212. PCILO and CD. Conformational Study of Sulpiride, a Dopamine Antagonist

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Summary

The conformational behaviour of sulpiride has been investigated by the quantum mechanical PCILO method and by CD. measurements. The results indicate the predominance of a folded, intramolecularly H-bonded conformer, with extended conformers being only slightly less stable. The pharmacological relevance of these results is briefly discussed.

1. Introduction. - Substituted *o*-anisamides (substituted *o*-methoxybenzamides, **1**) are a group of dopamine (**2**, DA) receptor antagonists of current interest [1], the mechanisms of action of which are not fully understood. One of the first benzamide drugs was sulpiride (**3**), the active enantiomer of which is (−)-(*S*)-sulpiride [2] [3].

Among a variety of DA receptors, benzamide drugs seem to act selectively as antagonists on D-2 receptors, *i.e.* a population of DA receptors not linked to adenylate cyclase [1] [4-7]. Structural properties of benzamide drugs must account for this selectivity. Their 3-dimensional properties, *i.e.* their conformational and topographical features, become of importance in rationalizing and improving DA receptors selectivity. In a previous work [8], the conformational behaviour of metoclopramide (**4**), another substituted benzamide, has been investigated by the quantum mechanical PCILO method using two models. Metoclopramide is thus believed to have only limited conformational freedom due to the presence of two intramolecular H-bonds acting as conformational locks (**4**). In this preferred conformation, metoclopramide is characterized by a distance of 5.95 Å between the centre of the aromatic ring and the N-atom, *i.e.* practically 1 Å longer than in the fully extended dopamine conformers. This suggests different topographical properties of DA agonists and antagonists acting at D-2 receptors [8].

In the present study, we investigate the conformational behaviour of sulpiride using theoretical (PCILO) and experimental (CD.) methods. The results essentially confirm those obtained with metoclopramide (**4**) [8].

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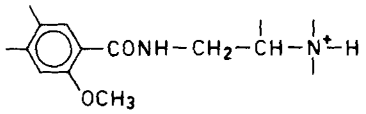

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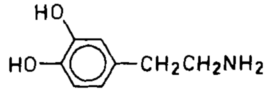

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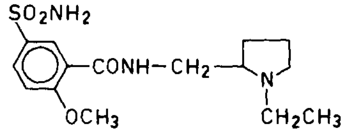

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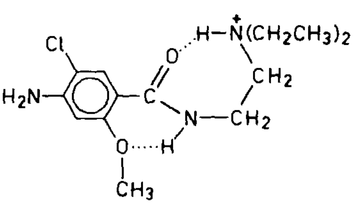
2. Theoretical conformational analysis. - The quantum mechanical method used is an all-valence-electron semi-empirical procedure, the PCILO method (perturbative configuration interactions using localized orbitals) [9]. This method has been used successfully for several years in the theoretical conformational analysis of numerous biomolecules and drugs (*e.g.*, [10]). A model molecule, *N*-(1-methyl-2-pyrrolidinylmethyl)-*o*-anisamide (**5**) is used in the calculation. This choice is dictated by the assumption that aromatic substituents in the 4- and 5-position influence only negligibly the conformation of the basic side chain, an assumption proven correct by the NMR. conformational analysis of metoclopramide (**4**) and analogues [11].

The molecule **5** is taken in its protonated form which is predominant under physiological conditions (see later). Standard bond lengths and valency angles are used, as obtained from crystallographic data [12]. Only two degrees of conformational freedom are considered, namely rotation about the NC–CN$^+$-bond (τ_1) and rotation about the CC–NC (=O)-bond (τ_2) (**5**). The pyrrolidine ring is blocked in a low-energy, envelope conformation [13], while the aromatic amide group is kept locked in an intramolecular H-bond with the methoxy O-atom, in agreement with previous calculations [8].

The torsion angle τ_1 and τ_2 are defined according to *Klyne & Prelog* [14]. They were varied by 30° increments, or 5° steps in the vicinity of energy minima. Formula **5** displays these two angles in their zero value. The conformational energy map of *N*-(1-methyl-2-pyrrolidinylmethyl)-*o*-anisamide is given in *Figure 1* showing topological features of interest. One global and three local energy minima are apparent, their salient properties being summarized in the *Table*.

