn., Chapman and Hall, London, 1984, Vol. 4, pp. 70–95.
[22] J. J. P. Stewart, *J. Comput.-Aided Mol. Des.* **1990,** *4,* 1.
[23] N. El Tayar, G. J. Kilpatrick, H. van de Waterbeemd, B. Testa, P. Jenner, C. D. Marsden, *Eur. J. Med. Chem.* **1988,** *23,* 173.
[24] U. Norinder, T. Högberg, *Acta Pharm. Nord.* **1989,** *1,* 75.
[25] M. Clark, R. D. Cramer III, N. Van Opdenbosch, *J. Comput.-Aided Mol. Des.* **1989,** *10,* 892.
[26] J. Gasteiger, M. Marsili, *Tetrahedron* **1980,** *36,* 3219.
[27] M. J. S. Dewar, E. G. Zoebisch, E. F. Healy, J. J. P. Stewart, *J. Am. Chem. Soc.* **1985,** *107,* 3902.
[28] W. M. F. Fabian, *J. Comput. Chem.* **1988,** *9,* 369.
[29] A. L. Baumstark, P. Balakrishnan, M. Dotrong, C. J. McCloskey, M. G. Oakley, D. W. Boykin, *J. Am. Chem. Soc.* **1987,** *109,* 1059.
[30] R. K. Pierens, A. J. Williams, *J. Chem. Soc., Perkin Trans. 2* **1980,** 235.
[31] J. J. P. Stewart, *J. Comput. Chem.* **1989,** *10,* 209.
[32] J. J. P. Stewart, *J. Am. Chem. Soc.* **1989,** *111,* 221.
[33] T. de Paulis, H. Hall, Y. Kumar, S. Rämsby, S. O. Ögren, T. Högberg, *Eur. J. Med. Chem.* **1990,** *25,* 507.
[34] T. de Paulis, N. El Tayar, P. A. Carrupt, B. Testa, H. van de Waterbeemd, *Helv. Chim. Acta* **1991,** *74,* 241.
[35] T. Högberg, P. Ström, T. de Paulis, B. Stensland, I. Csöregh, K. Lundin, H. Hall, S. O. Ögren, *J. Med. Chem.* **1991,** *34,* 948.