The two folded *(gauche)* conformers ($\tau_1 = 90°$), and particularly the preferred conformer ($\tau_1 = 90°$; $\tau_2 = 330°$) have (N$^+$,O=)-distances indicative of an intramolecular H-bond [13], which appears as the factor governing the conformation of the side chain, as in metoclopramide (**4**) [8]. In these two *gauche* conformers,



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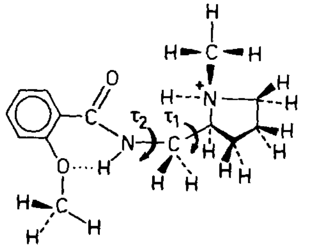

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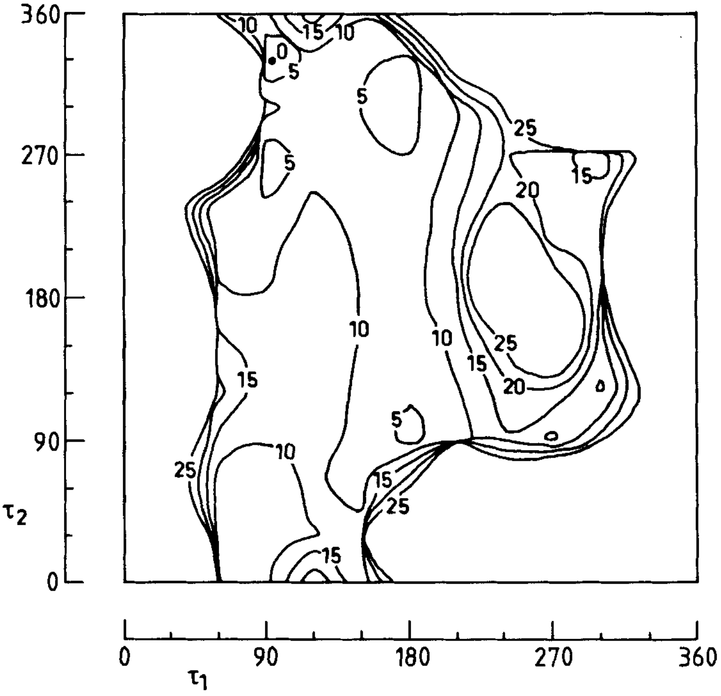

Fig. 1. *The conformational energy map of* N-*(1-methyl-2-pyrrolidinylmethyl)*-o-*anisamide.* Definition of torsion angles in formula **5**. The isoenergy contours are in kcal/mol.

the distance between the centre of the aromatic ring and the basic N-atom is calculated to be *ca.* 6.2 Å, compared to 6.3 Å found for metoclopramide in the crystal state [15].

As opposed to metoclopramide analogues [8], energy minima are also found for extended *(trans)* conformers ($\tau_1 = 180°$) (see *Table*). These conformers differ even more from dopamine (**2**) than do the *gauche* conformers, since the (N^+/aromatic centre)-distance is now larger than 7 Å. The energy difference between the folded ($\tau_1 = 90°$) and extended ($\tau_1 = 180°$) conformers *(Table)* are rather moderate and do not lead to conclusions at this stage, considering the uncertainties of the method.

Table. *Topological features of* N-*(1-methyl-2-pyrrolidinylmethyl)*-o-*anisamide* (**5**) *as a function of the torsion angles* τ_1 *and* τ_2

τ_1	τ_2	Energy (kcal/mol)	Distances in Å		
			N^+/arom. centre	N^+/molec. plane	N^+/O=
90	330	0	6.16	0.03	2.56
90	270	1.96	6.29	2.13	3.45
180	315	2.79	7.12	1.32	4.00
180	100	4.21	7.24	1.75	4.69

3. Circular dichroism. - The circular dichroism of (+)-(*R*)-sulpiride ((+)-**3**) is shown in *Figure 2* as a function of pH. No detectable differences appear in the spectra recorded between pH 2-8, these spectra showing two positive *Cotton* effects (294 and 245 nm) and a negative one at 212 nm. As the pH is increased from 8 to 10, the three CD. bonds progressively decrease. Above pH 10, only a small and poorly measurable band at *ca.* 210 nm remains.

The variation of the three bands is plotted as a function of pH in *Figure 3*, showing indeed the same pH dependency for the three bands and suggesting these curves to reflect the protonation/deprotonation of sulpiride. From the three curves in *Figure 3*, a spectroscopic pK_a of 9.13±0.16 is calculated. The pK_a of sulpiride (**3**) determined by potentiometry (see experimental part) was found to be 9.12±0.04, indicating that the pH-dependent variation in the CD. spectrum of sulpiride reflects a ionization-dependent conformational equilibrium. Such an equilibrium is compatible with the presence of an intramolecular H-bond existing in the protonated form but disappearing upon deprotonation.

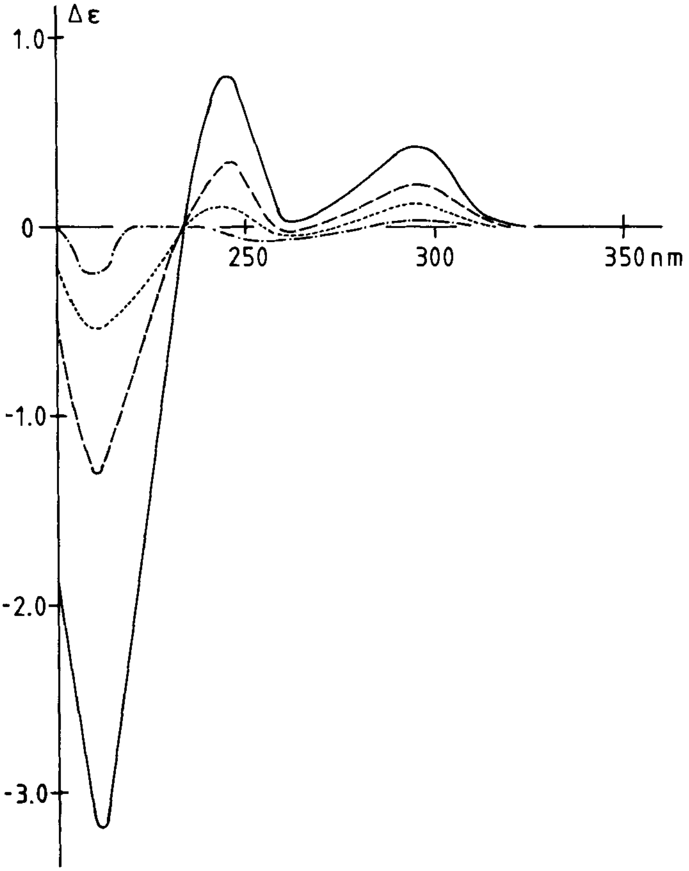

Fig. 2. *The CD. spectrum of (+)-sulpiride in water at pH values of 2-8 (——), 9.17 (- - -), 9.5 (····) and 10.2 (-·-)*

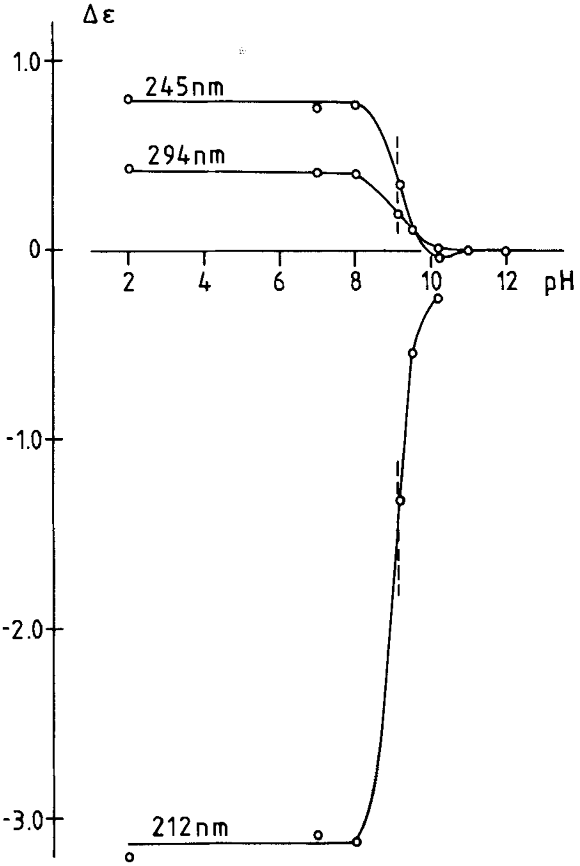

Fig. 3. *A plot of Δε vs. pH for the three CD. bands of (+)-sulpiride.* The potentiometrically determined pK_a is indicated at 9.12 by a vertical broken line.

Therefore the present CD. study confirms the finding of quantum calculations that the preferred conformation of protonated sulpiride is a folded, intramolecularly H-bonded, form. The CD. spectra however do not rule out the simultaneous presence of a population of extended conformers.

4. Discussion. - The protonated form of sulpiride (**3**) is the physiologically significant one, and the above PCILO and CD. investigations indicate the predominance of a folded, intramolecular H-bonded conformer. However, the conformational calculations indicate extended conformers to be less stable by only 3-4 kcal/mol *(Table)*. Such an energy difference can be expected to be even smaller if hydration factors are considered. It is therefore difficult from the present results to determine the pharmacologically active conformation of sulpiride, *i.e.*, that conformation which is topographically complementary to the dopamine receptor(s) antagonized by the drug. A similar ambiguity is apparent when other neuroleptics are considered [15] [16], making it difficult at this point to draw conclusions regarding the conformation-activity relationships of these flexible compounds.

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Experimental Part

1. Potentiometric pK_a determination. - The pK_a of sulpiride was determined at 25° and a ionic strength (I) of 0.1 using a *Metrohm* E 535 Dosimat and a E 536 potentiograph. The water used was CO_2-free. For the calculation, the method of *Benet & Goyan* [17] was used. This method, which is based on a non-logarithmic linearization of the titration curve, has been improved by *Leeson & Brown* [18] in order to take into account the dilution occurring during the titration. A *Diehl* Alphatronic desk calculator was used for the calculation.

2. Spectroscopic measurements of (+)-(*R*)-sulpiride. - The UV. spectra were measured on a *Beckman* 25 spectrophotometer, and the CD. spectra on a *Roussel-Jouan Mark* III dichrograph. The temperature was 21 ± 1°.

At pH 2.0 (HCl 0.01N). UV. (nm, ε): 290 (2300), 212 (32600). - CD. (nm, $\Delta\varepsilon$): 294 (0.430), 245 (0.805), 212 (−3.22).

At pH 7.00 (phosphate 0.1M). UV.: 290 (2300), 212 (32600). - CD.: 294 (0.415), 245 (0.751), 212 (−3.07).

At pH 8.12 (phosphate 0.01M, I=0.1). UV.: 290 (2300), 212 (31400). - CD.: 294 (0.405), 245 (0.771), 212 (−3.12).

At pH 9.17 (NH_4Cl/NH_4OH 0.01M, I=0.1). UV.: 289 (2200), 212 (29800). - CD.: 294 (0.225), 245 (0.351), 210 (−1.32).

At pH 9.51 (NH_4Cl/NH_4OH 0.01M, I=0.1). UV.: 288 (2150), 212 (29500). - CD.: 294 (0.112), 245 (0.107), 210 (−0.536).

At pH 10.18 (NH_4Cl/NH_4OH 0.01M, I=0.1). UV.: 288 (2150), 210 (28500). - CD.: 300 (0.029), 250 (−0.078), 210 (−0.24).

REFERENCES

[1] *P. Jenner & C.D. Marsden*, Life Sci. *25*, 479 (1979).
[2] *M. Hofmann, G.C. Jommi, O. Montefusco, P.F. Spano & M. Trabucchi*, J. Neurochem. *32*, 1547 (1979).
[3] *P. Jenner, A. Clow, C. Reavill, A. Theodorou & C.D. Marsden*, J. Pharm. Pharmacol. *32*, 39 (1980).
[4] *J.W. Kebabian & D.B. Calne*, Nature *277*, 93 (1979).
[5] *P. Laduron*, Trends Pharmacol. Sci. *1*, 471 (1980).
[6] *J.W. Kebabian & T.E. Cote*, Trends Pharmacol. Sci. *2*, 69 (1981).
[7] *P. Sokoloff, M.P. Martres & J.C. Schwartz*, N.S. Archiv. Pharmacol. *315*, 89 (1980).
[8] *A. Pannatier, L. Anker, B. Testa & P.A. Carrupt*, J. Pharm. Pharmacol. *33*, 145 (1981).
[9] *S. Diner, J.P. Malrieu, F. Jordan & M. Gilbert*, Theor. Chim. Acta *15*, 100 (1969).
[10] *B. Pullman*, 'Quantum Mechanics of Molecular Conformations', Wiley, London, 1976.
[11] *L. Anker, B. Testa & J. Lauterwein*, in preparation.
[12] *L.E. Sutton* (Ed.), Tables of interatomic distances and configuration in molecules and ions, The Chemical Society, London (1965).
[13] *B. Testa*, 'Principles of Organic Stereochemistry', Dekker, New York, 1979.
[14] *W. Klyne & V. Prelog*, Experientia *16*, 521 (1960).
[15] *M. Cesario, C. Pascal, M. El Moukhtari & L. Jung*, Eur. J. Med. Chem. *16*, 13 (1981).
[16] *J.P. Tollenaere, H. Moereels & M.H.J. Koch*, Eur. J. Med. Chem. *12*, 199 (1977).
[17] *L.Z. Benet & J.E. Goyan*, J. Pharm. Sci. *56*, 665 (1967).
[18] *L.J. Leeson & M. Brown*, J. Pharm. Sci. *55*, 431 (1966).